Anaerobic Digestion of *Saccharina Latissima*

Birmingham City University
Faculty of Computing, Engineering and the Built Environment
School of Engineering and Built Environment

Roshni Paul

Supervisors:
Prof. Lynsey Melville
Dr. Sri Suhartini
Dr. Michael Sulu

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“The more you know, the more you know you don't know.”

Aristotle

Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where states otherwise by reference or acknowledgment, the work presented is entirely my own.

Name: Roshni Paul

Date: 15/11/18

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Abstract

Anaerobic digestion, AD, of brown macroalgae biomass species *Saccharina latissima* (*S. Latissima*) was investigated in this research study for its biochemical methane potential in batch and continuous operations, co-digestion performance and techno-economic feasibility. Environmental conditions of the cultivation site were observed to have a significant effect on the organic and inorganic content of the samples from different locations. For S.Latissima harvested from three different locations, the specific methane production of biomass from Strangford Lough was found to be $0.393\, L\, CH_4/kg\, VS\, added$, while biomass from Ventry Harbour had a methane production of $0.391\, L\, CH_4/kg\, VS\, added$. The lowest of the specific methane production was shown for the biomass from Isle of Seil with a value of $0.265\, L\, CH_4/kg\, VS\, added$. Growth cycle of the macroalgae was found an important indicator to optimise harvest times to target highest storage carbohydrates in the biomass. Highest methane production was obtained for summer biomass which showed highest carbon percentages and C/N ratios. Biomass characteristics of wild and cultivated *S. Latissima* was found to be significantly different. The specific methane potential of the macroalgae biomass exhibited an inverse relationship with the volatile solids and ash content. Co-digestion with *S. Latissima* in a 70:30 ratio resulted in higher rates of methane production and methane yields. Co-digestion of wheat and sugar beet – vegetable mix was found synergistic with *S. Latissima* with a specific methane production of 0.472 and 0.373 $L\, CH_4/kg\, VS\, added$ however antagonistic with pig manure with methane production of 0.172 $L\, CH_4/kg\, VS\, added$. The net percentage increase observed for wheat was 21.59%, and 15.95% of SBV mix however a decrease of -17.43% for pig manure while co-digested with S. Latissima. Semi-continuous digestion experiments showed that mesophilic temperatures were more stable with higher gas production while thermophilic digesters had higher volatile solids destruction. In the case of *S. Latissima* macroalgae biomass, addition of trace element solution only enhanced the methane concentrations in the biogas production. In the techno economic analysis, AD of *S. Latissima* as a monodigestion feedstock was found to be economically not feasible due to the high price of the macroalgae biomass. However co-digestion with sugar beet – vegetable mix with a gate fee of 29 Euros per tonne was found to be economically feasible with macroalgae biomass priced at 50 Euros per tonne.

Table of Contents

List of Figures

List of Tables

List of Equations

Nomenclature

Acronyms

AD	Anaerobic digestion
BMP	Biochemical methane potential
COD	Chemical oxygen demand
CV	Calorific value
QUB	Queens University Belfast
SAMS	Scottish Association for Marine Sciences
SMP	Specific methane potential
TRL	Technology readiness level
TS	Total solids
VS	Volatile solids
WW	Wet weight

1 Introduction

This research study titled “Anaerobic digestion of Saccharina latissima” intends to investigate the potential of the species *Saccharina latissima* for anaerobic digestion purposes. *S. Latissima* has previously been reported to be a feasible feedstock for AD due to its favourable biomass composition for easier degradation during microbial reactions of AD. However, the impact of environmental conditions, harvest times and growth type on the biomass is not completely understood to utilise the species. In addition, there have been few studies on the performance of *S. Latissima* during continuous studies and its techno-economic potential for large scale applications. The outline of this thesis is given in Figure 1.

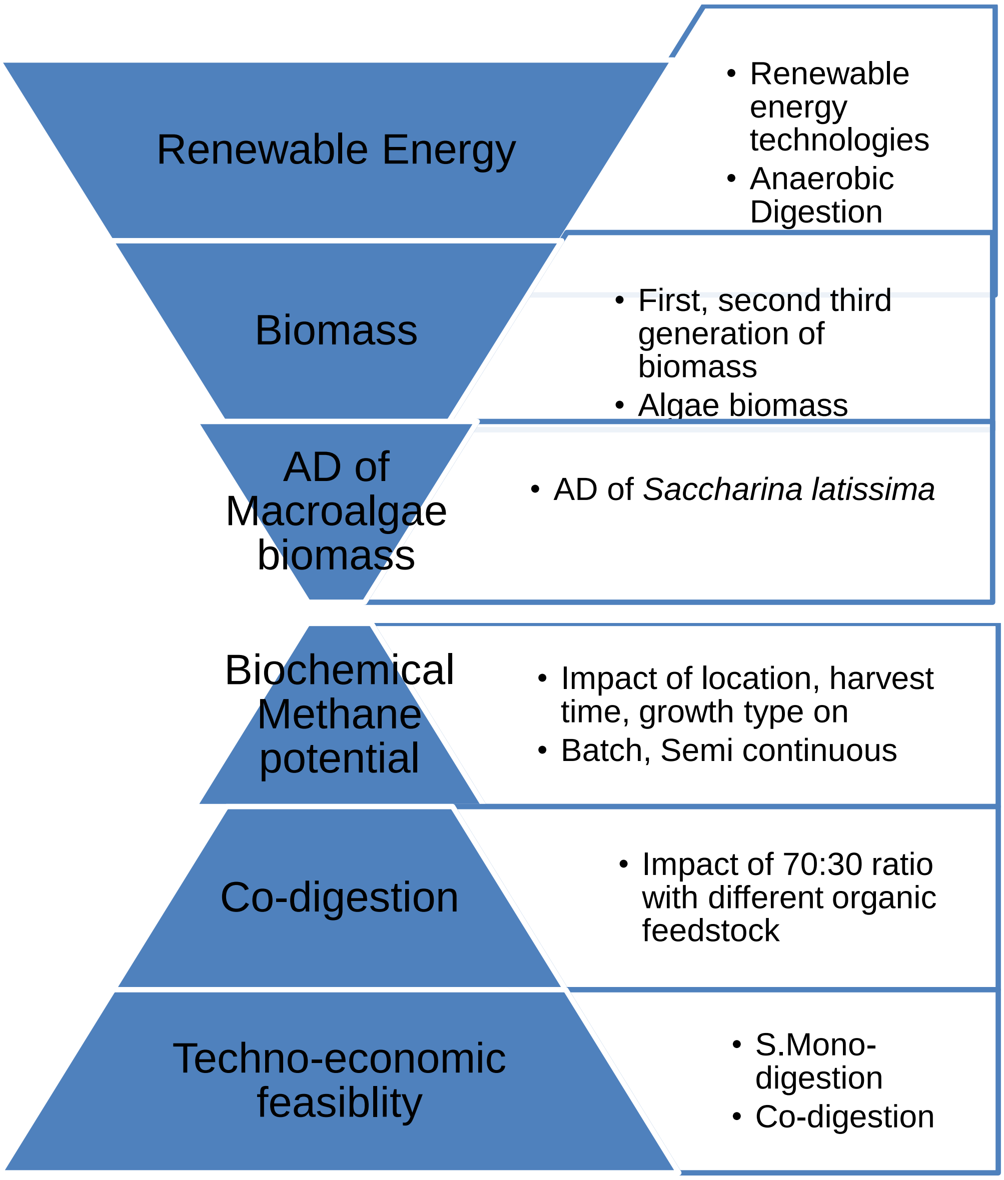

Figure 1 Thesis outline diagram

Aim and Objectives

The aim of this study is to optimise the anaerobic digestion of macroalgae biomass species *Saccharina latissima*, (*S. Latissima*).

The objectives for this study are as follows: -

- To determine the extent to which biomass characteristics vary with environmental conditions, macroalgae growth cycle and growth type
- To identify the differences in the biomethane potential of Saccharina latissima related to its characteristics varying with environmental conditions, harvest times and growth type
- To investigate the semi-continuous digestion performance of Saccharina latissima for three hydraulic retention times
- To identify the characteristics of Saccharina latissima for co-digestion with other organic feedstock
- To determine the techno-economic feasibility of Saccharina latissima as a mono-digestion and co-digestion feedstock

1.1 Renewable energy and Anaerobic Digestion

Energy demand has continued to grow globally in recent decades. The decline of natural resources, environmental deterioration due to climate change, the growing need for electricity and power, and the exponential growth of the human population has contributed massively to the energy crisis (Mercuri *et al.*, 2016). Increasing concerns over the impacts of greenhouse gases (GHG) emissions and global warming have also triggered the search for alternative cleaner energy resources. Global climate change can only be tackled by improving energy efficiency and reducing energy demand (Sorrell, 2015). The International Energy Agency (IEA) and other similar bodies worldwide are placing priority on reducing energy demand, and in Europe, the European Commission (EC) has proposed long-term targets for energy demand reduction. Countries all over the globe are introducing a range of policies to help achieve similar targets (Hasanuzzaman *et al.*, 2017, Sorrell, 2015).

An overview of renewable energy resources is shown in Figure 2.

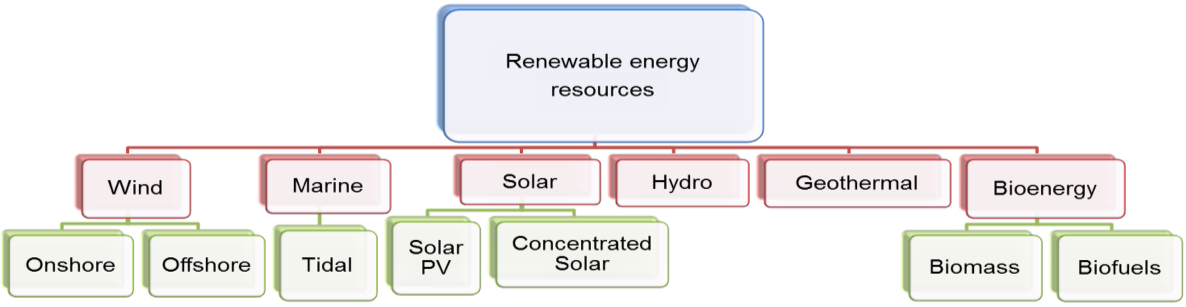

Figure 2 Renewable energy technologies

Source (Ellabban *et al.*, 2014)

In March 2007, the EU heads of state agreed to set a target of 20% of renewable energy use of the total EU energy needs by 2020 (Popp *et al.*, 2011). Very recently in 2018, the EU commission has reached a political agreement with a binding energy target for the EU for 2030 of 32% with a revision by 2023. The renewable energy progress report from the EU stated that in 2016, EU as a whole achieved a renewable energy share of 17% of the final energy consumption (EU Commission, 2018). In the UK, renewable electricity has had a specific delivery mechanism since 1990 and in 2003, the UK government White paper set out the bold vision of a sustainable energy future and an ambition to achieve 60% cuts in carbon dioxide emissions by 2050 (Mitchell and Connor, 2004). There has been a growing research since then with current focus on life cycle analyses (LCA) of new technologies and approaches to ascertain the sustainability of renewable technologies (Walker *et al.*, 2015).

Renewable energy technologies provide an alternative to fossil fuels, however designing and implementing them on a commercial level has technical and financial challenges associated with it (Martinez-Val, 2013). Transitioning of the fossil fuel-based economy to a green economy is an ongoing challenge and the higher penetration of renewable energy pathways is currently the backbone of green economy efforts (Gasparatos *et al.*, 2017). Therefore, more focus is drawn towards bioenergy or energy production from biomass or waste resources. Biomass overcomes some of the challenges of traditional renewable energy technologies by being geographically diverse and widely available, present in large quantities and with high potential for numerous energy vectors (gas, solid and liquid fuels) and utilisation pathways. Bioenergy has an important role to play if the UK is to meet its low carbon objectives by 2050. If biomass is excluded from the energy mix, then the cost of decarbonising our energy system would increase by £44 billion (Energy Technologies Institute, 2016). The UK renewable energy roadmap (2011) has also set out bioenergy as an important part of the UK government’s plan to meet the renewable energy directive objectives in 2020. The strategy defines uses of wastes, heating provided by biomass sources, biofuels for transport and use of sustainable biomass to generate electricity (DECC, 2012). Various pathways of utilising low carbon technologies to generate sustainable energy from sources like biomass will be discussed in the following sections.

1.2 Fundamentals of Anaerobic digestion

Anaerobic digestion (AD) is a commercial level conversion technology resulting in the production of renewable energy in the form of methane. Anaerobic digestion is a biochemical process where energy is released by the breakdown of substances in the absence of oxygen (Beavis and Charlier, 1987). Renewed interest in the renewable energy technologies has developed AD processes not only to produce methane to reduce the greenhouse gases emissions, but also as an effective solution for treatment and disposal of large quantities of organic wastes. AD provides a pathway for closed balanced carbon cycle with respect to atmospheric carbon dioxide and also utilise wastes and biomass as a significant renewable energy resource (Chynoweth *et al.*, 2001).

1.3 Products of anaerobic digestion

There are mainly 2 products of anaerobic digestion – Biogas and digestate.

1.3.1 Biogas

Biogas is a renewable gaseous fuel harvested from different varieties of organic waste as they break down under anaerobic conditions produces biogas (50 – 60%) and carbon dioxide (40 – 50%) by volume. In addition, the process produces trace gases such as ammonia, hydrogen sulphide, or nitrogen (Hornung, 2014). Currently biogas is mainly used to generate electricity for local use and for feeding into the national gas grid and fore renewable heat production through combined heat and power (CHP) mechanisms. When methane is injected into the national gas grid, then this leads to a replacement of natural gas and consequent reduction in GHG emissions (DEFRA, 2011).

1.3.2 Digestate

Digestate contains a solid fraction of undigested fibrous material and a liquid fraction high in nutrients such as nitrates and phosphates. Digestion of the biomass provides biogas, but the digestate is still high in organic content with dissolved nutrients that can be further utilised via nutrient stripping (N, P, K), or to produce secondary fuels including solid recovered fuel generation or use of fibrous materials extracted from the digestate in construction materials (DEFRA, 2011). Digestate can also be used as a bio-fertiliser. The composition of digestate varies considerably and this can impact the quality of the fertiliser applied on the land. This is crucial to the quality of the soil, the chemical and biological compounds in the digestate affecting the water resources, and crop cultivation (Hornung, 2014). The application of digestate is regulated by the framework of regulations – PAS (Publicly Available Specification, BSI 110). However, nitrogen vulnerable zone legislation, pathogen control and seasonal restrictions can limit the use of digestate on agricultural land. Therefore, a more recent focus has been in recycling the nutrients from digestate for growing newer resources such as algae and thereby enhancing sustainability within a circular economy (Stiles *et al.*, 2018).

1.4 Biochemical processes in anaerobic digestion

The AD process is mediated by microorganisms where organic matter is subjected to four interrelated and sequential steps (Horan *et al.*, 2011). Anaerobic digestion is a complex process which can be divided into four phases of degradation named hydrolysis, acidogenesis, acetogenesis, and methanogenesis according to the main process of degradation linked to each phase (Deublein and Steinhauser, 2011).

The mechanism of the anaerobic degradation occurs at the cellular level and involves varied microbial populations. The four main metabolic microbial groups that govern the biochemical reactions are hydrolytic fermentative bacteria, proton reducing acetogenic bacteria, hydrogenotrophic methanogens and acetoclastic methanogens (Zinder *et al.*, 1984). These groups of bacteria work in sequence, with the products of one group forming the substrates of the next group, where each group is linked to other groups in a chain like fashion (Gerardi, 2003). The four different stages of anaerobic digestion process are described below in Table 1 Four different stages of ADTable 1.

Table 1 Four different stages of AD

AD stage	Process & Duration	Governing bacterial population	Products	Significance	Reference
Hydrolysis	Complex biomass material is degraded into smaller counterparts. Carbohydrates - a few hours Proteins and lipids - a few days.	Hydrolytic bacteria or facultative anaerobes	Cellulosic material converted to simple glucose, proteins into amino acids, lipids into long chain fatty acids	Rate-limiting step in an AD process due to slow chemical decomposition of complex polymeric substances.	(Sorensen, 2011) (Deublein and Steinhauser, 2011) Gerardi, 2003)
Acido-genesis	Degradation of monomers formed in the hydrolysis stage into short chain organic acids C1 – C5 molecules. Quickest step in the AD process	facultative and obligatorily anaerobic bacteria	Volatile fatty acids such as butyric acid, propionic acid, acetate and acetic acid, alcohols, hydrogen, carbon dioxide	Lowering of pH and can lead to inhibition of the bacteria in the subsequent stages.	(Deublein and Steinhauser, 2011). (Vavilin et al., 2008) (Sørensen, 2011) (Hornung, 2014)

Acetogenesis	Fermentation of fatty acids into acetic acid, carbon dioxide, hydrogen and water.	homoacetogenic (acetate forming) bacteria	Acetic acid, carbon dioxide, hydrogen and water	Controls the quality and composition of biogas formed in the final stage	(Hornung, 2014) (Sorensen, 2011, Gerardi, 2003)
Methanogenesis	Formation of methane Can take up to weeks	methanogenic archaea - acetoclastic methanogens and hydrogenotropic methanogens	Biogas, Methane, ammonia	Longer retention times leading to inhibition and rate limiting step in the process	(Deublein and Steinhauser, 2011)

1.5 Factors influencing anaerobic digestion – Process parameters

The operation of an anaerobic digester is a complex process. Hence, these systems are very sensitive to small changes in conditions and each intermediate step is governed by strict requirements (Horan *et al.*, 2011). Optimised process control of anaerobic digesters is challenging because of the interrelationship between operational conditions and biomass composition. Operational parameters require constant monitoring and adjustment according to the biomass composition and the conditions within the reactor which can impact upon the bacterial populations (Gerardi, 2003). Factors affecting anaerobic digestion process are shown in Figure 3.

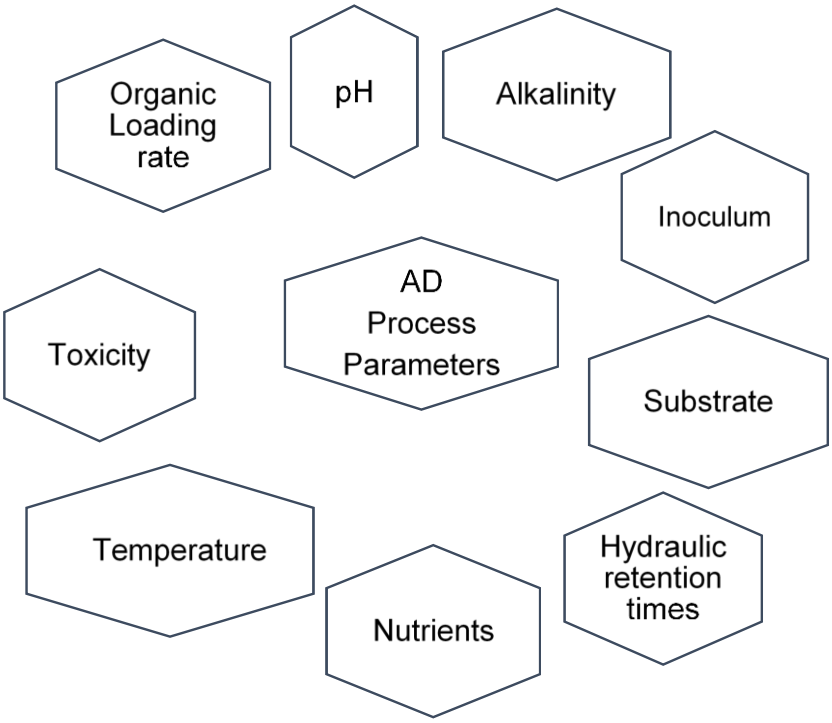

Figure 3 AD process parameters

1.5.1 Inoculum characteristics and acclimation

Anaerobic digestion functions in the degradation of the waste feedstock requiring a range of several types of bacteria and hence the use of an inoculum containing the optimal bacterial populations is crucial for the success of the degradation process (Angelidaki and Sanders, 2004). Primary and secondary sludge from an existing operational AD plants provide the bacteria required for hydrolysis and production of methane (Gerardi, 2003). The selection of suitable inoculum source is essential as the success of the start-up phase is crucial to reach stable operation and the type of bacteria found in the inoculum will also depend on the operational conditions and type of feedstock they have originated from (Fernandez *et al.*, 2001). The volume of inoculum added in an anaerobic batch system is usually dependent on the concentration of the feedstock but should be enough to avoid acidification and offer process stability (Hansen *et al.*, 2004). Acclimation is the process by which the bacteria and their communities adapt to a given set of environmental conditions. This stage is critical to any digestion as this affects the methane production rates and the activity of the bacteria in the presence of inhibitors (Chen *et al.*, 2008).

Once an anaerobic digester is seeded and is operating efficiently (acclimated), the feedstock can be fed into the reactor.

1.5.2 Feedstock characteristics

Characteristics of the feedstock relates to the physical, chemical and biological characteristics of the material including the composition of feedstock in its organic and elemental form which can either be favourable for AD or as toxic substances causing inhibition towards AD processes. The key to successful anaerobic digestion relies on the characteristics of the feedstock. This is measured by optimal biogas yields and the rates of organic degradation. The biochemical composition of the feedstock determines the quality of anaerobic degradation. The specific methane potential of a feedstock is influenced by a variety of factors such as: -

- Composition - The amount of carbohydrates, proteins and lipids in the feedstock. Feedstock rich in carbohydrates can be easily converted to volatile fatty acids (Jiang *et al.*, 2012). Lipid rich feedstock are also considered easily biodegradable but because of the accumulation of long chain fatty acids during hydrolysis of neutral lipids, they can also induce inhibition in the process.
- Presence of toxic substances - Proteinaceous feedstock might result in high ammonia levels by the degradation of nitrogenous matter (Chen *et al.*, 2008), and feedstock with lignin content reducing the hydrolysis rate and consequently reducing the extent of degradation (Mata-Alvarez *et al.*, 2000).
- Inoculum to feedstock (substrate) (I/S) ratio affects the biodegradability of an organic feedstock. Instability in the anaerobic processes can trigger acidification phenomena for a ration less than 0.5 gVS. Further studies have also shown that the biogas yield is in an inverse proportion to the I/S ratio in the range 1.6 – 5.0 gVS/gVS (Esposito *et al.*, 2012a).
- The main organic elements in an organic feedstock are C, H, O, N, and S and the minor inorganic elements are normally Si, Mg, Al, S, Fe, P, Cl, and Na. The major elements are present in higher percentages >1% while the minor elements in trace quantities (<1%). The elemental composition of a feedstock is a key factor as it can indicate the potential inhibitors for a successful anaerobic digestion (Vassilev *et al.*, 2010).

Apart from the feedstock characteristics, there are a number of key parameters that need to be monitored and controlled to ensure optimal conditions within the digester. These parameters (Figure 3) are however influenced by the feedstock choice and the degradation products evolved during the AD process.

1.6 Benefits and challenges of anaerobic digestion

Anaerobic digestion has its advantages as well as challenges. Anaerobic digestion is not only feasible in large scale industrial installations, but can also be applied to small scale applications, which makes it appropriate for community or domestic use in developed and developing countries and rural areas where energy supply is limited. It is a robust process and its benefits include treatment of high moisture containing biomass, and the suitability of various types of feedstock leading to higher process efficiencies (Appels *et al.*, 2011).

From a socio-economic perspective, centralised AD plants offer solutions to many challenges encountered in agriculture and rural districts. Digestate offers a nutrient rich fertiliser which can be applied to land and overcomes some of the issues of odour production from silage as well as application of manures to agricultural land in Nitrate Vulnerable Zones for example. The impact an AD plant has on the local community is significant as the possibility of local communities becoming energy efficient with clean renewable energy is higher which can also be in turn a strong motivating factor for AD implementation (Madsen *et al.*, 2011).

Based on the discussion in the previous sections, some of the key challenges for anaerobic digestion are given below.

- Limited understanding of the complex microbiological processes of AD
- Understanding utilisation of heterogeneous feedstock
- Developing biogas upgrading technologies to use biomethane and the digestate (Appels *et al.*, 2011).

The AD sector is growing in the UK but there are legislative barriers currently that needs to be overcome for successful AD practice (Madsen *et al.*, 2011). As discussed earlier there are opportunities for successful AD operation, however there still needs optimising practices such as pre-treatment techniques to achieve optimal yield of biogas from feedstock. The AD process can be optimised by improving the characteristics of the feedstock via pre-treatment techniques, or by controlling the different factors influencing AD and optimising the process parameters or by effectively changing the design of the reactors to enhance overall methane production from the process. An additional approach commonly adopted for AD optimisation is co-digestion of multiple feedstock. Co-digestion is digestion of more than one feedstock in combination, adopted to improve the characteristics of the composite feedstock. Additionally, co-digestion has also shown to enhance biogas production by increasing the nutrients or moisture deficiency that certain feedstock can have and when effectively treated anaerobically (Hornung, 2014). This can also have impacts on the commercial feasibility of a centralised facility where co-digestion is planned to be practiced with a base material (Callaghan et al., 2002). Therefore, it is important to understand the economic feasibility of the AD process to critically evaluate the performance of the digesters in their intended level of application.

Anaerobic digestion is widely used in European countries. Along with benefits such as pollution control, odour and pathogen level reduction, nutrient recovery and digestate production, energy production through biogas is of commercial interest provided the economics are favourable. Economic efficiency is dependent on investment and operating costs of the biogas plant and on optimum methane production. Biogas utilisation depends on biogas quality, and the levels of contaminants present in the biogas derived from the waste resources. Overall AD is reviewed as an economically favourable process however there is a need for more research to identify the best operational practices to enhance methane production from the chosen feedstock (Hublin *et al.*, 2014).

Despite advances in both design and configuration of AD systems and the optimisation of feedstock through pre-treatment, one of the prevailing challenges is security and consistency of feedstock supplies. The ambition to increase biogas yields lies in the availability of the easily digestible waste resources. The following sections will discuss the common and new biomass sources and characteristics of biomass feedstock used for AD. The section will also examine the advantages and disadvantages of using the selected feedstock for AD.

1.7 Biomass feedstock for anaerobic digestion

Biomass is a term defining any living or recently dead organic matter that can be converted to energy (McKendry, 2002a, Bracmort, 2015). Based on the characteristics of the raw materials, conversion processes and their features, biomass feedstock are categorised into first generation (e.g. food crops), second generation (e.g. energy crops) and third generation (e.g. algae) biomass feedstock and other feedstock (e.g. municipal solid wastes) (Srirangan et al., 2012).

The majority of the biomass which can potentially be used for anaerobic digestion can be classified into 2 main categories depending on the source from which it is derived.

- Virgin biomass

- Waste biomass

The different biomass feedstock used for anaerobic digestion is given in Table 2.

Table 2 Biomass feedstock used for AD

Feedstock type	Examples	Importance for AD	Reference
Virgin	First and third generation biomass resources	Can source from plants directly.	(Fantini, 2017)
Agricultural crop wastes	crop residues include wheat, wheat straw, grass, grass silage, vegetable residues, and purpose grown like sugar beet	Can optimise and reduce waste management. Average of 1530PJ/year is available for bioenergy production using crop residues	(EU Commission, 1999) (Lehtomäki and Björnsson, 2006, Parawira *et al.*, 2008) (Chandra *et al.*, 2012) (Scarlat *et al.*, 2010)
Industrial wastes	Food processing waste, and other organic solid wastes from industrial processes such as brewing etc.	Largest component of waste streams in the UK and Europe	(Khalid, 2011) (Zhang *et al.*, 2008)
Municipal solid waste	Digestible organic fraction (kitchen waste, grass cuttings, etc.), or indigestible fraction (synthetics, plastics) and an inert fraction (stones, sand, glass etc.)	Effective for energy production with waste treatment and environmental benefits	(Braber, 1995) (Chen *et al.*, 2008) (Cuetos *et al.*, 2008) (Hartmann and Ahring, 2006) (Benabdallah El Hadj *et al.*, 2009) (Tian *et al.*, 2015)
Manure	Chicken litter, cow manure, swine and other dairy sources	Up to 1.4 billion tonnes of livestock manure available in the EU.	(Foged *et al.*, 2012) (Makara and Kowalski, 2015) (Ward *et al.*, 2008)

Virgin biomass include wood, plants, leaves (lignocellulose) and crops and vegetables (carbohydrates), algae etc. while waste biomass include solid and liquid wastes municipal, industrial, sewage and agricultural and animal wastes. Virgin or primary biomass is directly sourced from plants while waste biomass is derived from the different biomass derived products (Fantini, 2017). This coincides with the classification of first, second and third generation of biomass feedstock where first and third generation sources can be included as virgin sources while second generation sources are considered as waste biomass. Predominantly, anaerobic digestion processes have been used as a waste management technology. AD has been used for sewage sludge treatment in the UK since the mid-twentieth century and has now been used to treat a wide variety of wastes including food waste, farm waste and industrial wastes. In the UK there are approximately 266 AD operational sites, 214 AD plants (about 40%) treating organic waste (Evangelisti et al., 2014). The AD plants in operation range in scale and scope and include on-farm, industrial, demonstration, commercial or form part of integrated waste management facilities. The variety of feedstock treated via UK AD plants include crops and farmyard manures (e.g. chicken manure, agricultural slurries, chicken litter, dairy manure), pig slurry and food waste, Rye, maize silage, brewery waste, municipal and business food waste, fruit and vegetable wastes, grass, brewery effluent, distillery wastes, and energy crops (WRAP, 2017).

While there is an abundant supply of organic material generated as waste from a variety of sources its availability and suitability for AD is greatly influenced by a variety of factors including geographical location, seasonality, processing and contamination. All of these factors influence the consistency of the resource and as described previously this can evolve challenges for conversion using biological processes such as AD. As first and second generation feedstock are mostly derived from edible sources, and utilises potable water and arable land, current biomass utilisation focuses on the third generation feedstock such as algae, which require neither of the requirements of potable water and land and still can be attractive for bioenergy production.

1.8 Algae biomass

Algae biomass is considered as an integral part of the third generation biomass resource overcoming the drawbacks of the first and second generation biomass resources (Vassilev and Vassileva, 2016). Algae biomass represent the future feedstock for third generation advanced biorefinery. This requires large investments in R&D to provide effective solutions for logistic, technological and economic issues associated with the biomass to become competitive (Sanna, 2014). Algae biomass includes both microalgae and macroalgae biomass that can be grown in either fresh or saline water for use as a feedstock for bioenergy (Sialve *et al.*, 2009). The focus of this research is the utilisation of macroalgae biomass for bioenergy (namely biogas) production. Therefore, the literature review herein will focus on the characteristics, components and bioenergy production from macroalgae biomass.

1.9 Macroalgae biomass

Macroscopic algae or macroalgae (seaweed) are an important part of marine ecosystems. These plants have provided a crucial ingredient for food for humans as well as animals and can be used as nutrient rich fertilisers for plants. They also contain compounds that can be processed into high value products such as phycocolloids (agars, alginates and carrageenans), cosmetic ingredients, food supplements or as a high value gourmet food ingredient in the Western world (Milledge *et al.*, 2014b).

Different uses of macroalgae are shown in Figure 4.

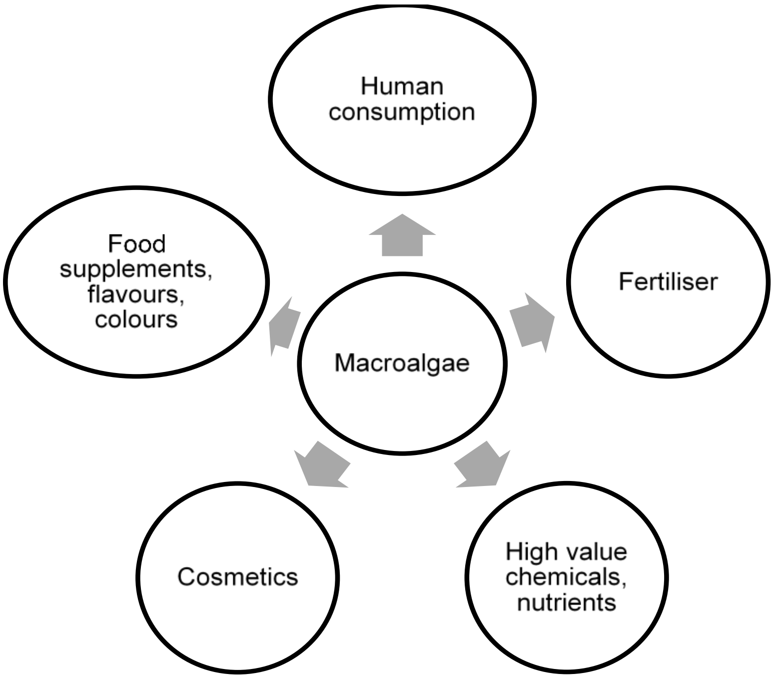

Figure 4 Different uses of macroalgae

Source (Milledge *et al.*, 2014b).

Macroalgae is commercially valuable for their extracts, agar, alginates and carrageenans used for food flavours, colours and nutrients. They have been commercialised in the food industry, as texturing agents and stabilisers, however agar has long been used in microbiological and electrophoresis media simultaneously. In recent years, as a result of market changes and demands, macroalgae costs have escalated and so has the competition in the market for better biomass species and derivatives from the biomass (Bixler and Porse, 2011).

Due to high demand, cultivation of macroalgae has become economically profitable for many countries. Globally, South East Asian countries are leading the market where a relatively low technological business model provides income, employment and foreign trade. The potential economic and ecological benefits have been widely acknowledged by governments, research institutions, and industry in recent years and this has led to increased investment and R&D. (Taelman *et al.*, 2015).

Macroalgae has been used to produce a variety of biofuels including biogas, bioethanol and pyrolysis oil etc. (Wei *et al.*, 2013). The possible conversion pathways are illustrated in Figure 5.

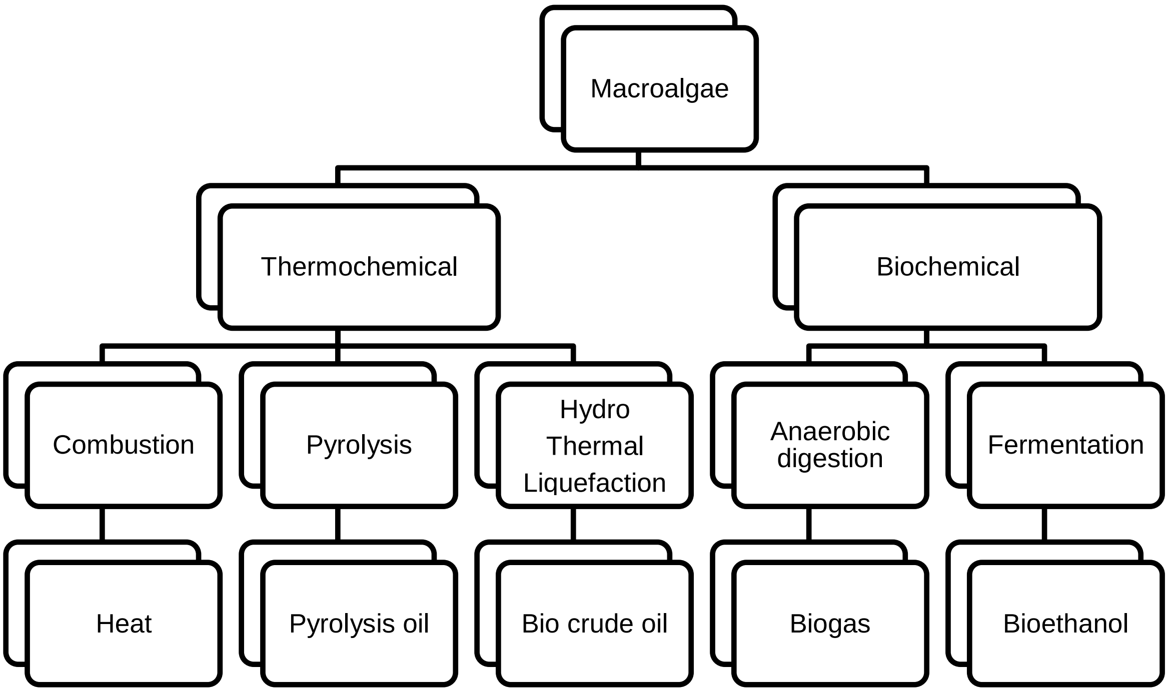

Figure 5 Conversion pathways for macroalgae biomass

Source (Wei *et al.*, 2013)

Thermochemical conversion routes e.g. combustion, gasification, and liquefaction and pyrolysis are still being investigated for macroalgae biomass. However, the efficiency of these processes are limited by the high moisture content in macroalgae biomass (generally >70% and these processes require feedstock with water content <50%WW).

Therefore, thermal processing requires some degree of pre-treatment to remove the excess water from the biomass (Ross *et al.*, 2008). Another interesting field of biofuel research is bioethanol production from macroalgae where the biomass showed significant potential for ethanol production with high carbohydrate content however would still require a pre-treatment stage to obtain higher yields (Borines *et al.*, 2013). To date the majority of research has focused on anaerobic digestion as the preferred conversion route for macroalgae as it can utilise the wet biomass and because of high methane yields and conversion rates obtained from the different species of macroalgae biomass (Hughes *et al.*, 2012, Allen *et al.*, 2015, Allen *et al.*, 2016). In order to optimise the biogas potential from macroalgae and explore the market opportunities for utilisation of this biomass as a biofuel feedstock it is important to understand the different species, fundamental structure, composition, and methods of cultivation.

Macroalgae closely resemble land plants and, as with other plant species, the localised environmental conditions where macroalgae grow are believed to influence the growth and productivity of macroalgae and its subsequent composition. The key environmental conditions that can influence this include seawater temperature, irradiation, nutrients, salinity, and conditions of the seafloor i.e. benthic characteristics, waves and tidal flows. These factors can alter species interactions in a marine ecosystem. Hence, marine ecosystems are constantly monitored for observing any changes of adaptation or shifts in their habitats due to direct or indirect effects of environmental factors associated with it. In temperate regions seasonal variation (i.e. variation of the environmental factors over time in a year) is also a characteristic feature which has an influential role on the balance of the system (Werner *et al.*, 2016). As with other biomass resources, for utilisation of bioenergy production, it is important to understand the characteristic properties and structure of macroalgae biomass.

1.10 Structure, growth cycle and composition of macroalgae biomass

1.10.1 Physical structure

Macroalgae are composed of multiple cells which organise to form structures resembling the roots (holdfast), stems (stipe) and leaves (frond) of higher plants (John *et al.*, 2011). The structure of the macroalgae is shown in Figure 6.

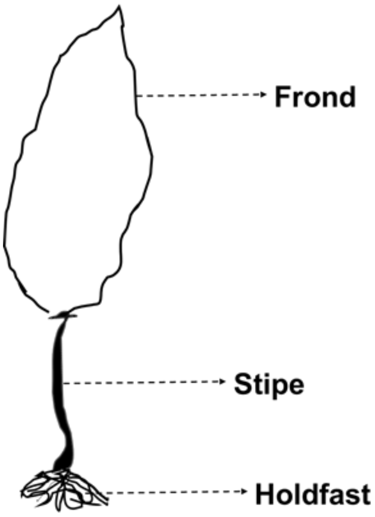

Figure 6 Structure of the macroalgae

Source (Bell and Redpath Museum, 1997)

The holdfast has a similar function to the roots in a terrestrial plant, the main use, however is to enable the biomass to attach to rocky surfaces. The stipe is very flexible in order to withstand the pressure from the waves and protect the biomass from breakage. The stipe connects the biomass with the blade or the upper part called frond. Similar to leaves in the terrestrial plant, the main function of the frond is photosynthesis and obtaining nutrients from the aquatic medium. The frond and stipe sometimes develop air bladders called floats which will help the stipe and the blades float near the surface. The whole macroalgae is sometimes referred to as thallus and the stipe with blades are also sometimes referred to as the frond in the literature (Arvanitis, 2016).

1.10.2 Reproduction and growth cycle

Growth and reproductive cycle of macroalgae biomass is given in Figure 7. They reproduce in vegetative cycle producing sporophytes and in sexual reproductive cycle forming gametophytes (Bell and Redpath Museum, 1997).

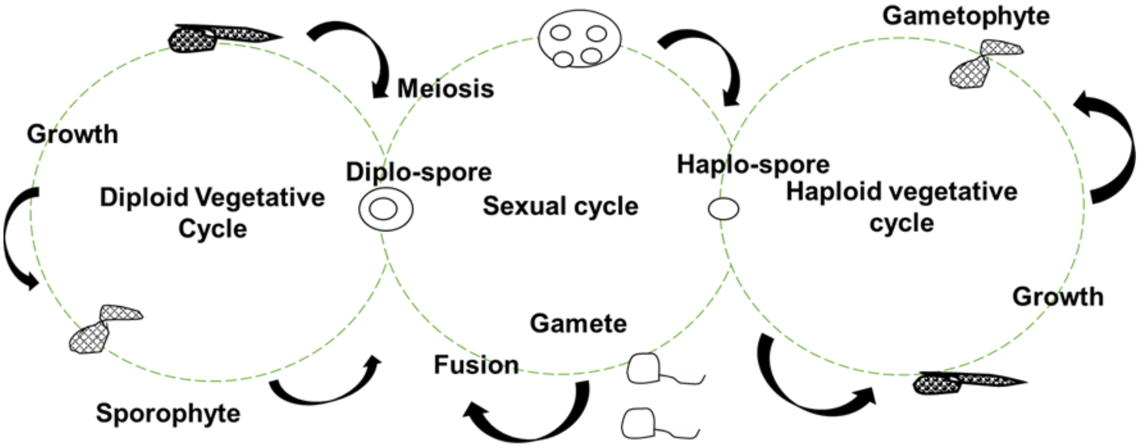

Figure 7 Growth and reproductive cycle of macroalgae biomass

Source: (Bell and Redpath Museum, 1997)

Macroalgae can reproduce via sexual reproductive methods through the joining of male and female gametes (Sexual cycle in Figure 7). The biomass begins their growth cycle as a spore released by the fertile sporophytes (diploid vegetative cycle) and develops into male and female plants called gametophytes (haploid vegetative cycle). When gametophytes become fertile, they release sperm and egg that fuse to form a germling. While growing, the biomass divides into many smaller pieces. The process of spore formation starts over again once the germling is a fully-grown, mature biomass. In cultivation systems, biomass is seeded from spores or through vegetative propagation where sections of seaweed are cut and attached to long strings where their growth is supported. The suspended plants are usually in their early growth stages in winter months, attaining full growth in six to eight months ready for harvest in summer. When fully grown, these are harvested by removing most of the biomass including the holdfast or by leaving a small amount of biomass that will grow again into another biomass plant. However, many macroalgae biomass cannot be grown from the cuttings as they have to undergo a complete reproductive cycle. To enable a full developmental cycle of the macroalgae biomass, the transitions from spore to gametophyte to sporophyte germling is usually carried out in land-based facilities called hatcheries with controlled conditions including, light, water temperature and nutrients (Schiener, 2014, Murphy *et al.*, 2015).

1.10.3 Organic composition

Macroalgae are photosynthetically efficient plants that inhabit most seas and oceans and can contain large amounts of proteins and carbohydrates (van Hal *et al.*, 2014). The different components of different macroalgae biomass are given in Table 3.

Table 3 Components of macroalgae biomass

Macroalgae Species	Division	Fats (g/100g)	Proteins (g/100g)	Carbohydrates (g/100g)	Fibre (g/100g)
Alaria esculenta (Winged Kelp)	Brown algae	3.6	18	40	39
Saccharina japonica (Kombu)	Brown algae	1.1	7	55	3
Fucus vesiculosus (Bladder wrack)	Brown algae	3.6	6	55	4
Ascophyllum nodusum (Knotted wrack)	Brown algae	1	10	52	6
Palmaria palmata (Dulse)	Red algae	1.7	22	45	33
Ulva Sp. (Sea lettuce)	Green algae	0.6	24	40	5

As it can be noted from the table, the fats, proteins, carbohydrates and fibre content vary within species and across divisions. Hence the differences could be potentially because of the cellular composition of the biomass across division and also due to the environmental conditions where the species have grown. On a percentage basis, water is the main constituent of the biomass, and on a dry basis organic matter represents between 62 – 87% dry weight. Organic constituents of the biomass are dependent on the growth cycle of the seaweed (Hierholtzer, 2013). Carbohydrates are the main constituent of macroalgae biomass and the content of the green, red and brown macroalgae ranges between 25 – 50%, 30 – 60%, and 30 – 50% (dry weight) respectively. The main carbohydrate in red algae is Floridian starch, and in green algae it is amylase or amylopectin (starch) (da Silva Marinho, 2016). The main carbohydrates in red algae is Floridian starch, and in green algae it is amylase or amylopectin (starch) (Anastasakis, 2011). Brown macroalgae biomass also contain some unique carbohydrates in their biomass called laminarin, mannitol, and alginate. None of these components are found in lignocellulosic or microalgae biomass.

These compounds have commercial value across a variety of markets (Ghadiryanfar *et al.*, 2016). Organic in nature, these carbohydrates are accumulated by the biomass in their earlier growth stages for structural aid in the waters and later growth stages as storage sugars.

The other constituents of macroalgae biomass include proteins, lipids and fatty acids. The protein content of macroalgae biomass ranges between 3 – 21%, 10 – 26%, and 8 – 47% of dry weight in brown, green and red macroalgae species respectively. Amino acids of glycine, alanine, arginine, proline, glutamic acid and aspartic acids are also found in macroalgae biomass. Macroalgae contain low concentrations of total lipids (approx. 0.61 – 6.48% dry weight) however, high proportions of polyunsaturated fatty acids. Macroalgae is valued as a highly nutritious dietary supplement due to the presence of these nutrients however in western countries, utilisation of macroalgae as food is still very less explored (da Silva Marinho, 2016).

1.10.4 Inorganic composition

Inorganic composition of the macroalgae biomass is diverse and can make up to around 55% of its dry weight. It comprises minerals and trace elements which the plants absorb during its growth cycle. Minerals are an integral part of the biomass their function being to assist the biomass in its developmental stages, i.e. for the growth of fronds, maintenance of stipes against tidal pressure (da Silva Marinho, 2016).

These inorganic constituents are absorbed from the surrounding water medium and occur as light metal salts in the biomass. These salts are found in varying concentrations in different macroalgae species include sodium, calcium, potassium, magnesium, barium and strontium (Arvanitis, 2016). Besides the essential macro nutrients of C, H, O, N, and S and the micro minerals (light metals), macroalgae biomass is also shown to assimilate heavy metals such as inorganic arsenic, lead, cadmium and mercury undesirable in the biomass if intended for human consumption. Other elements of Iodine, Iron, copper, chromium, selenium, zinc, manganese are also found in the biomass in trace quantities (da Silva Marinho, 2016).

1.11 Variation in composition in macroalgae biomass

Compared to microalgae, macroalgae biomass shows higher volumetric production rates and biomass densities. The diversity in the chemical composition of macroalgae biomass (carbohydrate based) also makes it a complementary biomass source to microalgae (lipids based) for a biorefinery (van Hal *et al.*, 2014).

However, there are challenges in realising the fuller potential of macroalgae biomass. This is mainly due to the variation observed in their chemical composition (i.e. inorganic and organic) which is reported to vary with species population, season, and geographic distribution (Marinho *et al.*, 2016). In addition, the carbon and nitrogen content of the macroalgae biomass have also been found to vary with carbon content increasing during spring and summer (with highest values at the end of summer and lowest in winter). Nitrogen concentrations have exhibited the opposite trend with the lowest values recorded in late spring and highest values observed in late winter. The high carbon values for summer may be attributed to the high photosynthetic activity during summer time leading to the accumulation of carbon in proportion to the carbon consumed for metabolic purposes. This increases the overall carbon to nitrogen ratio in the biomass. Similarly, during spring and winter, photosynthetic activity is lower as the carbon is being used for the production of new tissues. As new tissues are formed, the biomass is rich in nitrogen content leading to a lower carbon to nitrogen ratio in the biomass (Arvanitis, 2016).

As the macroalgae biomass moves through different stages in its growth cycle, organic composition of the biomass is also found to be varying with lower carbohydrate (laminarin, alginic acid and mannitol) levels in winter and higher during summer and autumn (Hierholtzer, 2013). Maximum levels of protein occur during winter and early spring followed by a constant decrease till the end of summer with minimum values. On a dry weight basis cellulose content of the biomass also varies throughout the year with maximum values in spring and decreasing in summer with increased mannitol levels. In winter the macroalgae will exhibit higher concentrations of inorganic compounds e.g. micro elements and light metals compared with lower concentrations in summer. This is because during winter months the plants tend to accumulate these elements for the development of their rigid cellular structure to survive lower water temperatures as opposed to focusing their efficiency in photosynthetic activities producing carbohydrates which occur in summer (Arvanitis, 2016). Even though much of the variability in the composition of the biomass is correlated to seasonal change (and is often referred to as seasonable variability in the literature), changes in environmental factors, (such as water temperature, salinity, irradiance, depth at which the biomass grows throughout the year) are also shown to have significant impacts on the composition and therefore on quality of the biomass produced (Hierholtzer, 2013). Given that the composition of biomass can be influenced by a number of factors it is therefore important to carefully consider the sites chosen for macroalgae cultivation.

While there are limitations surrounding control over climatic changes localised factors such as wave exposure, benthic conditions (rocky, sandy sea bed), salinity, tides etc. can be measured and to some extent considered when adapting cultivation systems to grow suitable species for a given location (Hierholtzer, 2013, Schiener, 2014). Similarly, understanding the local environmental conditions enables operators to choose optimal sites for future cultivation. The cultivation techniques commonly adopted for macroalgae biomass are described in the next sections.

1.12 Cultivation of macroalgae biomass

Globally cultivated macroalgae biomass production is estimated at 23.8 million tonnes (wet weight) in comparison to 1.1 million tonnes harvested from wild stocks. Compared to the global production figures, macroalgae production in Europe is negligible, at around 1%. Norway and France are the main macroalgae producers in Europe with a combined annual production of 181,565 tonnes mostly harvested from wild stocks (Marinho *et al.*, 2016).

All three types of red, green and brown macroalgae biomass are found in the UK coastlines where environmental conditions are favourable for the respective species. In temperate seas, brown species dominate the biomass but the pattern of species distribution differs among geographical regions. Brown macroalgae biomass e.g. *Laminaria* species and *Ascophyllum* are common species found on the British coastline (Hierholtzer). The reported yield of brown biomass produced from rope cultivation globally is estimated at 80 – 400 tonnes (wet weight) per hectare per year, where highest values are found in Asia while Europe has a lack of yield values for up scaled sites (da Silva Marinho, 2016).

Among the three varieties; red, brown and green algae, brown algae are the faster growing and is commonly termed kelp. These kelps commonly comprise up to 60% (as dry weight) carbohydrates. Studies have shown that brown macroalgae also demonstrates variability in its macromolecular compositions across various seasons and has the highest carbohydrate concentrations in autumn and lowest in winter. Similar trends are also reported for inorganic composition of the brown biomass with variations in minerals and salt content (Jiang *et al.*, 2016).

1.13 Cultivation types of macroalgae biomass

Macroalgae cultivation can be carried out on shore, near shore or off shore determined by distance of the system from land. On shore cultivation methods include in shore coastal farms, and ponds. Pond culture systems are the preferred method for specialty market products as there is greater control over the environmental conditions. Near-shore is the most prevalent method in its near commercial phase while off shore is still considered to be in experimental phase. Near shore systems are simple to construct, easy to manage, low cost, and are easily accessible at low tide. Off shore farms are those that are constructed in deeper water, requiring growth structures that are anchored to the ocean floor, or floating lines requiring positioning devices. This method is favoured in weaker water currents or with too deep water in which fixation the bottom lines is too difficult. Near shore cultivation results in faster growth rates but farm managing is difficult and the seaweed is susceptible to damage and degradation to weather, waves and boats (Ghadiryanfar *et al.*, 2016). On shore cultivation is proven to be costly as land-based cultivation requires additional nutrients for biomass growth. However, the capital cost, production cost, maintenance etc. of off-shore cultivation is also found to be higher than the market price for the biofuel from the macroalgae as the biomass contains considerable amount of water (Jiang *et al.*, 2016).

1.14 Cultivation processes of macroalgae biomass

The cultivation process can be divided into two main phases. Phase 1, the hatchery phase and Phase 2, the grow-out phase. The initial cultivation is conducted in a controlled hatchery prior to seeding and grow out phase in open waters (Roesijadi *et al.*, 2010). The process is cyclical because the cultivation procedures start straight after harvest and as the selection of fertile seaweed is complete, the hatchery cultivation processes can be initiated. The macroalgae biomass cultivation stages are shown in Figure 8.

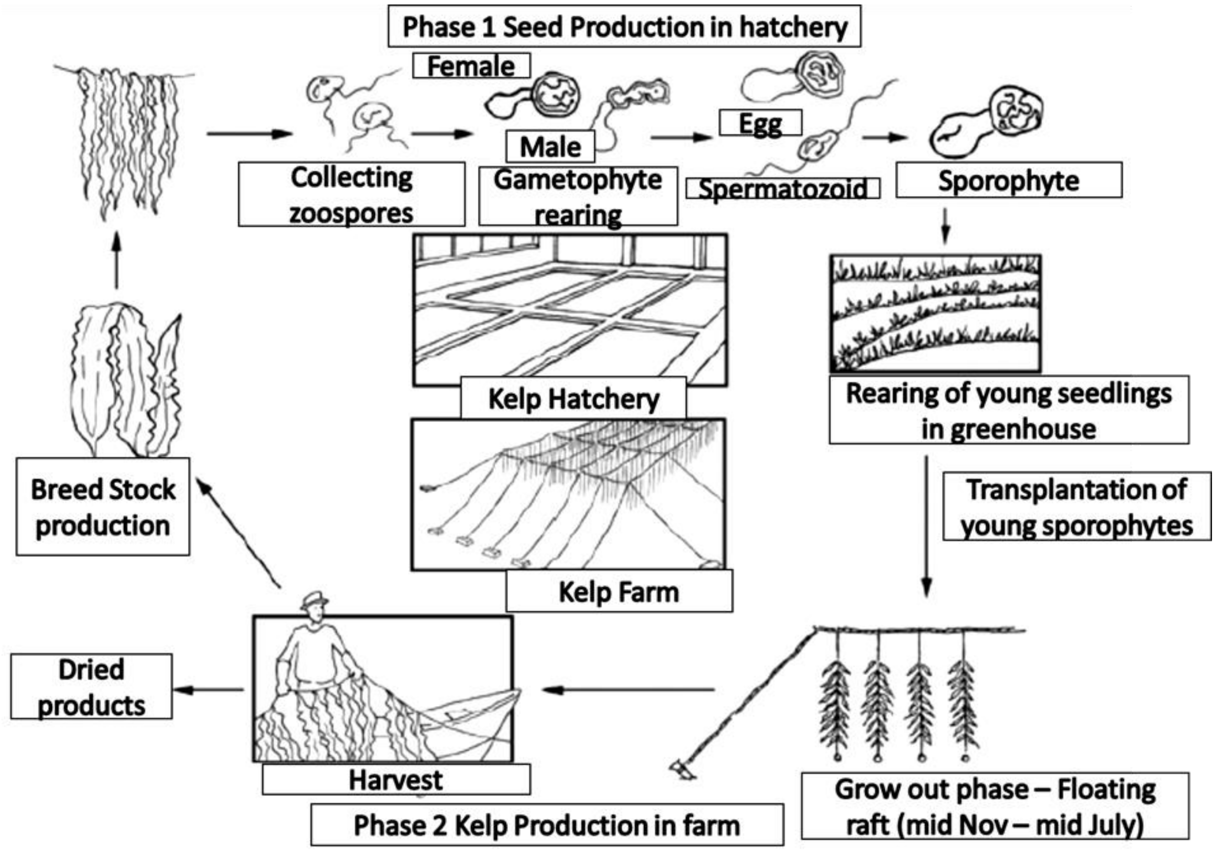

Figure 8 Macroalgae biomass cultivation stages

Source: (Roesijadi *et al.*, 2010)

Once the collection of fertile seaweed is complete, summary of the activities in the hatchery stage are as follows: -

- Release of spores from fertile material
- Development of culture under controlled lab conditions
- Induction of reproduction under altered lab conditions
- Spraying of fertile cultures on to suitable substrate (Culture string)
- Development of culture on the string substrate under lab conditions.

Summary of grow out phase include the activities such as: -

- Deployment of culture on long line or other systems at sea
- Development of seaweed in approximately 6 – 7 months
- Harvest of seaweed.

(Ghadiryanfar *et al.*, 2016).

1.14.1 Hatchery and seeding

The hatchery phase consists of collecting the spores from the fertile macroalgae biomass, induction of reproduction with supplemented nutrients in the growth medium and development of spore cultures into seedlings under laboratory conditions (Jiang *et al.*, 2016). Hatchery seedlings of 1 – 2mm length are ideal for transfer out to sea (Schiener, 2014, Jiang *et al.*, 2016). Hatchery seedlings of 1-2mm length are ideal for transfer out to sea (Schiener, 2014). These seedlings of considerable length are then attached to longer lines (strings attached with buoys or air floats for support) which are then deployed at sea (Jiang *et al.*, 2016).

1.14.2 Planting out at sea

Generally divided into two types, seaweed planting out (or deployment) is done either vertically or horizontally, depending on the way in which the ropes, containing the seedlings, are attached and suspended in the sea level air floats and anchor lines (Jiang *et al.*, 2016). Techniques used for macroalgae cultivation are quite comparable to mussel farming techniques. The horizontal rope holds together a number of buoys floating on the sea surface level. From each buoy a vertical rope with the seedlings are then immersed to the sea (Hierholtzer, 2013). The depths of these ropes are then adjusted to a suitable length and then left untouched to allow for growth. Periodic measurements of lengths are carried out only after a few months (Jiang *et al.*, 2016, Schiener, 2014). Techniques used for macroalgae cultivation are quite comparable to mussel farming techniques. The horizontal rope holds together a number of buoys floating on the sea surface level. From each buoy a vertical rope with the seedlings are then immersed to the sea (Hierholtzer, 2013). The depths of these ropes are then adjusted to a suitable length and then left untouched to allow for growth. Periodic measurements of lengths are carried out only after a few months (Schiener, 2014).

1.14.3 Transport

The transportation route is dependent on the distance of the farm from the land. Transport of biomass is mainly via boats which run on fossil fuels. Transportation is required at two intervals; one after the hatchery stage for the transport of seedlings to the farm and later during harvest stage carrying fully grown biomass from the farm to the land. Transportation costs increases as farm distance increases from the coast as longer distances require increased use of fossil fuels and associated emissions. The greenhouse gases emissions attached to the macroalgae cultivation is mainly associated with the transportation costs. The transport of macroalgae by boats is also weather dependent and journeys are often postponed during bad weather conditions often postponing the harvest times of the macroalgae biomass. On the other hand, land based cultivation does not require the same transportation and also are not influenced by physical conditions which affect offshore growth for example weather, waves, tidal changes etc. however they are associated with still higher installation and maintenance costs (Ghadiryanfar *et al.*, 2016).

1.14.4 Harvest

Traditionally macroalgae have been harvested from natural/wild stocks, however, these resources are being depleted by overharvesting and non-scientific harvesting practices and are therefore considered unsustainable (Sanderson *et al.*, 2008). In addition, harvesting from wild stock also affects the natural ecosystems causing detrimental effects on the organisms depending on these biomasses for their growth and survival (Hughes *et al.*, 2012). Harvesting of the cultivated biomass is usually performed in spring or early summer months. Fishing boats aided with mechanical cutters will be driven out to the farm to harvest the fully grown biomass. The long lines are lifted up to the sea surface and stripped off the lines manually (Ghadiryanfar *et al.*, 2016). Summer biomass becomes infested with epiphytes, therefore food quality biomass is normally targeted for harvest in spring in months of April and May (Marinho *et al.*, 2016a). As cultivation techniques are optimised, harvesting of the biomass can also be controlled by improving stocks with genetic strain collection, and by harvesting mono cultures instead of co harvesting unnecessary species. The harvesting times can also be modified to target the best chemical composition of the biomass required for either bioenergy production or high value products development (Hierholtzer, 2013).

In comparison to other traditional biomass sources, macroalgae offer advantages of higher productivity and no competition for arable land and potable water compared to traditional land crops. However, based on current information surrounding the costs and benefits, offshore cultivation of the biomass in the North Sea remains economically unfeasible if biomass is targeted for the extraction of one particular high value product e.g. animal feed ingredient. Hence, a cascading bio-refinery approach should be adopted for better utilisation of the ingredients of the biomass for high value products and the residue for the production of biofuels (Bikker *et al.*, 2016). As brown macroalgae biomass is the most common and under exploited biomass in the UK, its suitability as an anaerobic digestion feedstock will be the focus in the literature review section to follow.

2 Literature Review

Macroalgae biomass has been the focus for bioenergy production as a third generation energy source in recent years. Macroalgae is a dominant plant group which are farmed in approximately 50 countries in the world. Globally, farmed aquatic plants contributed 28 million tonnes to total world aquaculture production in 2015. Asia contributes the most and Indonesia is the major contributor in the sector where production in 2015 was 10 million tonnes. In Asia the biomass is predominantly used for human consumption (Cochrane *et al.*, 2009). However, in Europe, native species including brown (Phaeophyte) macroalgae commonly known as Kelp, have been cultivated at large scale and researched for their suitability as biofuel source.

As Kelp or brown algae biomass is the native species in the UK, the literature review for this study is focused on this and in particular the species *Saccharina latissima*, *S. Latissima*. This study involved a comprehensive review of peer reviewed journals accessed via Google Scholar using key words such as anaerobic digestion, macroalgae biomass, Europe, Brown algae, co-digestion, Kelp, *S. Latissima* and techno-economics feasibility of anaerobic digestion. The number of papers used for this study, the categories and focus time period of research are given below.

The initial research literature focused on the time period from 2000 until 2018. Google scholar results showed 161,000 research papers including patents and citations on anaerobic digestion while the term ‘brown algae’ search resulted in 300,000 results. The search on ‘anaerobic digestion of algae’ showed 18500 results while ‘anaerobic digestion of brown algae’ resulted in only 23 papers. ‘*S. Latissima*’ showed a result of 3060 papers while semi continuous digestion of *S. Latissima* showed a total of 377 results. Global experimental determination of ‘seasonal variation in Kelp’ showed a result of 18200 results while seasonal variation and chemical composition of Kelp in Europe for AD purposes resulted in only 1630 papers. The advanced search on Google Scholar allows for more focused research with the terms either anywhere in the article or with the title and also to differentiate between review and experimental papers. Therefore, the literature search was then focused in terms of the year of publication and species utilisation of *S. Latissima* for AD in particular.

As the time period focused on the last 5 years from 2013-2018, interestingly it could be noticed that more papers on *S. Latissima* were published during these years than in the previous 12 years. The advanced search on “Anaerobic Digestion” of “*S. Latissima*” only showed a result of 3 papers with these key words in the title, however

43 papers with “Semi continuous digestion” where the species is used for comparison with another species for AD purposes. Biochemical methane potential of *S. Latissima* in Europe with experimental results showed 115 papers while anaerobic co-digestion with the focus on co-digestion showed 98 papers. Interestingly 119 results were shown for the search of anaerobic digestion of *S. Latissima* in North West Europe with 93 results for experimental studies. However, upon analysing the papers, more than 50% of the papers were review papers with very few experimental studies involved. Also, the search included any study where *S. Latissima* was included in the text of the papers to give such large numbers. In scrutiny, it could be seen that the important areas of study focused on *S. Latissima* in the last five years was their feasibility for AD, biogas potential, inhibition studies for sodium or potassium levels, nutritional value, life cycle assessment in comparison to first and second generation biomass sources and an additional species for comparison during semi continuous digestion. No studies have been found to be during this review that analysed the variation of the biochemical composition of the macroalgae biomass in North West Europe for AD purposes with supporting experimental data on the impact of environmental factors or harvest times. No papers/ studies were identified which evaluated semi continuous digestion of *S. Latissima* comparing thermophilic and mesophilic conditions for biomass grown in North West Europe. Similarly, there were no techno-economic nor LCA studies identified on the co-digestion potential of *S. Latissima* with experimental results. The review also found that there was a gap in the literature for studies comparing the characteristics, and growth conditions between the wild and cultivated biomass of *S. Latissima*.

In summary, a total of 165 papers were reviewed as a part of this literature review search with particular focus on the recent papers on *S. Latissima* published from 2013 to 2018 out of which only less than 30 papers covered areas of *S. Latissima*, anaerobic digestion, semi continuous digestion, co-digestion, and life cycle assessment with experimental results. Therefore, the focus is on the extent to which environmental conditions, growth cycles and cultivation type impact on biomass characteristics and on subsequent valorisation pathways for macroalgae. In addition, European project reports, governmental panel reports from e.g. IPCC (Intergovernmental panel on climate change) & DEFRA (Department for environment, food and rural affairs), IPCC & DEFRA, were also reviewed and referenced to gain a better understanding of the wider challenges and opportunities of utilising *S. Latissima* as a biofuel resource.

Recent theses published on *S. Latissima* between 2013-2018 were also referenced as a part of this study.

2.1 Macroalgae biomass characteristics

Climate change has a direct impact on the physical and chemical properties of water resources worldwide and oceans are no exception. The Food and Agricultural Organisation of the United Nations (FAO) published a report in 2016 identifying the complex challenges of climate change adaption which the aquaculture sector must address. This report identified a variety of physical (temperature anomalies, Sea surface temperature changes, precipitation anomalies, rising sea levels, floods, drought, cyclones), chemical hazards (salinity changes, pH changes, low oxygen levels), and biological hazards (eutrophication, pathogens and parasites, pollution) cause direct and indirect serious threats to the sector. These factors have to be addressed via short- and long-term studies to identify and understand how the impact upon macroalgae species, their habitats and communities in a cumulative way (Fao, 2016).

Macroalgae have a variety of physiological adaptation techniques which are triggered as a result of changing environmental conditions. Kelps in particular are known to possess very high phenotypic plasticity allowing them to adapt to a wide range of fixed and varying environmental conditions. Kelp undergo physiological adjustments to preserve cellular growth and biochemical composition in response to seasonal and nutritional cues (Kerrison *et al.*, 2015). This has a direct effect on the composition and characteristics of the biomass and the associated yield and quality of chemicals which can be extracted and converted to bioenergy. In addition, understanding the complex interrelationships between environmental conditions and macroalgae growth ensures that future deployment and utilisation of macroalgae are sustainable and commercially viable.

The following sections will provide a review of the current knowledge surrounding macroalgae and the extent to which environmental conditions impact and its growth and physical, biological and chemical characteristics.

Bioenergy production requires large quantities of biomass to be commercially viable. The transition towards wider utilisation of macroalgae for bioenergy purposes, therefore, requires a greater understanding of specific, ubiquitous species. In addition, the efficacy and efficiency of conversion processes are influenced, to some extent, by the consistency and security of biomass feedstock supplies. To this end it is important to identify the key factors which impact on composition and characteristics, particularly those characteristics which are favourable for biogas production. From the literature review the following factors were determined to impact on biomass characteristics and composition:

1) Local Environmental Conditions
    a) Sea water temp
    b) Benthic conditions
    c) Salinity, Irradiance and Depth
    d) Tides and currents
    e) Pollution and Fouling
2) Growth Cycle (and therefore the point in this cycle when biomass is harvested)

Growth / cultivation conditions (whether biomass is artificially cultivated or naturally occurring as kelp forests)

Kelp is one of the most ubiquitous species found in the North Atlantic species and specifically in the British Isles. Of the Kelp, the most common species are *Laminaria digitata*, *Saccharina Latissima*, *Alaria esculenta*, and *Laminaria hyperborea* (Yesson *et al.*, 2015). Most current studies focus on *Laminaria digitata* as countries such as the UK in North Western Europe have long stretches of coastlines surrounding their main lands where the species is cultivated for extracting high value products such as pigments, gourmet and nutritional products. *S. Latissima* is equally interesting but there are only a few cultivation sites in the UK and it is currently grown as a trial species in most cultivation farms. In addition, there are no industrial scale farms in the UK where currently *S. Latissima* has any reported yearly yield. There are only estimated yields based on studies carried out on similar species like *Laminaria digitata*. Hence this gives researchers a good opportunity to study the species requirements and thereby optimise the cultivation parameters, growth conditions, harvest practices for *S. Latissima*.

2.1.1 Impact of local environmental conditions on the characteristics and composition of macroalgae

As discussed previously many environmental parameters that control successful macroalgae growth are influenced by both natural and human factors. Natural factors such as water quality, photosynthetic active radiation, temperature, salinity, and concentrations of inorganic nutrients includingCO_2, absence of environmental toxins, seasons, wind, rainfalls, tides, and human activities such as aquaculture and wastewater discharges are specific to each geographical location. Environmental conditions in proximity to the farm site location are therefore important to the success of the cultivation of kelp (Peteiro and Freire, 2013). The following table (Table 4) will now review literature on the environmental parameters recorded for macroalgae biomass.

Table 4 Review of lieterature on environmental factors

Environmental factor	Main findings	Comments	References
Sea water temperature (Temperature of the surrounding waters where Kelp cultivation occurs)	Conservation studies identified temperature as a key factor for Kelp growth. Preferred temperature is around -1.5°C and not surviving beyond 18°C. Increasing temperatures led to the shift of Kelp populations towards lower temperature regions.	Kelp is vulnerable to climate change related temperature shifts in the seawaters. Further conservation studies required on Kelp forests in Europe.	(White N. & Marshall, 2007) (Gao *et al.*, 2013) (Merzouk and Johnson, 2011) (Philippart *et al.*, 2011) (Smale *et al.*, 2013, Fernand *et al.*, 2017)
Benthic Conditions Biotic and abiotic characteristics of the seafloor present at varying depths of marine ecosystem	Characteristics gradually changes with time, and can impact on the marine ecosystem.	None of these data available for macroalgal growth in Europe including spatial mapping for Kelp in the UK.	(OSPAR, 2017) (Frid *et al.*, 2009) (Araujo *et al.*, 2016) (Walls *et al.*, 2017)

	Variations as a result of external factors such as climate change.	Requires more study in relation with IMTA systems	
Salinity Variety of inorganic concentrations in seawater	Salts include Na, K, Ca, Mg, Ba, and Sr. Fluctuate during the year.	Salts are important for macroalgal growth and development.	(Ding *et al.*, 2013) (Adams *et al.*, 2011, Gunaseelan, 1997, Black, 1950, Carpentier *et al.*, 1988)
Irradiance Light intensity from varying day length	Higher in summer and lower in winter. Growth rate and irradiance found to have a liner relationship	Saturation of Kelp growth occurred at photon flux density above 70 µE/m^2/s.	Fortes and Luning (1980) (Walls *et al.*, 2017)
Water Depth Different water levels in sea	Kelp reported to grow in sublittoral zone a little above the tidal mark up to the depth of eighteen metres	Cellulose content of Kelp found to vary with season and water depths.	(White N. & Marshall, 2007) (Walls *et al.*, 2017)
Tides and Currents Hydrodynamics in the sea	Higher densities of frond found in reduced current regions.	Can be detrimental causing the Kelp lines to wash off the biomass, also limits the fouling agents on fronds.	(Walls *et al.*, 2017, Peteiro and Freire, 2013). (Walls *et al.*, 2018)
Pollution and fouling	Fouling agents including hydroids, snails, blue mussels,	Pollution from anthropogenic activities.	(Walls *et al.*, 2018) (Ward *et al.*, 2014) (Fernand *et al.*, 2017)

Presence of epiphytes and bryozoans	bryozoans, epiphytes settle on the fronds during summer.	No study conducted to assess the effect of pollution on Kelp cultivation farms, IMTA systems etc.	

In summary, environmental conditions such as seawater temperature, benthic conditions, salinity, irradiance, water depth, tides, currents, pollution, fouling can all directly and indirectly influence the growth and characteristics of macroalgae and are therefore important considerations when selecting the best geographical location for the cultivation of biomass. Understanding how these factors influence characteristics is also important when optimising cultivation conditions to achieve biomass with preferred characteristics. However, this review has identified that macroalgae growth is complex and characteristics are not determined by one factor alone but by a combination of several environmental factors.

The findings cited here were from individual, isolated studies evaluating growth and characteristics in relation to one particular environmental factor in a particular location. The focus of these studies has been ecological and environmental preservation rather than for bioenergy production. In addition, the studies were performed, in general, on brown algae biomass or Kelp rather than specifically *S. Latissima*.

Therefore, there is an evident gap in the literature whereby further research is required to better understand the interrelationship between biomass growth conditions and subsequent characteristics in order to both select appropriate sites and conditions for cultivation as well as predict the impact of environmental change on future biomass quality. This is particularly relevant for species such as *S. Latissima* which grows in abundance in UK waters and therefore offers the greatest potential as a feedstock for AD.

2.1.2 Impact of growth cycle on the characteristics and composition of macroalgae

The literature on the biochemical profiling of macroalgae biomass is found to be broadly referred to under the generic term ‘seasonal variation’. This is attributed to the difference recorded in their biochemical constituents harvested throughout the year. However, the variation in the biochemical contents of biomass occurs seasonally owing to the growth stage of the biomass (Ghadiryanfar *et al.*, 2016). Therefore, this review maintains the view that the term seasonal variability does not reflect the seasons of spring, summer, autumn and winter, rather the biomass response to environmental conditions in their composition at different points of time throughout their growth cycle. Seasonal variation and corresponding variability in the in the characteristics of the biomass in its growth cycle becomes important when considering the best time to harvest the biomass for bioenergy purposes. *S. Latissima*, the brown kelp species has been studied in the literature for seasonal variation in its biochemical composition however not in relation with AD.

Macroalgae biomass resembles plants and have distinct life stages during different times of the year. *S. Latissima* is reported to be a perennial species which includes a period of maximum growth in the first half of the year followed by a period of reduced growth during the summer months with sori (reproductive spores) formation from late autumn until early winter (Azevedo *et al.*, 2016). As discussed in the introduction chapter on biomass, *S. Latissima* also exhibit complex and diverse lifecycles with combinations of sexual and asexual reproductive pathways which is very important to understand to develop appropriate cultivation techniques and also determine the best harvest times for the biomass intended for various purposes including bioenergy.

Seasonal profiling of macroalgae biomass has been attractive to researchers in order to quantify their unique carbohydrate contents. Macroalgae biomass in general, consists of large amounts of carbohydrates (almost 60%) and the concentration of these storage products are reflected in the biomass biochemical composition and inorganic content (Roesijadi *et al.*, 2010). For *S. Latissima* species (which belongs to the brown algae group) the major storage product is primarily laminarin and mannitol. Earlier studies in the literature have reported the concentration of laminarin range between 2 – 34% of the algal dry weight and mannitol exists around 2% in the cells (Davis *et al.*, 2003, Bold and Wynne, 1978, Lewis and Smith, 1967). The cell wall of the species has cellulose, alginic acid (10 – 40% DW) and fucoidin (5 – 20% DW) (South and Whittick, 2009).

Another aspect of biochemical profiling was to assess the concentration of these components in different parts of the biomass. The physiology of the macroalgae biomass is similar to plants, the bottom part called holdfast (similar to roots), and the part above named stipe (similar to stem), and leaf life structures are called frond (Arvanitis, 2016). Being physiologically similar to plants, it was assumed that different parts of the biomass will have different concentrations of these components. However, it was not quantified until research carried out by Black *et al.* in 1950s. The study showed that each part of the seaweed contains different compositions of its constituents like laminarin, mannitol, alginic acid, and ash where the part of the blade of *S. Latissima* nearer the stipe had a higher mannitol and ash content and lower protein and laminarin content. Laminarin, protein and alginic acids were found higher in the upper parts of the blade. However cellulose levels were found steady in all parts of the blade (Black, 1948).

There are studies in the literature which have equally explored the changes in the organic and inorganic constituents of the biomass termed seasonal variation. A study by Adams *et al.* (2011) found that the organic constituents in the form of alginic acid, mannitol and laminarin are usually lower in winter and spring, whereas the level are high in summer and autumn. In another study, ash and nitrogen levels were also found to fluctuate during the year, whereas sulphur concentrations were found stable with values typically four times lower for brown seaweed when compared to red seaweed (Hierholtzer, 2013, Rupérez *et al.*, 2002, Adams *et al.*, 2011).

Despite these reported studies, the most cited study performed on European Kelp is still the seasonal chemical profiling done by Black (1950). However, a more recent study was carried out by Schiener *et al.* (2015) on Kelp species including *Laminaria digitata*, *Laminaria hyperborea*, *Alaria esculenta* and *Saccharina Latissima* for 14 months. The study analysed the biomass to identify seasonal variations and thereby predict the best harvest times for these species. For the species, *S. Latissima* the carbohydrates (alginate, laminarin, mannitol and cellulose) represented up to 84% of the seaweed biomass. Alginate was found higher in winter months and lower summer (July). Laminarin (24 – 27%) and mannitol (12 – 19%) was found highest during the late summer to autumn months and lowest during the spring months. The same study concluded that as the total carbon content in the biomass was highest in autumn, the seasonal variation in the species should be targeted to harvest the best biomass for fermentation or other bioenergy derivation routes (Schiener *et al.*, 2015).

This can be explained by the growth cycle of the macroalgae biomass. *S. Latissima* is a perennial species which undergoes a period of maximum growth in the first half of the year followed by a period of reduced growth during the summer months with sori (reproductive spores) formation from late autumn until early winter (Azevedo *et al.*, 2016). In winter, the biomass is in its earlier growth stages and therefore contains more nitrogen or protein compounds for their cellular growth, and the carbon is utilised for producing the alginate content (structural carbohydrate) for better structural support of the biomass against the environmental conditions. Acquiring inorganic elements for their growth also continue to increase from winter till spring. As spring approaches with increased irradiance, the rate of biomass photosynthesis also increases however concentration of alginate starts to decrease. The photosynthetic activity of the biomass continues through spring to summer where the biomass utilises the carbon to produce more storage carbohydrates i.e. laminarin and mannitol. This continues until late summer to autumn months where the biomass prepares for their reproductive stages and survival of winter months (White N. & Marshall, 2007).

The seasonal variability of macroalgae biomass is reported to be an important factor however again these studies have their limitations. Even though the study by Black (1950) has been extensive, this study needs to be updated from a bio-refinery perspective. The work published by Schiener *et al.* (2015) had bioethanol production as its focus and this again is limiting if the biomass is intended for anaerobic digestion purposes. Also, from the studies reviewed it becomes clear that even though the terminology used for recording the biochemical profiling noticed in the Kelp species is 'seasonal variation', it is not the 'season' that seems to cause the difference in composition of the biomass. The view was supported by Black's research with observations made on the fucoidan and cellulose content. He reported that the concentration of fucoidan is not dependent on the seasonal variation rather on the tidal strength and is found higher in the species growing in intertidal regions. The cellulose content varies with the strength requirements of the biomass hence depends on the depth of immersion in the waters (Black, 1948). Hence this triggers the need for more studies to simultaneously observe the growth cycle of the biomass and their biochemical composition over a year at different sites.

In most cases, the reported studies have focused on the holdfasts rather than the fronds. This is because they also function as a sediment trap accumulating the organic nutrients for the growth of the biomass and also for any organism inhabiting the structure (Walls *et al.*, 2017). However, from an AD perspective, composition of the fronds is more important as they are more suitable as a biomass feedstock and holdfasts are usually discarded as they may be more difficult to digest due to the presence of cellulose and can cause potential for inhibition from heavy metals etc. Therefore, it is necessary to understand how the seasonal variation is observed in the fronds in comparison to the holdfasts as fronds are attractive parts of the biomass for high value products and bioenergy.

In addition, this review has identified that, seasonal profiles for the macroalgae biomass are generally focussed only on wild populations of macroalgae. It is essential to extend the profiling to cultivated biomass as this is a more sustainable and viable way of sourcing the macroalgae for bioenergy. Wild species can survive for an average of 10 years and plant age can vary significantly within a wild population, therefore in addition to growth phase, age of plant may be an important factor influencing the characteristics and therefore variability of biomass. (Walls *et al.*, 2017). From this review, it can be concluded that there remains a significant gap in knowledge and information surrounding the impacts of growth phase on the characteristics and composition of *S. Latissima* particularly in relation to its suitability for bioenergy production. Harvesting of biomass can be a time consuming and costly part of the process and as described previously, a greater understanding of these interrelationships will help to inform economic and operational decisions.

2.1.3 Impact of growth type on macroalgae biomass

As described, the key factors that influence the characteristics and composition of macroalgae include environmental conditions and growth cycle. It is important to ensure a secure and sustainable supply of biomass for bioenergy and other high value markets. Therefore, we must consider the advantages and disadvantages of artificially cultivated biomass compared to wild grown biomass in terms of techno-economic feasibility, environmental sustainability and also biomass yield and quality.

Sourcing wild biomass is not considered as a sustainable option (Hughes *et al.*, 2012). Harvesting Kelp from the wild habitats is not encouraged as it might affect the ecological balance in the marine environment. Colonisation of Kelp stipes and holdfasts make Kelp forests a hive of highly diverse flora and fauna. This varies with spatial location and time of the year (Walls *et al.*, 2017). Kelps have been mainly cultivated for iodine and alginate production in Europe (Guiry and Morrison, 2013, Nielsen *et al.*, 2016). The kelp cultivated biomass has increased from about 2 million tonne in 1990 to more than 8 million tonne in 2012 (Nielsen *et al.*, 2016). Cultivation ropes can also act as a habitat for a number of different organisms as both the long line structure and the kelp biomass can act as a platform for habitat and refuge for these species (Teagle *et al.*, 2017). However, the percentage of inhabiting fauna on the cultivated biomass is lesser when compared to the naturally occurring Kelp forests. Cultivated and wild *S. Latissima* can potentially vary in their characteristics. Wild Kelp, even though they occur as annuals differ in their maturity stages and age (White N. & Marshall, 2007). Cultivated species on the other hand are comparatively younger as they are grown and harvested in one complete cycle. Even at the same geographical location, growth pattern reflecting in the biomass composition of wild and cultivated species can be different owing to the varied environmental conditions they are subjected to. Wild Kelp are shown to grow a characteristic flattened or slightly conical holdfast attached to a rock while cultivated Kelp are seeded onto ropes for growth. This results in different morphology for the holdfasts for wild and cultivated species. The wild species will draw its characteristics from the surrounding oceans and benthic floors while cultivated species have suspended growth altering the environmental conditions in which the species grow (Walls *et al.*, 2017). Cultivated species will be less subjected to deeper oceanic currents however tidal waves activities can be substantial. In terms of irradiance, cultivated species will have more access to light being closer to the surface in comparison to the wild counterparts which can also increase the fouling in summer months (Teagle *et al.*, 2017). Pollution can also affect wild species considerably high in comparison to cultivated biomass as sedimentation can be high on benthic floors due to these activities (OSPAR, 2017). The coastal aquaculture is also shown to suffer from floods and heavy runoff of freshwater into cultivation sites which tends to lower the salinity levels which stimulate the growth of seaweed that suffocates the seaweed (Cochrane *et al.*, 2009). This poses a serious limitation to the harvesting time of the cultivated biomass to late spring or early summer, thus limiting the time for accumulation of storage carbohydrates in the biomass, leading to a lower quality and yield of macroalgae biomass (Fernand *et al.*, 2017). Therefore, the greater the difference in environmental conditions are, the greater the difference in characteristics will be between wild and cultivated biomass. Difference in cultivation methods can also impact on the biomass characteristics. Biomass grown in land based on shore cultivation systems will have highly controlled conditions however this method is not economically sustainable due to the high costs associated with the energy and nutrients required for sustaining biomass growth, hence not practiced widely (Ghadiryanfar *et al.*, 2016).

The choice of raw material is critical for the efficient production of biofuels, in the case of macroalgae the critical decision is the choice of suitable species. Different macroalgal species could be chosen for their production of low-cost fuel with the combination with high value components and/or bioremediation applications. Therefore, biomass quality obtained from cultivation of species like *S. Latissima* is essential if high value products are intended. However, in a recent review of macroalgae biomass for bioenergy production it is noted that even before the algal species and cultivation site are selected, a complex interaction of physical, chemical and biological factors of a potential site have to be considered (Fernand *et al.*, 2017). This can be informative to identify both the characteristics of natural Kelp forests and thereby estimate the quality of biomass that can be grown should the site be chosen for macroalgae biomass cultivation. In addition, even though numerous studies are available in the literature which evaluate the wild species for seasonal and locational impacts, they have focused on the holdfasts of the biomass and no studies have reported on the differences observed in the fronds of the wild and cultivated species. This is critical for energy conversion process or for high value products extraction as fronds are preferred over holdfasts and stipes.

While there exists a number of studies exploring the impacts of environmental conditions on macroalgae there remains gaps in the literature pertaining to the utilisation of macroalgae (particularly *S. latissima*) as a feedstock for biogas production. Therefore, the following sections will review the existing literature surrounding biochemical methane potential reported for species *S. Latissima*.

2.2 Biochemical methane potential of *S. Latissima*

Macroalgae species have been studied since the 1970s and have frequently been shown to be a suitable feedstock for anaerobic digestion (Murphy *et al.*, 2015). There has been studies in the literature focusing on the factors influencing the biogas potential of macroalgae biomass. Factors such as the species and composition of macroalgae that can impact on the efficacy and efficiency of biogas production using AD (Jung *et al.*, 2013).

Studies have also been performed to quantify the methane yields from different macroalgae species using theoretical and experimental methods. Theoretical methane yields from anaerobic digestion of macroalgae have been reported in the range of 0.14 – 0.40 m^3/kg VS however, the practical yields of the biogas from the macroalgae are experimentally found below their theoretical maximum (Milledge *et al.*, 2014a).

Reported studies in the literature include green species like Ulva and brown species of Laminaria digitata and Laminaria hyperborea. Methane yields of seaweed species have been found to strongly depend on the concentrations of storage carbohydrates and in the case of brown algae, among the storage sugars, laminarin and mannitol have been shown to have the highest biogas potential during digestion however alginic acid have a relatively lower methane yield. However, it is reported that many microorganisms are not able to digest the biomass completely under strict anaerobic conditions. Many microorganisms can hydrolyse laminarin easily and it can be easily degraded during anaerobic digestion, however alginates found in seaweed are reported to be more difficult to digest (Black, 1948, Adams *et al.*, 2011, Gunaseelan, 1997, Briand and Morand, 1997). For the species *S. Latissima*, studies are still evolving with testing for anaerobic digestion potential among other macroalgae species. The theoretical biochemical yield of *S. Latissima* has been reported to be $422\ ml\ CH_4/g\ VS$ with a theoretical methane percentage of 50% (Allen *et al.*, 2015). Some of the reported studies are shown in Table 5.

Table 5 Review of Biochemical methane potential studies

Species	Main findings	Comments	References
Ulva (Green macroalgae)	Suitable for AD due to its prolific growth, composition and degradation characteristics	Disadvantageous due to high sulphur content	(Briand and Morand, 1997)
Laminaria hyperborea and Laminaria digitata (Brown macroalgae)	Methane production of 80 L/Kg for L. digitata and 40 L.kg for L. hyperborea (batch test)	Higher methane potential than green and red species	(Hinks *et al.*, 2013, McKennedy and Sherlock, 2015)
Laminaria digitata	Methane production of $336\ ml/g\ VS$ obtained for batch tests	Mesophilic digestion produced 30% higher methane than thermophilic conditions.	(Vanegas and Bartlett, 2013)
Saccharina latissima	Methane production of $223\ ml\ CH_4/\ gVS$ was obtained in batch test.	Thermal pre-treatment was found effective for higher BMP.	(Vivekanand *et al.*, 2012)

Even though brown algae biomass has been a focus for AD, most of the reported studies are observed to be feasibility studies trying to determine the effect of operational parameters on the digestion of the biomass. Therefore, following sections will discuss the studies specifically reported in the literature for *S. Latissima* with reference to their location of collection, harvest times, and utilising either wild or cultivated biomass for anaerobic digestion.

2.2.1 Impact of location

The studies in the literature reporting the use of *S. Latissima* indicate their reference of collection from a particular location. These are not studies intended to study any impact of location on the biomethane potential of the species rather these are mainly feasibility studies to select the best feasible species from a number of locally available species where the study has been carried out. A comparison of methane potential obtained for the species from various locations are given in Table 6.

Nielsen and Heiske (2011) compared four macroalgae species harvested in Denmark for the suitability for anaerobic digestion and the study included *S. Latissima*. The study showed that *S. Latissima* was highly suitable for anaerobic digestion with a methane yield of $340 ml\, CH_4/\, g\, VS$ during thermophilic batch tests. However, as *Ulva lactuca* had a higher potential for cultivation under Nordic conditions, Ulva was selected for further studies by the authors (Nielsen *et al.*, 2011). In another study where *S. Latissima* was collected from Norway, a biogas production of $223\, CH_4/\, gVS$ was observed (Vivekanand *et al.*, 2012). Study, by Jard *et al.* (2012) compared anaerobic digestion potential of *Palmaria palmata* and *S. Latissima* collected in Brittany, France. Contrary to the previous study, it was observed that P. Palmata offered better methane production both in batch (500 ml) and semi continuous digestion tests (3L) due to its high volatile solids content and low cations content. Both the tests were carried out at mesophilic temperatures (35°C). Palmaria palmata showed a methane production of $257\, ml\, CH_4/g\, VS$ and *S. Latissima* showed a methane production of $209\, ml\, CH_4/g\, VS$ (Jard *et al.*, 2012). In Ireland, Vanegas and Bartlett compared the biogas potential of five Irish species and based on the results, *S. Latissima* and *S. Polyschides* offered the highest biogas production at mesophilic temperatures with $335\, ml\, CH_4/g\, VS$ and $255\, ml\, CH_4/g\, VS$ respectively in batch assays (120 and 1000ml). In another study conducted in Ireland, Allen *et al.* (2015) collected ten varieties of seaweed species in Cork, Ireland and tested for their biomethane potential which included *S. Latissima*. The BMP of the species showed a methane production of $341\, ml\, CH_4/g\, VS$ (Allen *et al.*, 2015).

Table 6 Methane Potential of *S. Latissima* from different locations

Location	Method	$ml\ CH_4/g\ VS$	Reference
Denmark	Thermophilic, Batch	340	(Nielsen and Heiske, 2011)
Norway	Mesophilic	223	(Vivekanand *et al.*, 2012)
France	Mesophilic, Batch	209	(Jard *et al.*, 2012)
Ireland	BMP	335	(Vanegas and Bartlett, 2013)
Ireland	BMP	341	(Allen *et al.*, 2015)

From briefly reviewing these studies, it can be seen that the species *S. Latissima* has demonstrated different methane potentials. This could be due to the fact that they were collected from different locations. The species has shown a BMP ranging from$209 - 341\ ml\ CH_4/g\ VS$. The biomass utilised by Vivekanand *et al.* was grown for one season in Trondheim, Norway (63°N, 10°E) by Seaweed Energy solution and was collected in August, 2010. As for the biomass utilised by Jard *et al.*, they were collected from Lézardrieux (Côtes d'Armor, Brittany, France) by Aleor seaweed farms. The biomass used in the Irish study by Vanegas and Bartlett was collected from a wild rocky outcrop of Streedagh beach, County Sligo, Ireland during low tide in September 2011. The biomass utilised by Allen *et al.*, however was collected from collected from beaches in Cork, South of Ireland (51°N, -9°E). The seaweeds were beach cast and harvested from their wild natural environment. The environmental conditions where this biomass were grown is different as it ranges from Norwegian Sea (Seaweed energy farm), Western part of Atlantic Ocean (Brittany), to Northern part of Atlantic Ocean (Sligo) and Celtic Sea (Cork). No information as to the environmental conditions where they were grown is provided by the studies. Therefore, there is a need to ascertain whether location is a significant factor and which environmental factors are critical for increasing methane production utilising macroalgae biomass.

2.2.2 Impact of season

As described previously, Kelp species are noted for their seasonal variation in their biochemical composition. There are few studies in the literature which have evaluated the impact of seasonal variation in the biochemical methane potential of *S. Latissima*.

Adams *et al.* (2011) explored the AD potential of *Laminaria digitata* harvested from three beaches - Barnacarry beach, Argyll and Bute, in the UK during the period from December 2007 till October 2008 for their anaerobic digestion potential. Methane production was observed for batch assays of 36 days. All samples followed a similar trend where there was a linear production of methane until the first five days of digestion and after which the methane production decreases. Samples from summer months produced a higher cumulative methane with harvest from July producing the highest amount of methane. This was attributed to the high laminarin and mannitol concentrations. The samples from the first 5 months of the year had lower concentrations of these carbohydrates and therefore lower methane yields. In addition, these samples also had higher alginic acids concentrations which could also have decreased the rate of hydrolysation in the biomass resulting in lower methane yields (Adams *et al.*, 2011).

There are few studies in the literature that investigated the effect of seasonal variation on the biochemical methane potential of *S. Latissima*. However, more recently a study by Marinho *et al.* (2016) focussed on the seasonal variation of *S. Latissima* grown in Danish waters for its bioremediation, and bio-refining potential with succinic acid production with integrated multi-trophic aquaculture. The study noted that November was the preferable harvest time for the species, due to high protein content. November harvest would increase the nutritional value of the seaweed to be used as a protein ingredient for fish feed. However the focus was fermentation based succinic acid production and not anaerobic digestion of the biomass (Marinho *et al.*, 2016).

From the above discussion it becomes clearer that, there is a gap in the literature to assess biomass harvested at different times of the year for their biochemical methane production characteristics owing to their varying biochemical composition at the time of their harvest. However, the generic rule that can be noted from the review is that if biomass is intended for AD, high carbohydrate content in the biomass should be targeted hence summer to early autumn could be the best times of harvest for *S. Latissima*.

2.2.3 Impact of growth type

In the literature, where studies have been performed on *S. Latissima*, there is a limitation to compare the results as the studies have utilised either wild sourced biomass, or long line cultivated biomass or simply beach cast biomass. However, no study was found to have compared the variation in the methane production of any species obtained from the wild sources and cultivated sources from the same location. This could be because as wild and cultivated biomass is genetically identical, their biomethane potential are also considered to be identical. For the studies reviewed in the earlier sections, Allen *et al.* (2015) and Vanegas and Bartlett (2013) utilised wild biomass. In contrast, Jard *et al.* (2012), and Vivekanand *et al.* (2012) sourced their biomass from cultivated long lines. These researches did not emphasise on the growth conditions and their impact on the methane potential of the biomass rather they were more focused on testing the overall feasibility of the species for anaerobic digestion. However, they can be used as indicators of methane production from cultivated or wild biomass from that location. More importantly, as wild sources are unsustainable, these studies can inform cultivation practices to cultivate biomass with specific characteristics intended for anaerobic digestion.

An initial review of the literature has highlighted the inherent variability of macroalgae biomass and the potential impact of location and environmental conditions on the biomass growth and characteristics. However, there remains little information on the species *S. Latissima* which will be the focus of this study. While there is some limited data in the literature on the utilisation of *S. Latissima* for AD there is very little relating this to biomass cultivation conditions. This gap in the knowledge needs to be explored in order to effectively manage the use of this biomass. The species will be studied to explore the extent to which environmental conditions at a given location growth cycle and consequently harvest time and growth type (wild or cultivated) impacts on biomass characteristics and methane potential.

Batch studies have their limitations, and are generally used as an indicator of suitability of a biomass for AD. Continuous studies performed in literature would show the advantages and challenges of utilising the biomass in detail with a large array of AD parameters observed as part of such studies. Semi-continuous studies performed for macroalgae biomass and particularly for *S. Latissima* will be discussed in the next section.

2.3 Semi-continuous digestion studies of *S. Latissima*

Macroalgae species as noted earlier are studied for their methane potential (Murphy *et al.*, 2015). The majority of the reported studies in the literature are batch systems. Continuous studies are limited in the literature for macroalgae biomass and especially for *S. Latissima*. This is predominantly because of the finite knowledge on the technical process efficiency, longer retention times for continuous studies, and also the limited availability of the biomass. The AD technology in itself is promising but the challenges include the need for developed knowledge about the microbial community and biochemical processes of the anaerobic degradation (Appels *et al.*, 2011). High retention times required for the digestion also has serious impacts on the costs and hence on the economic sustainability of the large scale AD. In addition, technical viability of the algae biomass for AD depends on the selected algae strain indicating deeper research needs for the selection processes and production systems (Brennan and Owende, 2010). Macroalgae utilisation for bioenergy industry is still at its early developmental stages and is yet to overcome environmental and economic challenges before the industry is a sustainable option.

For the semi continuous digestion studies for macroalgae biomass in the literature, studies are reported mainly for green macroalgae whereas studies for brown algae are only a recent development. The reported continuous digestion studies are shown in Table 7.

Table 7 Review of semicontinous studies

Species	Main findings	Comments	References
Ulva (Biomass from Osaka Bay)	Methane yield of $0.10\ L\ CH_4/day$	Mesophilic conditions.	(Otsuka and Yoshino, 2004)
Laminaria hyperborea and Macrocystis pyrifera (Northumberland, Spring 2007)	Methane production of 0.23 – $0.26\ L\ CH_4/\ g\ VS$	6 L reactor, OLR of 1gTSS/L/d. 179 days of operation.	(Hinks *et al.*, 2013)
Macrocystis pyrifera and Dunaliella Antartica (1:1 w/w)	Methane production of 180.4 ml/g/dry algae/day obtained.	1L volume, 31 days HRT and OLR of 3g/ dry algae/day.	(Vergara-FernÃ¡ndez *et al.*, 2008)
Saccharina latissima	Methane production in the range of 0.243 to $0.510\ L\ CH_4/\ g\ VS$	3 L volume, mesophilic, 9 weeks HRT, OLR increasing from 0.8 to 2.	(Jard *et al.*, 2012)

Saccharina latissima	Methane production of $0.131\ L\ CH_4/kg\ VS$ was obtained.	50 L reactor, 91.3g fed/day, mesophilic condition	(McKennedy and Sherlock, 2015)

In the limited studies reported in the literature the species *S. Latissima* has showed an overall methane production which ranged between $0.150 - 0.400\ L\ CH_4/gVS$. However, as observed from the previous studies, the results are varied and reflect the fact that the seaweed was collected from different countries at different times of the year, with differing environmental conditions. The methodology of assessing the methane production with working volumes, inoculum, I/S ratio, etc. are also varied in different studies (Laurens, 2017). This requires a standard operating procedure for such continuous studies with detailed analysis to identify the potential of *S. Latissima*. However, the process can be challenging as biomass can vary accordingly with locations, seasons and growth type and therefore have its impact on the studies. The following sections will now discuss the various process parameters observed for continuous digestion studies utilising *S. Latissima*. This will provide a perspective of the biomass performance in such studies. The important parameters required for continuous studies will be discussed in Table 8.

Table 8 Parameters recorded optimal of S.Latssima

Factor	Optimal conditions for S. Latissima	Comments	Reference
pH	7.20 – 7.38	Optimal for pre-treatment for BMP	(Vivekanand *et al.*, 2012)
Alkalinity	10.6 ± 0.40 mg/l	Range for continuous operations	(Tedesco *et al.*, 2014)
Temperature	Mesophilic temperatures between 35 - 37°C	No comparison performed between mesophilic and thermophilic temperatures -	(Vanegas and Bartlett, 2013, Vivekanand *et al.*, 2012)

		continuous studies	
Carbon to Nitrogen ratio	10/1 – continuous studies 7 – for spring and 21 – summer biomass	Important to identify the biomass with higher C/N ratio for higher BMP	(Montingelli *et al.*, 2015) (Handå *et al.*, 2013) (Schiener *et al.*, 2015)
HRT	21 days, 22-24 days	Longer HRT needs to be tested for S. Latissima	(Jard *et al.*, 2012) (Montingelli *et al.*, 2015, Hanssen *et al.*, 1987, Sarker *et al.*, 2012)
OLR	1.2 – 1.65 gVS/l/day 0.5 – 3.0 gVS/l/day	Higher OLR needs to be tested for S. Latissima	(Jard *et al.*, 2012)
Volatile fatty acids	Level of acetate and butyrate higher when BMP was higher. Ratio of acetate:propionate:butyrate should be 6:1:3 or 7:1:2.	No VFA profile has been provided yet for S. Latissima	(Moen *et al.*, 1997) (Chang *et al.*, 2010) (McKennedy and Sherlock, 2015)
Nutrients in the biomass	Na, K, Ca, Mg, P Fe, Zn, Mn, Al, and Cu	Nutrients found in S. Latissima	(Schiener *et al.*, 2015)
Nutrients for digester	K, Ca, Mg, Fe, Zn	No studies reported for the effect of nutrient solution to S. Latissima	(Angelidaki and Sanders, 2004) (Demirel and Scherer, 2008)

Toxicity	Possibly from Na ions or polyphenol content in the biomass	No studies reported for toxicity factors in S. Latissima	(Schiener *et al.*, 2015)

In summary, this review shows that even though an array of parameters is studied during continuous trials, critical parameters affecting the digestion of *S. Latissima* are not yet fully understood i.e. optimum temperature, longer retention times, biomass degradability ($VS_{destruction}$), trace element addition etc. The feedstock characteristics have shown that the summer biomass of *S. Latissima* has higher potentials for methane production with optimum C/N ratio, and higher carbohydrate content. The micronutrients present in the biomass can also be suggested to be helpful for maintaining the balance (pH, alkalinity) of the digester without any further addition of trace elements for stability.

Therefore, the scope of this study was to understand the methane production profile of *S. Latissima* in a semi-continuous digestion operation. This was performed by comparing the effect of mesophilic and thermophilic temperature on AD performance of *S. Latissima*. In addition, the study also investigated any effect on methane production by the addition of trace elements for enhanced digestion of the macroalgae biomass. pH, alkalinity, SCOD, were analysed on all of the reactors. The results from continuous studies also researched the effect of longer retention times of 105 days over three retention times. The effect of toxic elements and salt inhibition were not the objectives of this study. Further work in the digestion studies can explore the VFA profiling for the digestion of *S. Latissima*, effect of micro and macro elements in the macroalgae biomass on methane production during continuous studies.

Although *S. Latissima* is shown to be promising for anaerobic digestion, the toxic metals in its biochemical composition can cause problems in further utilisation of the biomass in large scale applications. The presence and/or formation of recalcitrant materials such as polyphenols, cellulosic fibres, and lignin like components in the biomass can result in the reduction of biodegradability of the biomass during bacterial activities associated with anaerobic digestion producing lower biogas quantities. In addition, the seasonal variability also affects the growth of the biomass and hence the availability of the feedstock for biogas production (Jard *et al.*, 2013). Therefore, even though anaerobic digestion has a mature platform for a newer biomass like algae, still further studies are required to optimise the reaction processes to increase the methane yield using either pre-treatment techniques or co-digestion methods or using a combination on simpler pre-treatment methods and co-digestion feedstock (Jiang *et al.*, 2016).

2.4 Macroalgae as a co-digestion feedstock

Due to some of the problems associated with mono digestion of macroalgae biomass such as high salinity, and the presence of compounds for e.g. polyphenols, cellulosic fibres, and sulphide concentrations in some species there are still barriers to achieve the full potential of AD utilising the biomass (Ward *et al.*, 2014). In comparison to microalgae, macroalgae have been studied for co-digestion in the literature. Ulva and *Gracilaria* species are found to be the most reported in the literature for co-digestion studies (Cecchi *et al.*, 1996, Costa *et al.*, 2012, Nielsen *et al.*, 2011, Sode *et al.*, 2013). Reported studies on co-digestion is given in Table 9.

Table 9 Review of co-digestion studies

Species	Co-digestion feedstock	Main findings	References
Ulva and Gracilaria (Venice lagoon)	Sewage sludge	Mesophilic, I:S – 1:4, 11 days HRT Optimised waste management	(Cecchi *et al.*, 1996)
Ulva and Gracilaria	Waste activated sludge	15% algae: 85% sludge Optimised biogas production (26% higher)	(Costa *et al.*, 2012)
Ulva species (Denmark)	Cattle manure	No increase after 50% concentration	(Nielsen *et al.*, 2011)
Laminaria digitata and S. Latissima	Bovine slurry	Decreased ammonia inhibition	(Vanegas and Bartlett, 2013)
Ulva	Gracilaria	Balances C/N ratio to between 20-30	(McKennedy and Sherlock, 2015)
Ulva	Waste paper	Balances C/N ratio for increased BMP	(Yen and Brune, 2007)

As discussed in the table, co-digestion has benefits in terms of improved waste management, enhancing methane yields, decreasing inhibition effects of particular feedstock and balancing of carbon to nitrogen ratios of the mixed feedstock.

Co-digestion is a practical way of incorporating new feedstock such as macroalgae into AD processes. Technologically, anaerobic digestion is promising as a sustainable option for waste treatment and energy production given its capacity to treat a range of materials. The following section will discuss the co-digestion studies reported specifically for *S. Latissima*.

2.4.1 Co-digestion of *S. Latissima*

There have been a number of studies recently reviewing the scope on the co- digestion of algae (Montingelli *et al.*, 2015, Jung *et al.*, 2013, Mata-Alvarez *et al.*, 2011, Nielsen *et al.*, 2011) however only around six papers were identified specifically evaluating S latissima which indicates that further work is required (Vivekanand *et al.*, 2012, Sarker *et al.*, 2012, Vasilaki and Garcia, 2013, Gurung *et al.*, 2012, Matsui and Koike, 2010). The main findings from the co-digestion studies are given in Table 10.

Table 10 Review of S. latissias for co-digestion

Feedstock digested with S. Latissima	Main findings	References
Wheat straw	Optimal blend was 75:25 Increased BMP $275\ mL/g\ VS$ for co-digestion batch studies. Increased digestibility for straw.	(Vivekanand *et al.*, 2012)
Bovine slurry (Ireland)	BMP of $244\ ml\ CH_4/g\ VS$ was observed for batch BMP	(Vanegas and Bartlett, 2013)
Cattle manure	Thermophilic digestion at 50°C preferred for BMP with improved digester conditions	(Sarker *et al.*, 2012)

Shrimp residues and sewage sludge	Higher BMP of $0.261\ m^3 CH_4/\ kg\ VS$ obtained	(Vasilaki and Garcia, 2013)
Fish viscera	BMP of $0.166\ L\ CH_4/\ kg\ VS$ obtained with better COD conversion to methane, longer retention times needed for co-digestion. Digestate produced is nutrient rich and complex	(Gurung *et al.*, 2012)

This review has demonstrated that most of the existing studies are batch lab-scale experiments. There are very few pilot scale studies reported for co-digestion. Only one study reported the pilot scale co-digestion of *Laminaria* and *Ulva* mix with milk residues in Japan. The process was successfully demonstrated at pilot scale with a period of 11 weeks of operation. The methane yield was found between 10-12 m^3/day where steady production of gas was observed from week 6 (Matsui and Koike, 2010).

In summary, there are isolated research studies reported in the literature utilising *S. Latissima* for co-digestion. There have been very few studies on co-digestion of organic feedstock with *S. Latissima*. In the reported studies, the maximum bio methane yields have ranged from $0.204\ to\ 0.380\ m^3\ /\ kg\ VS$ indicating that co-digestion using *S. Latissima* could be favourable in existing AD plants because of the similarity of organic composition to other organic feedstock already being used. In relation to *S. Latissima*, there are few technical barriers reported yet for the process, however the presence of salts (Na, and K) and polyphenols and their impact on long term digestion is yet to be studied in detail before the large-scale implementation of the macroalgae biomass. Anaerobic co-digestion should be hence a feasible option to overcome the drawbacks of mono digestion of either the traditional organic feedstock or of *S. Latissima* and therefore improve an AD plant's overall economic feasibility.

2.5 Techno-economic feasibility of macroalgae for anaerobic digestion

In addition to technical feasibility it is important that economic assessments are conducted to determine the commercial viability of projects and illustrate how to harness the true potential of biomass to meet the energy production goals and emissions targets at a national level. Macroalgae, which can be mass produced in temperate climates is also currently viewed as an interesting biomass at a national level in the UK. However, its viability for AD in full scale operation is still in question (Levidow and Papaioannou, 2013). That is precisely where economic feasibility studies would allow the potential users to identify the bottlenecks associated with the systems and potentially reduce the costs and energy input for their production and harvest, transportation, and develop overlap with the existing energy supply chains and conversion to fuels (Roesijadi *et al.*, 2010). There are mainly two kinds of studies reported in the literature – life cycle analysis and techno-economic analysis.

2.5.1 Life cycle analysis studies of *S. Latissima*

Algae biomass (both macro and micro algae) have been studied for their overall potential to contribute towards sustainability. For AD using microalgae biomass, costs were strongly correlated to electricity consumption and suggestions were made to reduce the mixing costs to improve the efficiency of AD processes (Collet *et al.*, 2011). On analysis about energy utilisation from AD coupled with microalgae production units were considered, research has suggested that if methane generated from AD is used in the facilities of microalgal biodiesel production, theoretically it can lead up to a 33% reduction in the production costs. The carbon emissions can also be reduced up to approximately 75% if biogas electricity is utilised instead of traditional grid electricity. Therefore, for microalgae, if biogas can be utilised for the energy required for microalgal cultivation, dewatering, extraction and trans-esterification process, then it has the potential to make production of biodiesel from algae more viable by reducing the overall cost of production per unit of biodiesel (Harun *et al.*, 2011).

Life cycle assessment of *S. Latissima* has not been reported in the literature so far. Life cycle assessments on macroalgae production systems were carried out by the EnAlgae project in Ireland and France for two different cultivation systems for the species *S. Latissima*. From their results it was observed that for Ireland, the long distance between the hatchery and the grow-out phase in the sea and the transport fossil fuel usage had the highest impact on the environmental footprint. While in France it was the complex use of different materials (material intensive, plastic tubes) for the infrastructure of the cultivation systems which had the largest environmental footprint. The impacts for freshwater, chemicals and nutrients were negligible at both sites. Therefore, the major recommendation from the study was to reduce the high use of fossil based resources either for transport (by reducing the distance by locating the grow-out phase closer to hatchery) and for production systems (by using alternative materials or trying different production designs) for seaweed production in North West Europe (Sprujit, 2015, Parker *et al.*, 2015).

Life cycle analysis of pilot macroalgae growing facilities at Queen's university Belfast was performed during 2012 – 2013 for the brown species *S. Latissima* and *Laminaria digitata* produced using long line cultivation systems in Strangford Lough. In this case, the hatchery phase had the highest share for environmental impact ($kg\ CO_2$ equivalent) and similar results for NW Europe were observed for the fossil fuel consumption and materials usage. However, this study identified the bottlenecks for seaweed production which were primarily energy savings and material reduction used for cultivation systems for seaweed. In addition, the study also suggested that bioenergy in terms of biomethane alone from seaweed may not fully satisfy environmental criteria therefore process optimisation and up scaled settings should be targeted for improved LCA results (Parker *et al.*, 2015).

Even though the focus of existing LCA studies has been optimisation of the cultivation systems, new and improved systems with lower impacts have been tested for e.g. Integrated Multi-Trophic Aquaculture (IMTA) systems. The IMTA system is considered more sustainable due to the reduced requirement for materials, and nutrients, equal space requirements for the growth of shellfish and seaweed and the by-products of one process being utilised as an input for the other's growth. Also, grown commodities such as pigments, additives, are highly valued in the market so that the economics are more favourable. In the UK, Scottish Association for Marine Sciences, SAMS have conducted numerous IMTA studies quantifying the bioremediation potential of seaweed in the area. However, the majority of these deployments are only carried out for academic purposes and no assessment of the economic aspects of such systems in the UK are so far established (Murray *et al.*, 2013).

2.5.2 Techno economic studies of *S. Latissima*

Techno-economic (TE) analysis of the overall AD process has been reported in the literature. Zammalloa *et al.* (2011) analysed the techno-economic potential of AD and stressed on the importance of governmental support mechanisms such as feed-in-tariffs as it is shown as the major determinant of the net present value providing almost 96% of the revenues. The other factors found important for a techno-economic view point were productivities of the biomass, loading rate, number of operational days of the digester and associated operational costs (Zamalloa *et al.*, 2011). Techno economic viability of the macroalgae cultivation systems are reported from a few studies which indicate that optimisation is still required for the long line cultivation techniques utilised in European countries (Roesijadi *et al.*, 2008, Roesijadi *et al.*, 2010, Kraan, 2013).

Techno-economic assessment particularly focuses on improving the overall feasibility of the process utilising the biomass. An extensive TE analysis evaluating performance of the brown species *Laminaria digitata* in European market was conducted by Dave *et al.* (2013). The study performed the analysis on the ECLIPSE model assuming 8.64 tonnes dry biomass per day (feed rate) for a community based (AD capacity of 1.6 MW) CHP production unit. The findings of the study stressed that even though the current market is economically favourable for extracted high value compounds from the macroalgae biomass in Europe, a community-based CHP plant could be a favourable option in utilising green energy from the brown algae biomass. The study considered the annual variations of the biomass, with the line breeding near shore cultivation systems for utilising 8 tonnes of biomass per day (dry basis) for a moderately sized AD plant assuming an algal conversion rate of 64% within 15 days. The total macroalgae cost was assumed to be 50 euros/tonne. The plant generated 237 kWE (net) electricity and 367 kW heat. No government incentives were considered for this study, however the study recommended that support systems such as Feed in tariffs and Renewable Obligation Certificates could significantly improve the project viability. The economic viability of the plant was measured in terms of internal rate of return in connection with the different feedstock prices, total annual power generation and the capex expenditure. The study found that the major cost factors were associated with the feedstock cost, maintenance and operating costs, any additional treatment facilities cost for biomass utilisation (drying, pre-treatment etc.). Moreover, the study also stressed on the reliability of continuous supply of feedstock, hence suggesting co-digestion with other biomass and wastes such as sewage sludge, food waste etc. as a potential solution (Dave *et al.*, 2013).

More recently Konda *et al.* (2015) performed a techno-economic assessment on *S. Latissima* to understand the cost drivers and identify the economic potential of the biomass at industrial scale. Despite the advantages over other biomass feedstock for reduced water usage, pollution control, etc., the actual success of the macroalgae based biorefinery was reliant on the economic performances of the processes which were converting the biomass into fuels or products. The study focused on ethanol production from macroalgae biomass with an industrial scale facility of 2000 MT/day dry biomass processing capacity. The preliminary results showed that the maximum allowable price for the seaweed was 28$/MT (dry) in order to produce ethanol at 2.2$/gal or less with a production cost ranging between 21-112$ /MT (depending on the species and the cultivation method employed). The same study found that *S. Latissima* to sugars platform is economically viable, easily scalable, and efficient, however downstream technologies i.e. purification and effective product recovery systems are still required to make the chemicals from the biomass cost competitive to the petroleum derived products. The other main critical factor identified through the study was the reduction in the macroalgae feedstock price and the need for the development of supply chain and logistics for the utilisation of such biomass (Konda *et al.*, 2015). Therefore, there is definitely a need for more studies to evaluate the techno-economic feasibility of species *S. Latissima* for its utilisation for AD.

From this review it also becomes clear that a biorefinery based approach is essential for an economically viable, scaled up system for biogas production from macroalgae biomass. Regardless of economic impacts, from a sustainability point of view it is important to consider metrics such as carbon, nutrient, and water balances, recycle opportunities and delivery sources, which are all location specific. For a commercial level realisation and economic viability of microalgae biomass utilisation, the use of spent biomass beyond biogas generation is recommended alongside creating high value by-products with market sustainability of such co-products (Davis *et al.*, 2011). For macroalgae biomass, the system should have a balance of high value products, and bioenergy from co-digestion with other available waste resources alongside production of biogas and digestate for the use as fertiliser (Ramírez, 2015). Bio-methane from macroalgae is definitely shown to be one of the promising systems for bioenergy production for future. Research suggests that this can be achieved by integrating the seaweed cultivation similar to IMTA techniques, using innovative designs for cultivation systems, ensuring optimal species and composition, reusing digestate and utilising renewable electricity to power the plant operations (Czyrnek-Deletre, 2017). So from the discussion it is also clear that techno-economic studies for macroalgae biomass is a developing field where most of the studies have focused on the feasibility of the species in terms of cultivation, and producing high value compounds or its suitability for a particular energy derivation path way. Therefore, from an AD perspective, efforts should be focused on decreasing the feedstock cost, increasing the methane yield of the biomass and identifying low cost effective pre-treatment facilities and suitable co-digestion strategies for macroalgae utilisation for AD. Hence, in this study, techno-economics analysis is chosen as an approach to identify the effect of AD technology on the overall economics of the process – i.e. the benefits and adverse effects of AD, monodigestion and co-digestion on economics utilising *S. Latissima*.

3 Methods

This chapter provides details of the general materials and methods used for the experimental aspects of this study. This includes detailed description of experimental and reactor designs, analytical methods, and materials used for this research. Details on the reliability, repeatability and precision of the analytical methods are also provided within each section.

3.1 Materials - Feedstock and Inoculum

3.1.1 Feedstock

The feedstock utilised for this research study involved macroalgae (sourced from various locations and detailed below) and organic feedstock supplied by local partners including agricultural crop waste residues, pig manure and brewery spent grain. The macroalgae species was evaluated for its anaerobic digestion potential as a single and co-digestion feedstock with the traditional organic AD feedstock described above. The details of procurement, processing and storage of each feedstock is detailed in the sections below.

The macroalgae species used in this study is *S. Latissima* (*S. Latissima* and was, obtained from Northern Ireland (Belfast), Southern Ireland (Ventry Harbour) and Scotland (Figure 9).

Figure 9 Source of seaweed used in this study

Source Google Maps

The macroalgae (seaweed) utilised for seasonal studies was obtained from the wild (natural) harvest at Strangford shore and cultivated samples were received from the Queen's Marine Laboratory long-line site in Strangford Lough, Queen's University, Belfast, (QUB). The seaweed samples from QUB were obtained from November 2015 to December 2016 including an early spring sample in April 2016 and summer sample in June 2016. The seaweed samples for comparative locational studies was received from Scottish Association of Marine Sciences (SAMS), Oban, Scotland and Dingle Bay (DB) seaweed cultivation farms, County Cork, Ireland. The wild samples and cultivated harvests from SAMS were received in June 2016 and December 2016. Cultivated samples from Dingle bay farms were obtained in June 2016. The frond (leaf like structure of macroalgae) view of seaweed *S. Latissima* is shown in Figure 10.

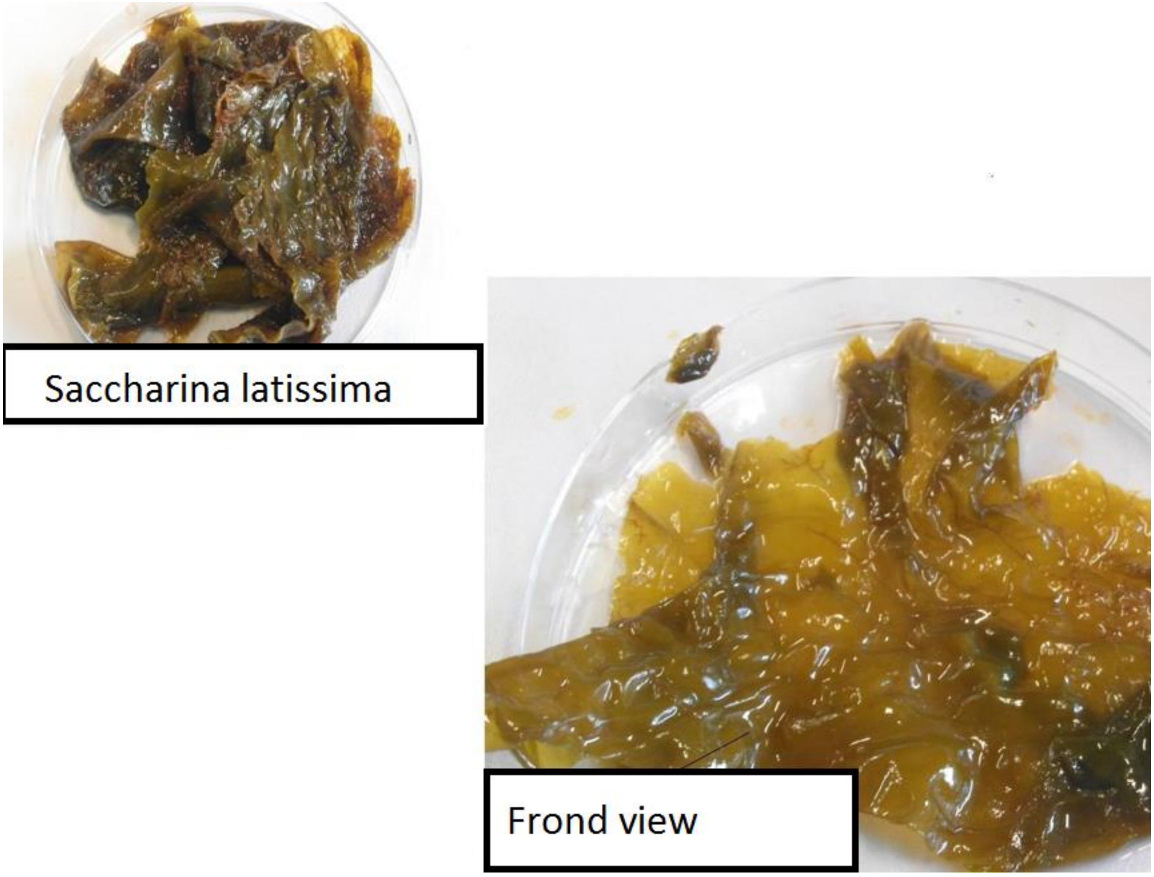

Figure 10 *S. Latissima* – frond view

The samples collected from SAMS, DB and QUB are listed in the table below (Table.1). A schematic of the process pathways used during this study is shown in Figure 3. The samples were named as Strangford Lough (samples from QUB), Isle of Seil (samples from SAMS), and Ventry Harbour (samples from dingle bay).

The schematic process pathway used for this study is shown in Figure 11.

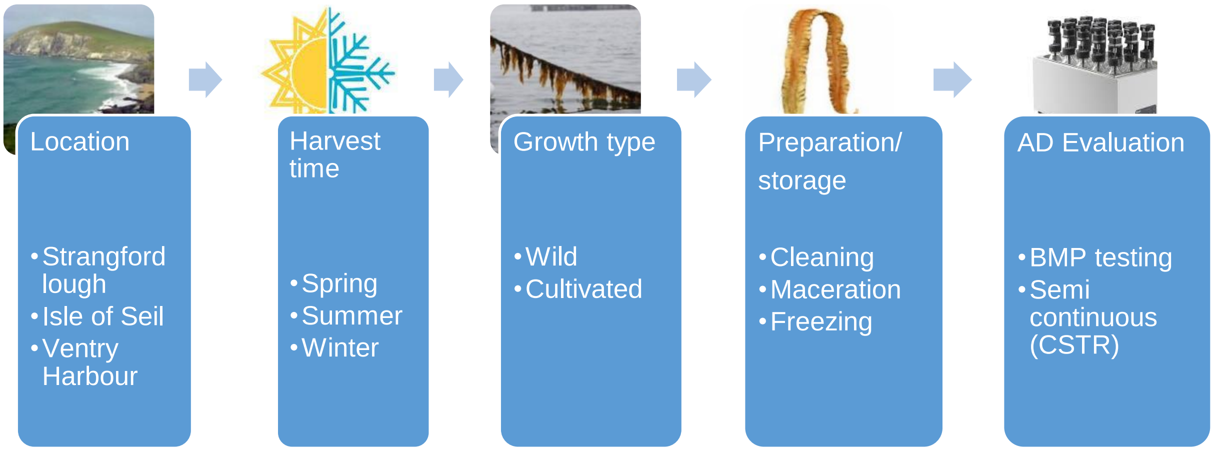

Figure 11 Schematic of process pathway for this study

The harvesting timetable for wild and cultivated samples used in this study is shown in Table 11.

Table 11 Harvesting time table used for this study

Supplier	Wild/Cultivated	Year/Season Harvest
Strangford Lough	Wild	Winter 2015
	Wild	Spring 2016
	Wild	Summer 2016
	Cultivated Wild	Summer 2016 Winter 2016
Isle of Seil	Cultivated Wild	Summer 2016 Winter 2016
Ventry Harbour	Cultivated	Summer 2016

The samples were harvested from the long lines, and transported immediately in sealed containers. On arrival at the BCU campus they were removed from the packaging, labelled and stored in the freezer at -20°C in zip lock bags for long term storage and for regular use for experiments. The storage method was followed as described in (Schiener, 2014, da Silva Marinho, 2016). The pre-treatment of the received seaweed prior to the experiments and storage are detailed under the section 3.2.

3.1.2 Organic feedstock

The organic feedstock that were used in the experiments included agricultural waste residues, pig manure and brewery spent grain. The agriculture crop waste feedstock and brewery spent gain was collected in March 2015, separately stored in individually sealed and labelled zip lock bags and kept in a freezer (-20°C) for storage until the experiments. The pig manure obtained was in dry, pelletised form. It was packed in an air tight container and stored until used for experiments.

3.1.2.1 Agricultural crop waste residues

The agricultural crop waste residues included corn silage, wheat residues, grass silage, and sugar beet-vegetable mix (SBV mix). This feedstock was collected from Vale Green Energy plant in Worcestershire, UK. The samples were collected from the storage area in the energy plant. The cemented shelters which served as storage area had large piles of crop residues and vegetables mix. These were stocked on the outside to allow daily feeding into the on-farm digesters. The crop residue samples were collected from the storage area and vegetable mix collected from the pile of daily feed for the digesters. These wastes were from the farm’s latest harvest having stored there for a few days. All of the samples were collected, separated, prepared and stored on the same day.

3.1.2.2 Pig manure

Pig manure was received from European Bioenergy Research Institute, (EBRI), at Aston University, Birmingham, UK. The pig manure pellets were ground to fine powder and not soaked prior to the BMP tests for this study.

3.1.2.3 Brewery spent grain

Brewery Spent Grain (BSG) samples were collected from a local brewery (Froth Blowers Brewing Company, Erdington in Birmingham, UK). The samples were received after the initial extraction operations in the brewery and separated from the wort liquid. The organic feedstock used for this research is shown in Figure 12 .

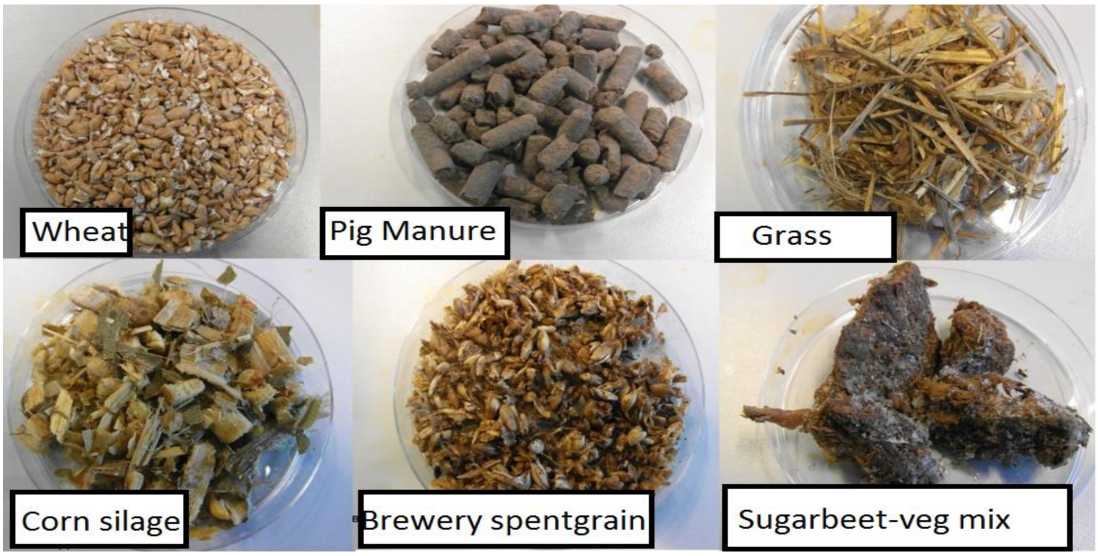

Figure 12 Organic feedstocks used in this study

3.1.3 Inoculum

The inoculum used for biochemical methane potential (BMP) tests and the semi continuous digestion experiments was collected from Severn Trent Waste Water Treatment Plant, (STWWTP), in Minworth, Sutton Coldfield, West Midlands, UK. The inoculum was collected from an active digester operating at 37°C. The inoculum was maintained at (37°C) in a temperature-controlled water bath until the start of experiments. The inoculum was tested for the pH, total and volatile solids content in triplicate on the same day of collection prior to further experiments. The processing and storage of inoculum for biochemical methane potential tests and semi-continuous tests are detailed separately in the following sections.

For BMP testing, the collected inoculum was sieved using a stainless steel sieve (size 5 mm) to separate any bulk impurities like glass or stones. The sieved inoculum was then degassed for two days in a temperature controlled water bath at 37°C (Suhartini, 2014). While, for semi-continuous trial, the freshly collected inoculum was directly filled into all reactor at volume of 2 L. The variability observed for the methane potential of the inoculum collected at various intervals will be discussed in the results section.

3.2 Pre-treatment of feedstock – Macroalgae biomass

3.2.1 Sample preparation

Following collection of seaweed, a number of steps were undertaken to produce a homogenised product suitable for long term storage.

3.2.1.1 Cleaning

The collected seaweed (as sent) was visually inspected on arrival for cleaning and removing holdfasts and foreign materials such as stones, shells, other seaweeds and invertebrates. The fronds of the wild samples of seaweed, when sparsely or irregularly covered with epiphytes, were retained as this is considered to be an unavoidable and natural occurrence on wild harvests (Forbord *et al.*, 2012).

3.2.1.2 Maceration

The second stage of sample preparation for samples was maceration. A household food blender (Bosch MCM 41, UK) was used to macerate the seaweed into smaller particles. The particle size of the shredded seaweed was approximately 1cm. The macerated seaweed was then mixed in a larger container to ensure a homogenous and representative sample could be taken. Samples were then divided into 0.5 to 1Kg portions for storage.

3.2.1.3 Storage

Each portion of the macerated seaweed was sealed in a plastic bag, labelled and stored in the freezer at -20°C. Prior to the analyses and experiments, the samples used for this study were taken from their storage and allowed to defrost to reach room temperature in the respective storage containers. Freezing the samples was not observed affecting the biodegradation of the biomass. The sample preparation steps were adopted from previous studies on *S. Latissima* published by (Schiener, 2014, Vivekanand *et al.*, 2012).

3.3 Experimental design

The following section describes the details of the preliminary experiments conducted to measure methane production using biochemical methane potential (BMP) tests and the semi-continuous digestion experiments conducted using lab scale continuously stirred tank reactors (CSTRs).

3.3.1 Biochemical methane potential tests

Anaerobic batch tests were conducted using Automated Methane Potential Test System (APMTS II, Bio Process Control, Sweden) (Bioprocess Control, 2017). The AMPTS II system provided an automated analytical procedure, real time data display and logging of accumulated bio methane volume, gas flow rate and analysis review. Prior to the BMP tests, the feedstock and inoculum were characterised for their pH, total and volatile solids content. The AMPTS II system consisted of 15 glass reactor bottles of 500ml volume, 15 x 100ml bottles respectively for each reactor bottle (containing 80ml of 3M $NaOH$ +0.4% Thymolphthalein pH indicator for the removal of CO_2 from the biogas), a thermostatic water bath and a gas volume measuring device. The gas measuring device was filled until the marked water level in the device (using deionised (DI) water). The tests were completed in triplicates for each combination of inoculum-substrate. Three reactors were used as blanks to ascertain the amount of biogas produced by the inoculum itself (blank) and three with the positive control cellulose to test the quality of the inoculum. The BMP tests were carried out for all the seaweed samples collected from QUB, SAMS, and DB as a single feedstock. The tests were also performed for all the co-digestion feedstock of agricultural crop waste residues, pig manure and brewery spent grain. A specific substrate to inoculum ratio (1:4) was used in each assay as per Angelidaki *et al.* (2009), and the working volume of the reactor was maintained at 400ml in order to ensure a sufficient headspace volume and therefore prevent any build-up of pressure. The AMPTSii system used for BMP tests is shown in Figure 13 .

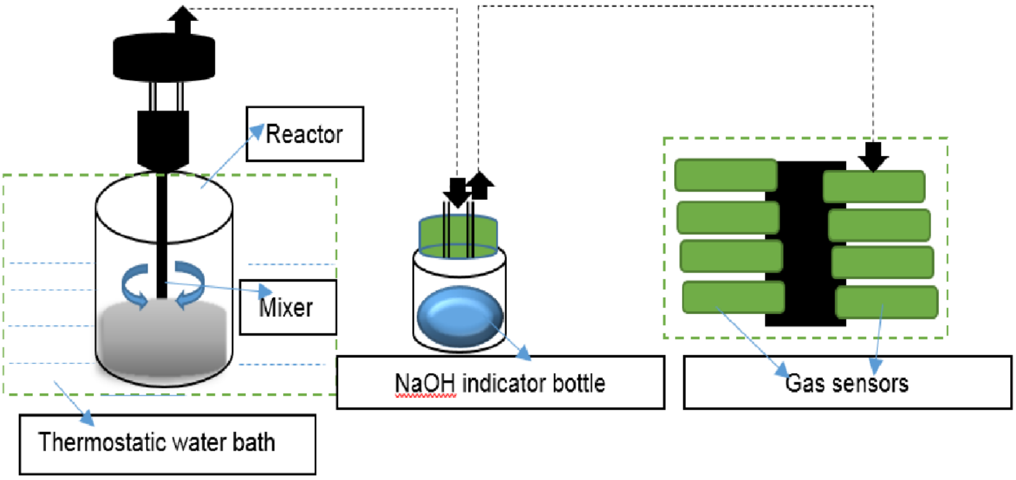

Figure 13 Automated Methane Potential System (AMPTS II)

The reactors were connected with the stirrers and placed in the incubation unit attached to the indicator bottles which are in turn connected to the gas measuring sensors (Figure 5). The tests were run for 30 days under mesophilic conditions (37°C). During the experimental run, the water bath was constantly checked for its water level and the indicator solutions were checked for any colour change (indicating CO_2 saturation). At the end of the experiments, data logging was stopped by pausing the individual cell, disconnecting the tubing connections with the indicator solutions, and from the gas volume measuring device and stirrers. The samples post BMP tests were all tested for pH, temperature, total and volatile solids.

3.3.2 Calculation of BMP

Biochemical methane potential is defined as the volume of methane produced per quantity of organic material added to the reactor. Therefore, the accumulated volume of gas from an experiment has to be divided by the amount of the substrate added into the reactor. However, to compensate for the gas production by the inoculum alone, its volume fraction of biomethane has to be subtracted from the total accumulated volume to get the true gas production from the substrate. Thus, BMP is expressed according to the equation below.

$$\boldsymbol{BMP = \frac{(V_s - V_I)}{m_{VSsS}}}$$

Equation 1: Calculation of BMP for the inoculum

Where V_s is the accumulated volume of biomethane produced by the substrate in the sample reactor, V_I is the volume of the biomethane produced by the inoculum in the sample reactor and $m_{VS,sS}$ is the amount of the substrate contained in the sample reactor.

The blank samples will generate only the amount of biomethane from the inoculum (V_B) and can be further on normalised to the biomethane production per unit weight of dry organic material in the inoculum. So to calculate the biomethane production from the inoculum in the sample reactor is given in the following equation.

$$\boldsymbol{BMP = \frac{V_s - V_I}{m_{VS,sS}} = \frac{V_s - V_B \frac{m_{VS,IS}}{m_{VS,IB}}}{m_{VS,sS}} = \frac{V_s - V_B \frac{m_{IS}}{m_{IB}}}{m_{VS,sS}}}$$

Equation 2: Calculation of BMP for the feedstock sample

As it can be seen from equation 9 the ratio between the amounts of organic material from the inoculum in the sample vs. the one in the blank is equal to the ratio between the total amount of inoculum in the sample (m_{IS}) and the one in the blank (m_{IB}). $m_{VS,sS}$ denote the VS amount of the substrate in the sample bottle, $m_{VS,IS}$ denotes the VS amount of the inoculum in the substrate bottle and $m_{VS,IB}$ denotes the VS amount of inoculum in the blank bottle.

The experiments are carried out in triplicate to ensure reproducibility and evaluate statistical significance: 3 reactors as blanks (inoculum alone), 3 reactors as positive control (cellulose) and the rest of the reactors containing samples (inoculum and substrate). The data generated from the report is also calculated for its standard deviation factor as it is commonly used to obtain the precision of the values obtained.

3.3.3 Semi continuous digestion trials

3.3.3.1 Design of the digester

Laboratory scale semi continuous anaerobic digestion experiments were carried out in a continuously stirred tank reactor (CSTR) as shown in Figure 14.

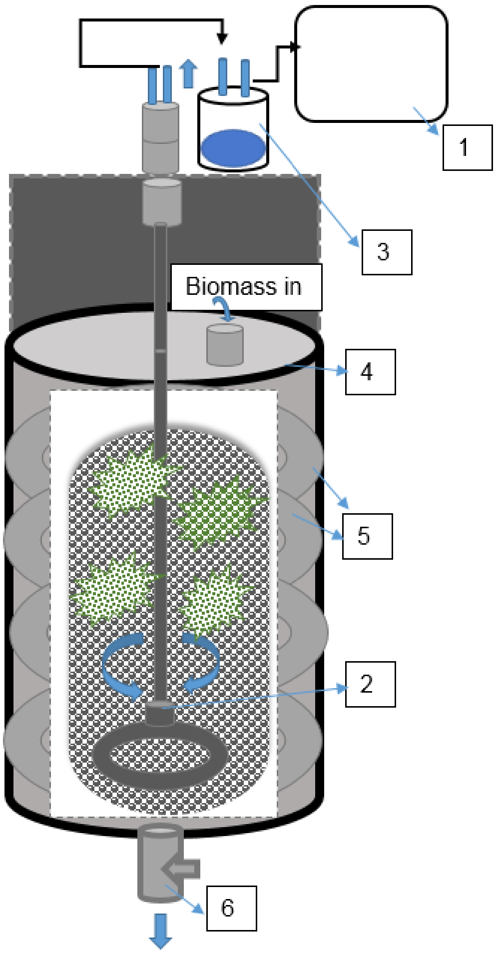

Figure 14 Continuously Stirred Tank Reactors – CSTR

1. Tedlar bag	2. Stirrer	3. $NaOH$ indicator
4. Reactor	5. Heating wire	6. Digestate port

The digester had a 3L total capacity with 2L working volume. Temperature was automatically controlled (via a PID temperature controller) and heating was achieved through an insulated electrical heating wire (Labelled 5) wrapped around the outside of the vessel. The internal temperatures of the digesters were monitored daily using a digital thermometer (Fisher scientific, UK). Continuous mixing was achieved through an automated mechanical stirrer (labelled 2) attached to a motor (RS, UK) controlled automatically. Feeding was done manually on a daily basis through the feeding port (labelled 4) located at the top of the digester. Similarly, the effluent (digestate) was withdrawn manually through the tap located at the bottom of the digester (labelled 6). Digestate was withdrawn until the level of the digesters were maintained to the working volume of 2L. Gas was collected using Tedlar bags (labelled 1) connected to each reactor with a capacity of 5L. Gas volumes were measured using the cylindrical gasometer based on the water displacement principle (Walker *et al.*, 2009). Alternate digesters were connected with $NaOH$ indicator bottles (3M $NaOH$+0.4%Thymolphthalein pH indicator) to monitor biogas production and methane production separately (labelled 3).

3.3.3.2 Experimental set up

Six reactors (duplicates of each), were used for the semi continuous digestion experiments. Three samples were evaluated for their performance during the experiments. An organic loading rate of 3gVS/l-d was used for the experiments. The organic loading rate was not increased to ensure digester stability. The experiments were run for 3 hydraulic retention times (a total of 106 days). The samples used were the cultivated summer harvest of *S. Latissima* from DB as a single feedstock. The digestate was tested for its pH, conductivity, alkalinity, COD, and dewaterability using CST measurements (all described in section 3.4 below). Gas volumes and digester temperatures were also monitored on a daily basis. Samples were stored for VFA analysis. Biogas produced from the reactors were collected in Tedlar bags (5L volume). The bags were measured daily for the biogas volume produced by the respective reactor. Gas composition analysis was conducted once a week to calculate biomethane percentage in the biogas produced.

The experimental set up and parameters are shown in Table 12.

Table 12 Semi continuous digestion experiments set up and parameters

Parameters	**Reactor 1**	**Reactor 2**	**Reactor 3**	**Reactor 4**	**Reactor 5**	**Reactor 6**
Temperature	M	M	M	M	T	T
Organic Loading Rate (OLR, g VS/l/day)	3	3	3	3	3	3
Daily Feed (g WW/day)	56.6	56.6	56.6	56.6	56.6	56.6
One Hydraulic retention times (HRT)	35.3	35.3	35.3	35.3	35.3	35.3
Total run of the digesters (days)	106	106	106	106	106	106
Trace element addition	No	No	Yes	Yes	No	No
Volume of the digesters (L)	2	2	2	2	2	2
*M – Mesophilic, T – Thermophilic						

3.4 Analytical methods

The analytical methods introduced here were used for the sample analysis and characterisation. The methods were for the BMP tests and the semi continuous digestion experiments.

3.4.1 pH

The pH values were measured using a Mettler Toledo pH meter with a built in temperature sensor (Mettler Toledo AG Analytical, Switzerland). The method is based on the voltage changes induced by the different concentrations of the hydrogen cations (H^+) measured by the electrode made of silver in the electrolyte solution. In order to ensure accuracy, the pH meter is first calibrated using the standard buffer solutions (pH 4, 7, 9). The precision, repeatability and accuracy of the measurements are highly dependent on the pH probe. The manufacturer guarantees an achievable accuracy of $\pm 0.05pH$ units.

3.4.2 COD

Chemical Oxygen Demand (COD) was measured using the barcoded cuvette tests – LCK 014 according to ISO 6060-1989 (Hach Lange, USA). Digestate samples were diluted to a dilution factor of 10. After mixing the diluted digestate solutions using a stirrer, 0.5 ml of the diluted samples were then added to the cuvettes to calculate the total COD. The diluted samples were then transferred to centrifuge tubes (2ml) and centrifuged for 10 minutes (14X1000 rpm). After centrifugation, the samples are then filtered for their supernatant. 0.5ml of the supernatant solution is then added to the cuvettes to calculate the SCOD of the tested samples. The cuvettes are then digested in a COD digester (Hanna Instruments, HI 839800 COD reactor) for 2 hours at 150°C. After the samples have cooled down to room temperature, the samples were evaluated for their optical densities using a spectrophotometer (DR2400 Hach) where the barcoded cuvettes were automatically detected for the tests and values are reported. The results obtained multiplying with the dilution factor were read as mg COD/l. The tests were performed in duplicates or triplicates for accuracy and precision of the obtained results. The preparation of the samples for dilution, and filtration thereafter were followed as per Suhartini *et al.*, 2014.

3.4.3 Total and Volatile solids

The quantification of total and volatile solids present in a sample was conducted according to the standard methods (APHA, 1992). Total solids (TS) were measured from a representative sample weighed in a porcelain crucible and dried up to a constant weight for up to 24 hours in the oven (Thermo Scientific Heraeus Oven, UK). The amount of sample taken for measurement was carefully recorded and total solids were calculated using the equation below.

$$\boldsymbol{Total\ solids = \frac{\{(A-B)*1000\}}{W\ sample}}$$

Equation 3: Calculation of total solids

Where A is the weight of the dried residue and the crucible at 105°C in gram (g), B is the weight of the crucible alone in g and W sample is the weight of the sample in g.

The samples after TS determination were further heated to a temperature of 550°C for 2 hours until a constant weight was reached. The concentration of volatile solids (Konda *et al.*) were calculated using the following equation.

$$\boldsymbol{Volatile\ solids = \frac{\{(A - C) * 1000\}}{W\ sample}}$$

Equation 4: Calculation of volatile solids

Where C is the weight of the sample and the crucibles at 550°C in gVS content can also be expressed as a percentage of TS.

3.4.4 Alkalinity

The measurement of alkalinity is based on the principle that the hydroxyl ions present in a sample formed as a result of dissociation or hydrolysis of solutes reacts with additions of the standard acid. Thus, the alkalinity is the acid-neutralising capacity and reported as the calcium carbonate per litres ($CaCO_3/L$) (APHA, 1992). In this study alkalinity was measured using automatic titrator (Titroline 5000 potentiometric titrator, Germany). The titrator was calibrated before the tests using the three standard buffer solutions (Titroline Buffer solutions). The alkalinity measurements were calculated from the values obtained from the automatic titrator when the sample was titrated with the acid until pH reached 5.7 and 4.3 for partial alkalinity and total alkalinity respectively. Sulphuric acid (0.001 N) was used as the standard acid reagent. Alkalinity expressed as mg $CaCO_3/L$ can be then calculated using the formula in the following equation.

$$\boldsymbol{Alkalinity = \frac{Vacid * N * 50000}{V\ sample}}$$

Equation 5: Calculation of alkalinity

Where V_{acid} is the volume of the standard acid used in ml, V_{sample} is the volume of the sample in ml, and N is the normality of the standard acid used.

3.4.5 Conductivity

Electrical Conductivity (EC) was measured using a portable conductivity meter (HI-99301, Hanna instruments, UK) at room temperature. The probe was immersed into the samples and left undisturbed until a constant reading is shown on the display. The probe had an accuracy of ±2% and working range of 0.00 to 20.00mS/cm. All readings were calculated for automatic temperature compensation with a built-in temperature sensor in the probe. The measured conductivity readings were shown in mS/cm.

3.4.6 Elemental composition

The seaweed samples and co-digestion samples were tested for their elemental composition including C, H, O,N, S. The tests were carried out in ESG Specialist laboratory (Burton-On-Trent, UK). The samples were air dried and ground prior to the analysis and analysed for the components using an elemental analyser and sulphur analyser.

3.4.7 Calorific values

Calorific values (CV) were measured for all the seaweed and co-digestion samples using a calorimeter (6100 Calorimeter, Parr Instrument Company, UK) using ISO 1928:2009 method of determination of gross calorific values by the bomb calorimetric method and calculation of net calorific values. The energy in the samples tested were observed as the high heating values (HHV). All samples were air dried and ground to fine powder. About 1g of the samples were then used for the tests. The calorimeter was first standardised using benzoic acid. After standardisation, the samples to be tested were put on the bomb head, fuse fire attached, and screw cap tightened. Then the bomb is filled with oxygen and inserted into the calorimeter with the bucket containing approximately 2litres of water. The tests were run in triplicate.

3.4.8 Biogas volume

The biogas volumes were measured using an in-situ gasometer working on the water displacement method. Gas was introduced from the Tedlar bags into the water in the gasometer column using the three-way valve for controlling the flow of the gas. The volume of the gas introduced was simply calculated by measuring the change in liquid height in the column. For multiple gas bags, the heights of the water level were recorded each bag until the bags were completely devoid of gas. The time, temperature and the pressure at the time of gas measurement were also noted on a daily basis.

The gas volume was measured in the height gasometer, where the gas was introduced into the closed column through the top valve while emptying the gas bag and it displaced the water into the container. The volume of the gas produced at standard temperature and pressure (STP) is then calculated using (Walker *et al.*, 2009) formula in the equation below.

$$\begin{gathered}
\boldsymbol{V_{STP} = \Big({}^{T_{STP}}/_{TatmP_{STP}}\Big) * (\big(P_{atm} - P_{H2O}(T_{atm}) - \rho bg(h2 + h0)\big)(Ah2 + Vh) -} \\
\boldsymbol{(P_{atm} - P_{H2O}\Big(T_{atm)} + \rho bg(h1 + h0)\Big)(Ah1 + Vh))) - \Big(P_{atm} - P_{H2O}\Big(T_{atm)} +} \\
\boldsymbol{\rho bg(h1 + h0)\Big)(Ah1 + Vh)\Big)}
\end{gathered}$$

Equation 6: Calculation of biogas volume in continuous system

Where V is the volume (m^3), P is the pressure (Pa), Vh is the headspace volume (m^3), H is the total height of the column, A, section of the gasometer (m^2), g is the gravitational constant (9.8m/s^2), ρ is density (kg/m^3), T_{stp} is temperature at standard temperature and pressure (K), T_{atm} is atmospheric temperature (K), h is the distance liquid surface form a datum (m) respectively.

3.4.9 Biogas composition

Biogas composition was analysed for the gas samples collected from the Tedlar bags connected to the respective reactor once a week. The gas was analysed for methane, carbon dioxide, oxygen and nitrogen percentages (%v/v). The gas analysis was performed at SOCOTEC specialist laboratory (Burton-On-Trent, UK). The tests were done using the bulk gas analytical method SMA 11a for analysing methane, carbon dioxide, oxygen and nitrogen using gas chromatography-thermal conductivity detection (GC-TCD) (SOCOTEC, UK, 2017).

3.4.10 Dewaterability

Dewaterability of the digestate samples were measured using the capillary suction time (CST) equipment (Type 304 M, Type 319 Multi CST, Triton Electronics, UK) as it has been established as a reliable method for sludge filterability and dewaterability. The digestate samples are stirred constantly for homogeneity and then pipetted into the test head assembly in the CST unit. The reset button is checked to be at zero reading. The volume required to complete the test is fixed by the reservoir volume at approx. 5 ml. The time (seconds) is noted from the display at the end of the test for the various samples tested.

3.5 Statistical analysis

The impact of location, harvest time and growth type on the biochemical methane potential of macroalgae biomass *S. Latissima* was analysed for statistical significance. The effect of different location, harvest times, and growth type were the variables analysed for statistical verification. ANOVA tests are normally used where comparison of means is tested for any difference between means of the different variables. Statistical verifications of the results obtained in this study was verified using ANOVA analysis. IBM SPSS statistical software version 22 was used for the analyses.

Statistical analyses were performed using the following steps.

Step 1: Test for null hypothesis using homogeneity of variance tests.

Step 2: Testing for equality of means using post hoc tests

Step 3: Bonferroni/Games Howell Test

Step 4: Statistically significance level (0.05, 0.001, and 0.1).

Homogeneity of variance test is an independent samples t-test stating that all comparison have the same variance. In our study, a Levene's test was conducted as the homogeneity of variance test. The Levene's test verifies the null hypothesis that variance is equal across groups. A p value less than 0.05 indicates the violation of the null hypothesis. Then the difference of the variance is statistically significant. When the Levene's test turned significant, Welch test, another robust test was run to test the equality of means. The results will be then confirmed using post hoc tests to identify the significant pairs.

Post hoc tests utilises multiple comparisons within the group to identify the significant pairs. Bonferroni tests is a preferred post hoc test when many independent or dependent statistical tests are run simultaneously. The Bonferroni is used also because it is highly flexible, very simple to calculate, and can be used with any type of statistical test (e.g., correlations)—not just post hoc tests with ANOVA. Bonferroni tests is ideal in this case where we have three variables to be tested for the different co-digestion substrates. Games Howell is also another post hoc test which is used with unequal variances and suitable for smaller group comparisons. Our BMP experiments were run in triplicates making the group size smaller for statistical comparisons. The choice of Games Howell is run in combination with Welch test as this test is preferred for smaller group sizes.

All results reported in the results chapters are reported following statistical verification. The significance level and the significant pairs of the results will be shown in the results chapters.

3.6 Techno economic analysis

A Techno economic analysis was conducted based on the biochemical methane potential of macroalgae co-digested with agricultural crop residues, manure and brewery spent grain. The methodology was adapted from a model provided by the EnAlgae (Interreg IVb, EU) project (Parker *et al.*, 2015). This techno-economic model (WP2A7.07) identified the political, economic, social and technological opportunities to promote the adoption of algal biomass within North-Western Europe. This model was specifically designed for considering the methane production from algae biomass and was therefore deemed appropriate for use in this study. The model was developed as a calculator to show the feasibility of AD for using algae biomass, and based on the findings from the EnAlgae report there existed a wide gap between a feasible business case and the AD of algae. Hence there was no verification study financed to verify the model as such.

Rationale for the adaptation of the EnAlgae model for this study:

The economic model considered co-digestion with products of either electricity with a combined heat and power (CHP) or green gas.

The investment parameters considered in the economic model included: -

- Cost of the digester,
- a CHP,
- a biogas process unit,
- A pre-treatment unit. (Can be included or excluded)

The substrates included in the model were predominantly for AD using co-digestion. Dairy manure was chosen as the base material and a maximum of 4 substrates could be chosen as co-digestion substrates. The full list of substrates is: -

Base material:

- Dairy Cow manure

Co-materials:

- Glycerine
- Silage maize
- Wheat straw
- Sugar beet
- Beet leaves
- Pig manure
- Maize straw
- Algae paste

In the developed EnAlgae model, a maximum of 4 co-substrates for the model could be chosen where the quantity of the co-materials should not exceed that of cow manure (Base material). The model assumes a ratio of 50-50% of cow manure and co-material. The scale of the CHP is adjustable in the model for the desired CHP level. The price of the algae paste and the co material can also be adjusted according to the choice of the co material chosen.

For this study, the model assumed a 52% (CH_4 content) and 48% (CO_2 content). A 36% electric efficiency was assumed for the CHP unit. The operational hours of the digester per year was assumed to be 8000 hours. The heating value of methane was approximated to be 36.5 MJ/m^3. An inflation rate of 2% per year was considered for the cost parameters in the model. The digester was given a life span of 25 years, 10 years for CHP, and 10 years for green gas processor, and 15 years' life span for the pre-treatment unit in the model. The scale of the digester at 1,000,000m^3 of biogas for a digesting time of 30 days.

The results from the economic model mainly focused on the return on investment percentage (ROI, %) and the payback time in years. The results also showed a comparison of the percentages of selling price and the cost price for the various substrates considered for co-digestion. The results would also give the comparison of total returns and total costs. A financial return for every 100 Euro in costs will also be shown on the results table.

The economics for *S. Latissima* harvested from three different locations, 4 different harvest times and wild and cultivated growth types were analysed in this study. The co-digestion feedstock of agricultural crop wastes, pig manure and brewery spent grain was analysed separately. The individual BMP of the co-digestion tests were inputted in to the model to estimate the effect of co-digestion on the biogas production unit of per gram of organic volatile solids digested.

4 Results – Macroalgae Characteristics

As discussed previously, the Kelp species, *S. Latissima* was chosen as the primary feedstock for this study. This chapter focuses on the impact of location (i.e. environmental factors of different sample locations), season (i.e. time of harvest) and growth type (wild sourced or cultivated) on the characteristics of the biomass.

Understanding the characteristics of a new biomass source such as macroalgae is important in order to assess feasibility and optimise conversion processes. Macroalgae biomass have complex and variable properties and research into utilisation of the biomass demands critical knowledge of different factors affecting its growth and cultivation throughout the life cycle of the biomass. The growth of Kelp for example is influenced by the physical and chemical conditions of the seawater. Various factors such as seawater temperature, salinity, water flow, availability of nutrients, carbon dioxide and sunlight, should be optimal (Kerrison *et al.*, 2015). Kelp biomass also exhibits seasonal variability as a result of its growth cycle and impacts of temperature and localised conditions (Schiener *et al.*, 2015). Gaining a greater understanding these variables will inform the design and operation of biomass cultivation and harvest practices. The following sections discuss the three main factors that are critical for *S. Latissima* – i.e. location (geographical location where they are grown), the time of harvest (season when the biomass is collected), and also primary source (wild or cultivated)

4.1 Sample collection

The biomass samples collected for this study are shown in Table 13. This study focused on the variability in the characteristics of the Kelp species, *S. Latissima* for any impact of environmental gradients in the location it was grown, the time of harvest and growth type.

Table 13 Samples collected for this study

Location	Winter 15	Spring 16	Summer 16	Winter 16
Strangford	W	W	W,C	W
Isle of Seil	-	-	W,C	W
Ventry Harbour	-	-	C	-

Samples were sourced from the three locations namely Strangford Lough, Isle of Seil and Ventry Harbour. Wild and cultivated samples were collected from Strangford

Lough and Isle of Seil while only cultivated samples were collected from Ventry harbour. The wild samples and cultivated samples collected from both these locations were growing closer to each other making sample collection manageable. The cultivated samples were only collected during their summer harvest while wild samples were collected to determine the impact of time of harvest on the sourced biomass. All samples were treated equally and systematically for the characterisation tests and any variability observed is confirmed using statistical analysis.

The sample locations and the environmental factors for the specific location are detailed in the following sections.

4.2 Sample Location

The seaweed samples of *S. Latissima* were collected from three different locations.

- Strangford Lough, Northern Ireland (Queen’s University Belfast)
- Isle of Seil, Argyll - Oban, Scotland (SAMS)
- Ventry Harbour - Dingle bay, Southern Ireland (Dingle Bay Seaweed Ltd)

The details of the three locations are given below.

4.2.1 Strangford Lough

The samples obtained from Strangford Lough were supplied by the seaweed growing facilities at Queen’s University Belfast (QUB), Northern Ireland, the Lough is located west of Jackdaw Island. Queen’s University Belfast had a leading role in a recent project (EnAlgae) providing a pilot site for the production of Kelp species *S. Latissima*. The cultivated samples obtained for this study were first developed in the hatchery situated at Queen’s Marine Laboratory in Portaferry which were then transferred to long lines in Strangford Lough for onward growth. The gametes sprayed on to the string were grown in water baths in the hatchery for 4-6 weeks at a temperature of 10°C. When the plants are approximately 5mm in length, they are then transferred to the site in a boat. The long lines are normally a 100 m long line connected to 10 buoys which float on the water surface which will keep them at uniform growing depth. The deployment normally happens at the end of summer (September to October) and they are harvested after 6 months when the biomass is around 3-4 meters in length. The wild samples used in the study were also collected from the wild habitats closer to the long line cultivation farms in Strangford Lough (Queen’s University Website, 2017).

The cultivation site in Strangford Lough is north/south oriented with 4 x 100m long lines running parallel to each other and along the water flow suspended approximately 1-2 m below the sea surface. The sea temperatures for Strangford Lough was recorded as low as 8°C in winter (January) and as high as 14°C in summer 2016 (World sea temperatures, 2016).

4.2.2 Isle of Seil

The samples obtained from Isle of Seil were supplied by the Scottish Association for Marine Science (SAMS), Scotland's largest and oldest independent marine science organisation. The samples were collected by the specialist marine consultancy SAMS Research Services Limited (SRSL). Based in Oban, on the Scottish west coast, SAMS has two experimental seaweed farms namely the Kerrera farm and Port a' bhultin farm. The main species tested in these farms were *S. Latissima* as a major Kelp species and *Alaria esculenta*, *Laminaria hyperborea* and other species like Palmaria palmata and Ulva. The farms vary in their size with Kerrera having 60m long lines set up similar to a mussel farm with a water depth of 5-25m. The Port a' bultin farm is the largest seaweed farm in the UK and is 30 hectares currently with a single 100 x 100 m grid system for growing seaweed with a capacity of 24 x 100 m long lines. The depth is around 15 – 25 m. The samples obtained from this study were collected from Isle of Seil, Argyll with the similar cultivation site observed in Kerrera farm. The latitude and longitude for the collection site is 56.32N 5.58W. The wild samples were also sampled from the wild sources near to the long lines in the collection site (SAMS Website, 2017). The average sea temperatures in Oban is observed between 8.2°C in winter (January) to 13.7°C in summer in 2016 (World sea temperatures, 2016). The surface salinity in the region is observed to be 34-34.75% (DEFRA, 2011). SAMS has a history of seaweed cultivation and Isle of Argyll, Oban seems to be an interesting collection site for the selected species, *S. Latissima*. The characteristics of the seaweed samples collected would signify its potential to be a renewable energy feedstock.

4.2.3 Ventry Harbour

The third location where the biomass samples were collected in this study was a seaweed farm named Dingle Bay Seaweeds Ltd, County Kerry in Western Ireland. It is a Small to Medium-size Enterprise (SME) operating in Ireland. It is one of Europe's largest commercial seaweed farms established since 2009. The farm is an active partner in many studies involved with Irish Sea fisheries board since its establishment.

Since 2011, the Kelp species, *S. Latissima* has been a trial species for aquaculture in the farm under the provision of a licensed sea trial site. In Northern Ireland, where the samples were collected from Strangford Lough the hatchery facilities were owned and operated by QUB, in the west of Ireland. Dingle Bay Ltd had access to Daithi O' Murchu Marine Research station in the South west Ireland, County Cork. It provided the company with access to filtered seawater, air supply and an insulated, constant temperature unit to monitor and control over the life cycle of the species tested if required. The cultivation site in the sea was reached by boat and it took up to 45 minutes to reach the cultivation site (Edwards and Watson, 2011). There are two dominant harbours in Dingle bay namely Ventry Harbour and Dingle Harbour. The cultivation site is based at Ventry harbour. This site also utilised longline cultivation with the typical float and rope plan similar to Strangford and SAMS cultivation longlines. The cultivation site operated by Dingle Bay has an area of 18 hectares in Ventry Harbour (Werner and Dring, 2011). The farm consists of 3 parallel units of 280 m linear longlines suspended approximately 1.5 m below the sea surface (Walls *et al.*, 2017). The average sea temperatures for Dingle Bay (Ventry Harbour) was observed to be as low as 10.1°C in winter (January) and as high as 15.7°C in summer in 2016 (World Sea Temperatures, 2016). Only cultivated *S. Latissima* was sourced from Dingle Bay farm in the summer 2016.

A summary of the different sample locations used for this study is given in Table 14 .

Table 14 Summary of the location factors

Location	Area (hectares)	Long Line conditions	Deployment – Harvest (Month)
Strangford Lough	7.3 ha	4 x 100m	September-June
Isle of Seil	1 ha	60 m	September-June
Ventry Harbour	18 ha	3 x 280 m	October-June

As the seaweed industry in Europe is expanding, attempts are being made to diversify the aquaculture sector. As a result, new methods and techniques (which do not involve wild harvest of Kelp and other species) are becoming the focus of emerging research. The importance of habitat and environmental conditions on the characteristics of wild Kelp biomass is demonstrated in the literature, however the impact of these factors on cultivated biomass is still very limited.

The cultivation sites at Ventry Harbour, Strangford Lough and Isle of Seil will provide information on how Kelp habitats and the various environmental gradients at these cultivation sites impact on the characteristics of the biomass collected from these sites. Holdfasts have been researched as the appropriate part of Kelp species for variation due to habitats in wild and cultivated biomass. However, it remains unclear the extent to which variation is observed in the fronds of wild and cultivated Kelp species. This research attempts to highlight this gap in the literature.

4.3 Environmental conditions

As described previously the environmental conditions in which the macroalgae grows can influence its general characteristics. A number of key parameters were evaluated as part of this study and the results are presented below.

4.3.1 Temperature

Temperature is a key variable in controlling the distribution of kelps (Müller *et al.*, 2009). The seawater temperature could be a controlling factor for the variation in physical and biochemical composition for the biomass from the three sites studied in this research. The average sea temperatures for the study regions were compared to observe any variations. The sea temperatures were collected from the online sea temperature resources (Sea temperature, 2017). The average sea temperatures (2016) are shown below in Figure 15.

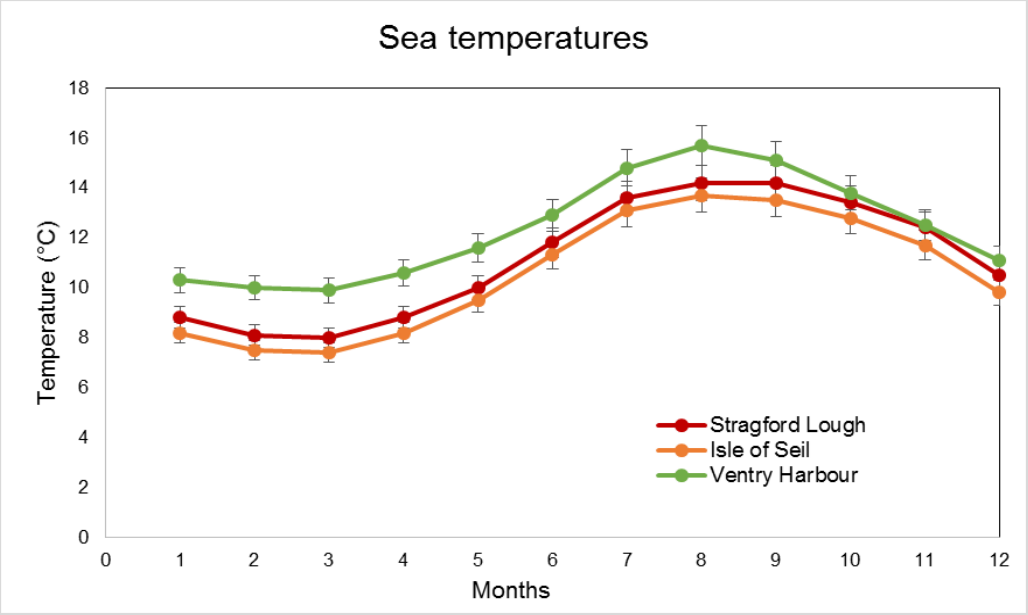

Figure 15 Average Sea Temperatures for three locations

It can be observed that Scottish water temperatures (Isle of Seil) were slightly colder than Irish waters (Strangford Loch and Ventry Harbour). For the samples from Ireland, august was the hottest month with a temperature of 14.2°C in Strangford Lough and 15.7°C in Ventry Harbour site. March was the coldest month in Strangford Lough (8°C) and Ventry Harbour (10°C).

For samples from Scotland, Isle of Seil had the highest temperature of 13.7°C in August and 7.4°C in March.

It was also noted that the south of Ireland (Ventry Harbour) had a higher summer temperature than the north of the country. Ventry Harbour had the hottest and Isle of Seil the coldest seawater conditions among the three sites compared in this study.

4.3.2 Benthic Conditions

As discussed in the literature review (Section 2.1.1.1), benthic conditions are important for the growth of macroalgae biomass. Kelp forests in Europe have shown to follow migration pattern owing to complex pattern of dynamic between environmental factors including the benthic conditions, however still Kelp forests in the UK are understudied (Araujo *et al.*, 2016). The benthic conditions of the three locations of this study are discussed below.

4.3.2.1 Strangford Lough

The Strangford Lough Ecological Change Investigation by Roberts *et al.*, (2004) reported that the lough is almost landlocked with separation from the Irish Sea by the Ards Peninsula to the east, with a connection to the open sea via an eight-kilometre-long channel called Strangford Narrows. The majority of the lough is less than 10 meters in depth. The benthic conditions in the lough has developed during the Ice age and the sediment type traces back from the Narrows into the main body of the Lough influenced by geology, tides, currents, and exposure to wave action (Roberts *et al.*, 2004).

4.3.2.2 Isle of Seil

The Isle of Seil (10 miles from Oban) is set within the Firth of Lorn off the west coast of Scotland. It is bounded to the east by the Seil Sound, a designated shellfish water protected area, and to the South and west by the Firth of Lorn special area of conservation. There are more specific shellfish harvesting areas through Seil Sound and towards the northern part of the island. This island is part of the island group of scarba, Luing, Kerrera and the Gravellachs. The mainland cost of south west Scotland is dissected by a series of sea lochs oriented along a south-west/north-east axis. Isle of Seil is a part of this highly dissected coast and therefore subject to wide range of physical conditions affecting the habitat diversity of the region. Since having a west facing coast, the island is almost fully exposed to the force of the Atlantic winds. The area is recorded for its complex hydrography with a diverse range of wild habitats for rich and varied marine life in the west Scotland. The tidal patterns narrow at Clachan sound between the mainland and the Isle of Seil where earlier studies in the eighties by Smith and Nunn *et al.* (1985) have even reported dramatic changes in mollusc fauna species composition resulting from natural causes (Smith and Nunn, 1985). Unusual growth of algae species in the strong tidal streams are also reported in the same area. Another interesting study Smith (1984) observed that the rock formations leading to the formation of large number of crevices and high exchange of water movement in the area to be significant factors in increasing species diversity (Levin and Smith, 1984). Therefore, it can be seen that the cultivation site in Isle of Seil is highly hydrodynamic in nature with high currents and tides and also rocky in nature when compared to cultivation site in Strangford Lough.

4.3.2.3 Ventry Harbour

Ventry Harbour is a large bay situated in the southwest corner of County Kerry. The harbour is a moderately sheltered and shallow depth waters facing the south east with the wide mouth opening to Dingle Bay. The licensed cultivation site is facing northwest to southeast and located to the westerly side of the Ventry Harbour. The depth underneath the farm is approximately 6m at the north-western end and then it gently slopes to 20m at the eastern edge of the farm at mean low water spring tide (Walls *et al.*, 2017). Due to the constant movement of waters because of the harbouring of the ships and boats, the harbour and the adjoining areas are recorded to be moderately hydrodynamic in nature but lesser compared to the other two locations.

In summary, among the three sites Isle of Seil was observed to be highly hydrodynamic with the greatest of depth and Ventry Harbour with the lowest depth and moderate hydrodynamic activities in their cultivation sites. The key factors to be noted from the environmental conditions around the cultivation sites are the water depth, benthic conditions and exposure to winds which will also influence the tides and currents among the three sites.

4.3.3 Tides and Currents

No measurements of the tides and currents were taken at the time of deployment or harvest for this research. However, the average of the tidal range and currents of the three different locations recorded in literature have shown that the Strangford Lough area is tidal in nature with a 4m tidal range, a low current of 40 cm/s, and exposed to wind throughout the year. The water depth varies between 3-7m at mid tide, 0.9-2 m wave heights in winter low tide (Sprujit, 2015). For Isle of Seil, higher tidal currents were observed with semi-diurnal tidal current of 77cm/s during spring tides (SAMS website, 2017). Even though higher currents are observed in the region the cultivation site is closer to shore and therefore is moderately sheltered from the south westerly winds from the Atlantic. Among the three, the tidal range of the Dingle Bay farm was reported to be largely variable with the range between 0.6 and 4.0m (Walls *et al.*, 2017). This could be attributed to the constant movement of waters due to the harbouring of the ships in Ventry Harbour.

4.3.4 Pollution, Sedimentation and Fouling of the biomass

Measurements for indicators for pollution, sedimentation or fouling were not taken during this study. However, a previous study conducted by Roberts *et al.* (2004) demonstrated that there was a well-defined seasonal maximum of nutrient concentration (indicated by nitrate concentration) in December and January. The major influents of nutrient into the Loch are primarily due to agricultural practices rather than industrial practices in the area, and also the sewerage treatment plants. The Irish Sea is the largest source of nitrogen and silicate loading to Strangford Lough. Higher nitrogen and phosphorus concentrations were found in the north of the Lough during the winter and south of the Lough during the summer. However Strangford Lough is not classified as either a sensitive or polluted water body under the regulations such as the EU water framework directive (2000) which has been recently adopted as the water framework directive in the UK (Roberts *et al.*, 2004).

The Scottish test site has in operation a wastewater treatment works on the east of the Island discharging to the shellfish water around Seil Sound since 2008-2009. Even though a proposal for a new and better planned sewerage systems for the Isle of Seil is under consideration, the Firth of the Lorn to the south and west of the Isle of Seil is already reported as good environmental status' under the Marine Strategy framework directive (Olenin *et al.*, 2010). This implies minimal pollution limits in the seaweed cultivation farms where the samples were collected. However, the main source of industrial effluent to the region was reported from pulp mills effluents and the resulting organic gradient enrichment has also been observed in the Firth of Lorn but only in limited detail in terms of the levels of information. Ventry Harbour has been prone to different dumping activities and this has resulted in pollution from tourists, and travellers alike (E-oceanic site, 2016). These prolonged dumping activities can lead to alterations in the benthic floor leading to potential impact on macroalgae cultivation sites. Sedimentation is believed to be an active process in all three sites and pollution being an equally important issue in all the sites due to different anthropological activities. As pollution levels of neither of three sites were quantified, it can only be said that if polluting activities continue, either from sewage or tourism or agricultural activities it can have negative impact on the marine ecology and thereby on the cultivation sites.

In addition, all biomass obtained from the three cultivation sites had bryozoans (fouling agents) attached to their fronds in summer. On personal communication with the cultivation site workers, it was confirmed that the fouling started to occur in the biomass late spring – early summer and reached its peak towards late summer.

A summary of the different environmental conditions for three locations are given in Table 15.

Table 15 Summary of different environmental conditions for three locations

Location	Water depth (m)	Tides and currents	Sea temperature (°C)	Pollution Cause	Benthic conditions
Strangford Lough	3-7 m	4 m (tide) 30-40 cm/S	8-14	Agricultural	Rocky
Isle of Seil	5-25m	77cm/S (current)	8.2-13.7	Sewage	Rocky
Ventry Harbour	6-20m	0.6-4.0m (tide)	10.1-15.7	Tourism	Shallow embayment

4.4 Biomass characterisation

S. Latissima samples were characterised for their physical and chemical characteristics including solids (Total and Volatile), ash, and moisture content. Their compositional analysis observed through elemental analysis are also discussed. The seaweed samples were analysed separately as a single biomass feedstock from the specific sites at different time of harvests and under different growth conditions (including wild sourced biomass and long line cultivated biomass). Only the fronds of the macro-algae were utilised for the study hence no comparison is made on the different parts of the macro-algae biomass (stipes and holdfasts). All biomass samples were macerated using a blender prior to the sample analyses.

4.4.1 Impact of location on biomass characteristics

4.4.1.1 Solids, Ash, Moisture and Calorific values

The proximate characteristics for *S. Latissima* obtained from Strangford Lough, Isle of Seil and Ventry Harbour are shown in Table 16 .

Table 16 Proximate characteristics of *S.Latissima* from three locations

Seaweed ID	Total Solids (%WW)	Volatile Solids (%WW)	VS (%TS)	Ash (%WW)	Moisture (%WW)	Calorific Values (MJ/kg)
Strangford Lough	17.04	11.29	66.30	5.74	82.96	11.10
Isle of Seil	32.74	18.84	57.55	13.90	67.26	11.30
Ventry Harbour	15.08	10.60	70.30	4.48	84.92	07.30

S. Latissima samples compared from different locations were harvested in summer 2016. The calorific values of all three locations indicated high energy content with the sample from Ventry Harbour exhibiting the lowest among them. Macroalgae biomass is reported with high solids content. Studies documented for *S. Latissima* report a total solids content in the range 8.3 – 22% (%WW) and volatile solids in the range 44.6 – 73.8 (%TS) (Allen *et al.*, 2015). Similar trends were observed in this study as the total solids have been shown in the range 17% for Strangford Lough; 32% for Isle of Seil and 15% for sample collected from Ventry harbour as shown in Figure 16.

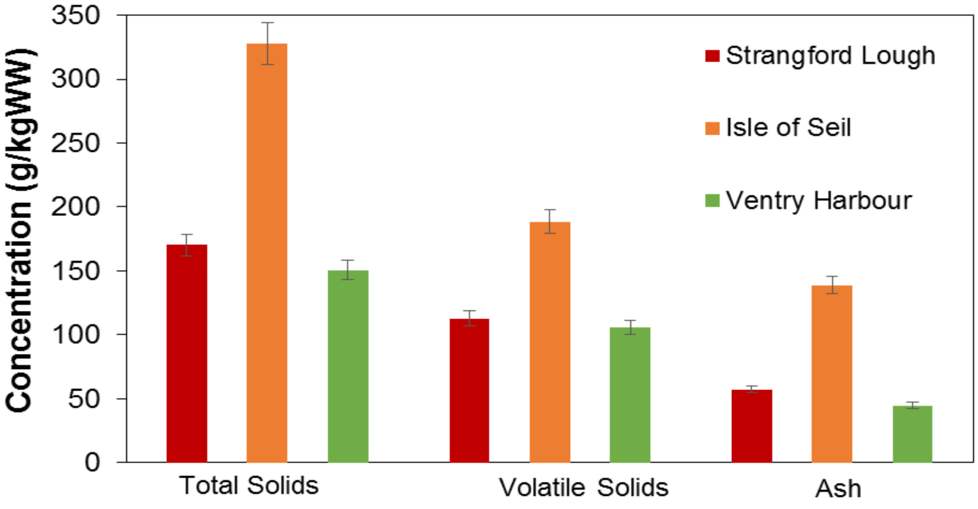

Figure 16 Total solids, volatile solids and ash for samples from three locations

From the figure, it can be seen that the samples from Isle of Seil showed higher percentages of volatile solids, total solids and ash in comparison to the other samples. The percentage difference observed between the total and volatile solids for Isle of Seil was 14% whereas for Strangford Lough was 6% and 5% for Ventry Harbour samples. Statistical analyses were performed to check whether these results were significant. ANOVA tests confirmed that samples from Isle of Seil had significant difference in their characteristics to those from Ventry Harbour and Strangford Lough. However, the analysis also confirmed that there were no significant differences in the characteristics of samples obtained from Strangford Lough and Ventry Harbour.

4.4.1.2 Elemental composition

The main elements in the macroalgae biomass C, H, N and S were plotted as percentage of their total solids. All of the samples had high concentrations of carbon in the biomass (28-30%). As observed in figure 2, the highest percentage of Carbon (C%) was found for the biomass samples from Isle of Seil with 29.27%TS, followed by Ventry Harbour biomass with 29.23% and finally Strangford Lough biomass with 28.53%. The elemental composition of *S. Latissima* from three locations is given in Figure 17 .

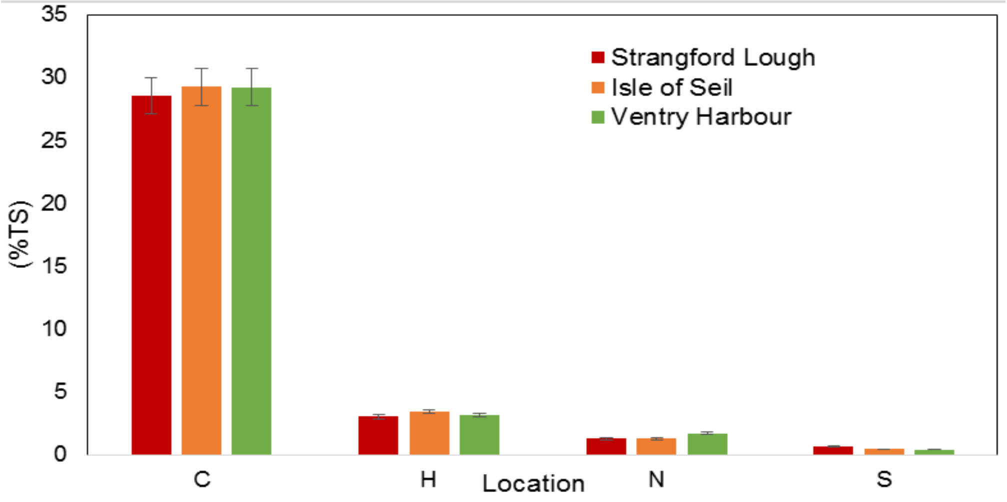

Figure 17 Elemental composition of *S. Latissima* from three locations

4.5 Impact of harvest time on biomass characteristics

Time of harvest for *S. Latissima* is an important factor as the biochemical composition of the biomass can alter with the different growth stages at different times of the year

(Azevedo *et al.*, 2016). In addition, for cultivated biomass, as they are grown in long lines they have a maximum growth of 1 year. However, for the wild biomass, as they are perennial the wild biomass can survive up to 5 years and harvesting biomass from the wild habitats can also affect the ecological balance in the marine environment (Teagle *et al.*, 2017, Walls *et al.*, 2017).

The characteristics of wild sourced *S. Latissima* sampled at different times of the year from Strangford Lough is compared in Table 17.

Table 17 Characteristics of wild *S. Latissima* harvested at different times of the year

Time of harvest	Total Solids (%WW)	Volatile Solids (%WW)	Ash (%WW)	Moisture (%WW)	Calorific Values (MJ/kg)
Winter/2015	02.98	1.70	1.39	96.92	09.50
Spring/2016	28.50	18.11	10.39	71.50	10.90
Summer/2016	25.77	17.84	08.13	74.03	11.40
Winter/2016	15.29	09.12	06.17	84.71	09.70

Four biomass samples collected in winter 2015, spring 2016, summer 2016, and winter 2016 were studied for their differences in characteristics. The highest energy content was observed in the summer biomass (11.4MJ/kg) and lowest in the winter biomass (9.7 MJ/kg).

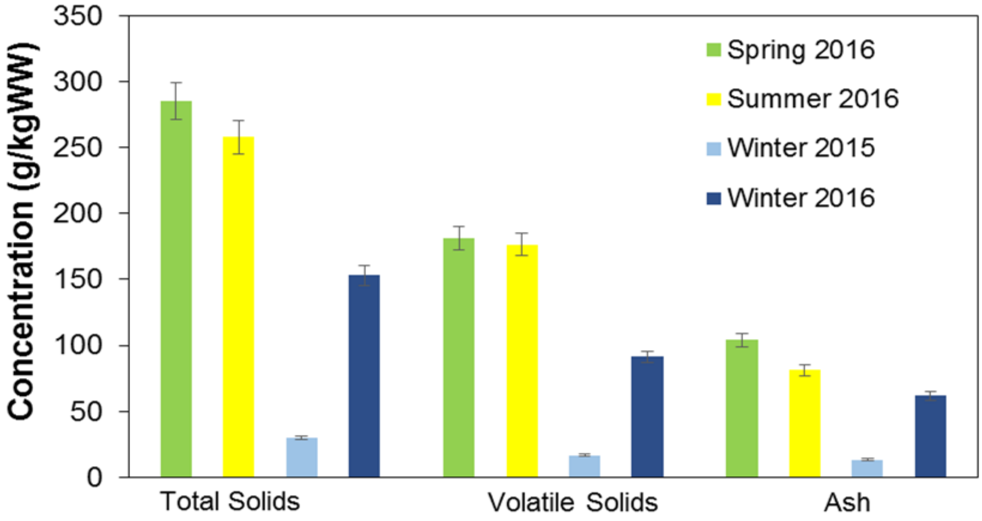

Figure 18 Total solids, Volatile solids and Ash for different harvest times

From Figure 18, it can be seen that the spring biomass exhibited consistently higher volatile solids, total solids and ash percentages. In contrast, winter biomass exhibited the lowest percentage of solids and ash concentration. ANOVA tests also confirmed that all four samples harvested at these four harvest time were significantly different from each other. This result shows that macroalgae biomass harvested at different points of the year will be significantly different in their characteristics. These findings are important when considering logistics of biomass utilisation as it can inform optimal harvest times for the macroalgae biomass based on the desired characteristics and valorisation routes. The elemental composition of wild *S. Latissima* harvested at different times of the year is shown in Figure 19.

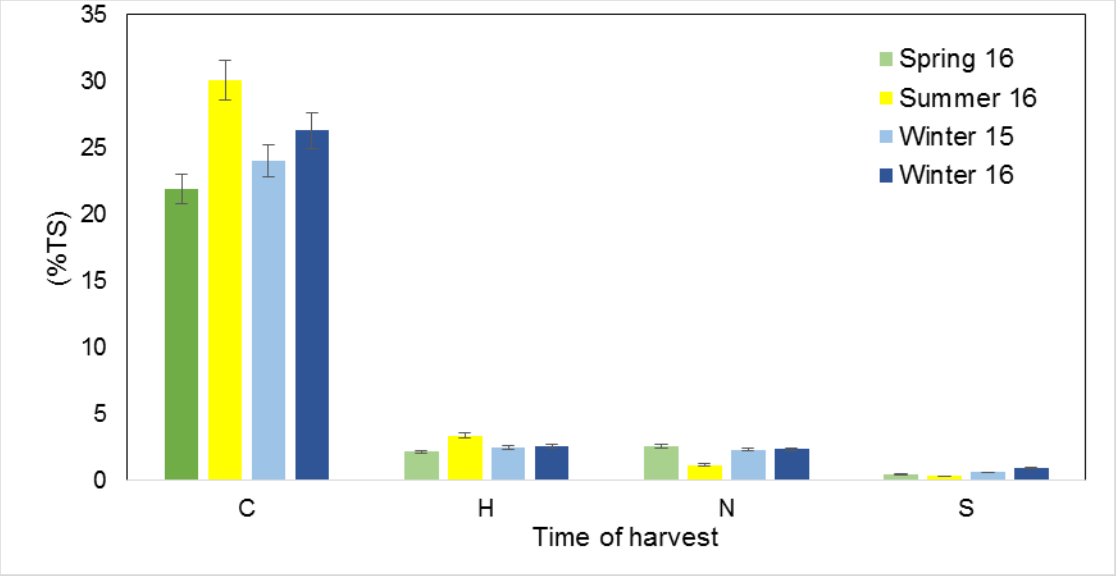

Figure 19 Elemental composition of wild *S. Latissima* harvested at different time of the year

It can be noted from the figure that for the wild biomass harvested at different times of the year, the percentage carbon (C) was highest in the summer 2016 biomass (30.09%) and lowest in the winter 2015 biomass (24%).

4.6 Impact of growth type on biomass characteristics

In order to better understand how growth conditions impact on the characteristics of macroalgae samples were taken of both wild sourced and artificially cultivated *S. Latissima* at the same time (Summer 2016). The characteristics of wild and cultivated biomass collected from Strangford Lough and Isle of Seil are given in Table 18. The calorific values (energy content measured as heat released on combustion) showed that the samples were not significantly different in terms of energy content in both wild and cultivated biomass.

Table 18 Characteristics of wild and cultivated biomass from Strangford Lough and Isle of Seil

Location	Type	Total Solids (%WW)	Volatile Solids (%WW)	Ash (%WW)	Moisture (%WW)	Calorific Values (MJ/kg)
Strangford Lough	Cultivated	17.04	11.29	05.74	82.96	11.1
	Wild	25.97	17.84	08.13	74.03	11.4
Isle of Seil	Cultivated	32.74	18.84	13.90	67.26	11.3
	Wild	54.97	39.25	15.72	45.03	10.8

The total solids, volatile solids and ash of wild and cultivated biomass from Strangford Lough is given in Figure 20. From the figure it can be seen that the wild sourced and cultivated biomass have varying total solids, ash and volatile solids concentrations. For Strangford Lough, the wild sourced biomass had consistently higher values across all of the measured parameters compared to the cultivated biomass. The volatile solids for wild sourced biomass were 36.71% more than the cultivated biomass. Similarly, the total solids for wild sourced biomass was 34.38% more than the cultivated biomass for samples from Strangford Lough.

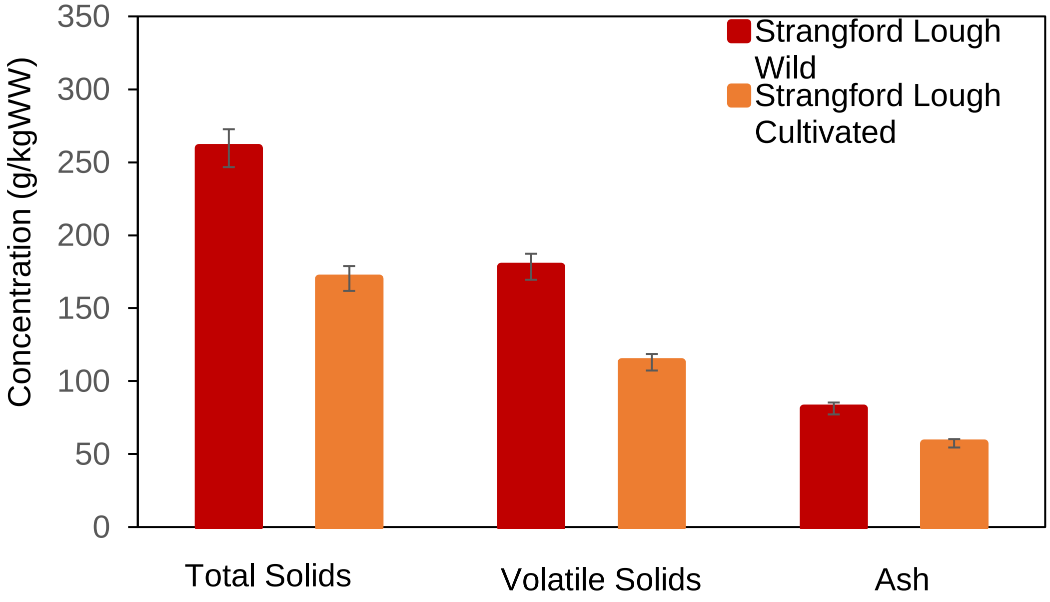

Figure 20 Total solids, Volatile solids and Ash of wild and cultivated samples from Strangford Lough

The total solids, volatile solids and ash concentrations of wild and cultivated biomass from Isle of Seil is given in Figure 21.

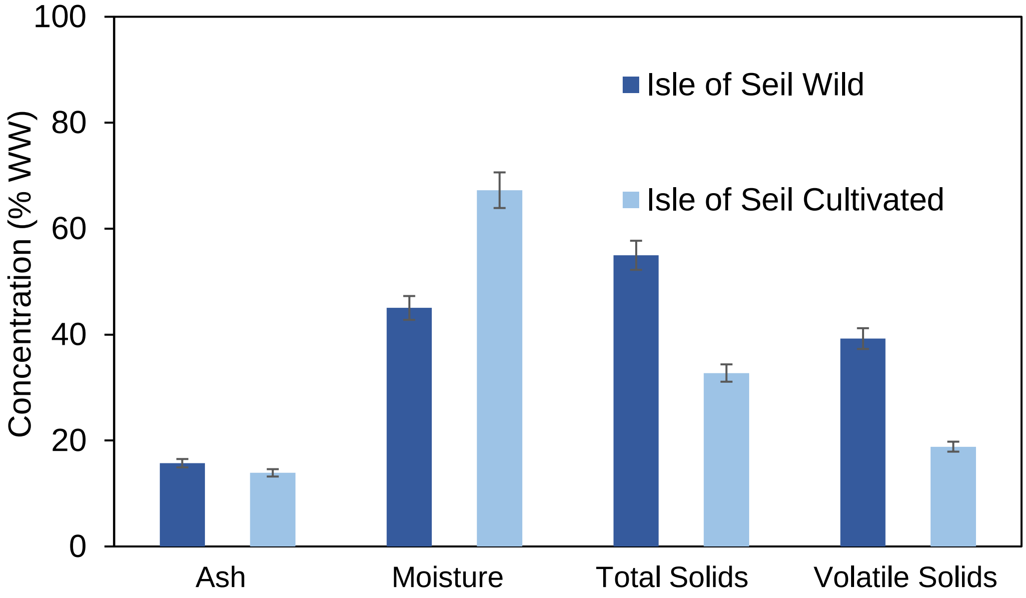

Figure 21 Total Solids, Volatile solids and ash of wild and cultivated samples from Isle of Seil

From the figure it can be seen that the wild sourced and cultivated biomass from Isle of Seil also have varying total solids, ash and volatile solids concentrations. Again, it can be seen that the wild sourced biomass had higher concentrations of TS, VS and ash compared to the cultivated biomass. The volatile solids for wild sourced biomass was 52% more than the cultivated biomass. Similarly, the total solids for wild sourced biomass was 40.44% more than the cultivated biomass obtained from Isle of Seil.

ANOVA tests indicated that there is significant variation between the wild and cultivated samples from each location. The characteristics were also significantly different between the wild sourced and cultivated samples from either location.

The elemental composition of wild and cultivated S. Latissima from Strangford Lough and Isle of Seil is shown in the figures below.

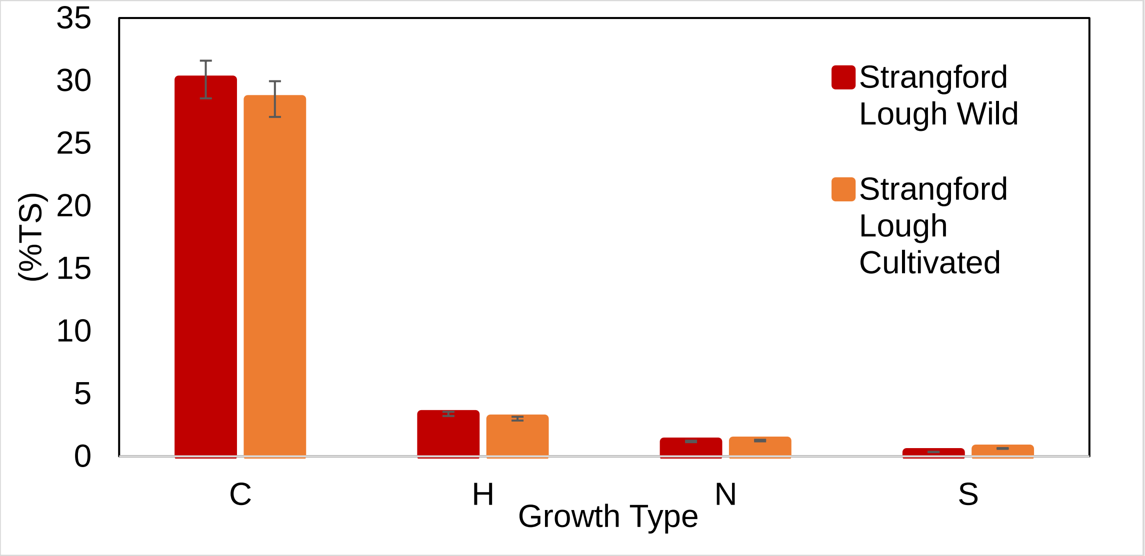

Figure 22 Elemental composition of wild and cultivated samples from Strangford Lough

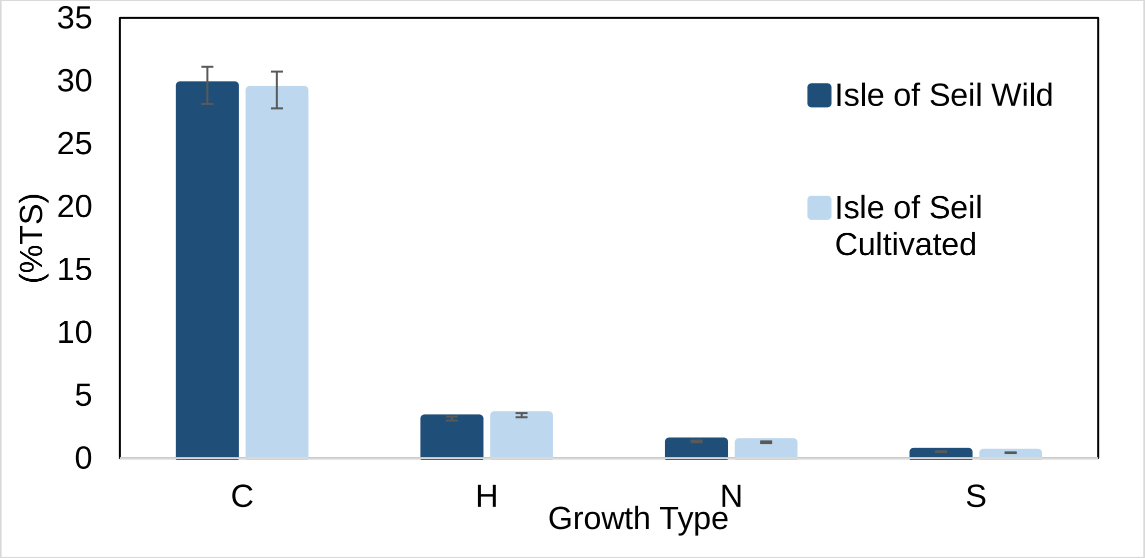

Figure 23 Elemental composition of wild and cultivated samples from Isle of Seil

As shown in the figures above, the % carbon for the two samples (wild and cultivated) was not significantly different (30% and 28% (C) respectively). A similar trend was observed for Isle of Seil, where wild samples exhibited 29.63 % carbon and cultivated exhibited 29.27 % respectively. The wild samples from Strangford Lough had 1.5 % more carbon than its cultivated counterparts whereas the wild Isle of Seil samples only had 0.36% more carbon than cultivated biomass. All results were reported for their significance following statistical analysis using ANOVA and showed that they were significantly different from each other in their characteristics.

In summary, it was observed that, environmental factors (such as sea water temperature, benthic conditions, tides and currents, pollution and fouling may have an impact on the characteristics of *S. Latissima*. samples obtained from the three different sites exhibiting significant differences in terms of TS, VS, Ash and elemental composition with samples from Isle of Seil having significant difference in their characteristics to those from Ventry Harbour and Strangford Lough.

In terms of season, the macroalgae biomass harvested at different points of the year also exhibited significantly different characteristics with the highest percentage carbon recorded in summer months and lowest in winter months.

For growth type, wild biomass from Isle of Seil and Strangford Lough had higher solids and carbon concentrations in comparison to the cultivated biomass. This indicates that growth type may (and particularly optimisation of growth conditions) needs to be carefully considered when choosing the best feedstock and cultivation techniques for bioenergy production.

4.7 Discussion

The observed characteristics of *S. Latissima* biomass in this study will be used to discuss the following research questions.

- To what extent do environmental conditions vary with location and how does this influence macroalgae biomass characteristics?
- To what extent do biomass characteristics vary with macroalgae growth cycle to inform optimum harvest times?
- To what extent do macroalgae biomass characteristics vary with growth conditions informing optimal cultivation techniques?

4.7.1 Impact of environmental conditions on biomass characteristics

In this study, *S. Latissima* from three different cultivation sites of Strangford Lough, Isle of Seil and Ventry Harbour were compared for biomass characteristics including solids (total and volatile), moisture, inorganic content and elemental composition. It was observed that out of the three locations, Isle of Seil samples showed higher percentages of volatile solids, total solids and ash. On statistical analysis samples from Isle of Seil were significantly different to samples from Strangford Lough and Ventry harbour. However, there were no significant differences in the characteristics of samples obtained from Strangford Lough and Ventry Harbour. The difference observed in biomass characteristics are analysed with respect to the environmental conditions where they were grown in each location.

One of the environmental conditions considered in this study was the water temperatures at the three different locations. From the literature, *S. Latissima* is reported to grow well between the optimal range of 5 and 17°C. The study by Lee and Brinkhuis (1988) studied the effect of seasonal light and temperature interactions in the development of *S. Latissima* in Long Island Sound and showed that in general the growth of the biomass improved with increasing temperature between 4 and 17°C, and at any temperature above 20°C growth was inhibited with temperature for optimal growth depending on the time of the year (Ae Lee and Brinkhuis, 1988). The temperatures recorded at all three sites in this study favour growth of the species during winter and summer months. No site recorded any spike or abnormal changes in the sea surface temperatures during the study. Out of the three locations, Ventry Harbour had the highest of the temperatures while Isle of Seil had the lowest temperatures. The temperatures recorded had a gradual increase over summer and subsequent decrease during winter months reflecting throughout the biomass life cycle. Also, none of the three sites had higher or lower temperatures than the optimal range. This shows that all three sites were favourable for Kelp cultivation in terms of the temperature requirements.

Previous research on *S. Latissima* from arctic and temperate ecotypes showed that their biochemical composition differs under changing temperature with higher content of total carbon, soluble carbohydrates, and lipids while lower nitrogen and protein content observed for the arctic samples (Olischläger *et al.*, 2014). In this study no quantification of carbohydrates, lipids or proteins were done for the samples, however out of the three locations, higher solids and ash content were observed for the samples grown in Isle of Seil with the lowest of the temperatures among the three locations. In addition, elemental composition of the biomass from three locations also showed higher concentration of carbon for Isle of Seil samples and lower for the Ventry Harbour samples. One of the reasons favouring the higher accumulation of carbon in Isle of Seil samples could be because of the lower temperatures and the evolutionary nature of Kelp species to prefer lower temperatures and thereby accumulating the solids as a matter of surviving the colder winter months in comparison to the milder temperatures at Ventry harbour.

The other environmental conditions compared for three different locations in this study were the tides and currents observed in three sites respectively. The hydrodynamic activities at Isle of Seil and Strangford Lough are considered to be greater than in Ventry Harbour. This can be influential to the difference in biomass characteristics of Isle of Seil and Strangford Lough where in order to resist water flow rates Kelp can allow morphological adaptations. From the literature it is observed that *S. Latissima* is a perennial species which is naturally found growing attached to substrates such as rocks and boulders. However, the adaptation of a flexible stipe as a morphological feature reduces the species' chance of being turned over by higher wave movement. A study by Gerard (1986) has found that plants subjected to higher flow rates can have significantly narrower blades and another study by Luning (1990) on *S. Latissima* from wave exposed sites showed short solid stipes, and short narrow thick fronds with closely wrinkled blades. On the other hand, plants from sheltered sites had thin blades with a smooth surface (Lüning, 1980). Therefore, it can be said that in order to allow morphological adaptations to balance external environmental conditions, the biochemical composition of the biomass would also be modified accordingly reflected in the overall solids, organic and inorganic content of the species.

The biochemical composition of *S. Latissima* found in the literature is summarised below in Table 19 (Schiener *et al.*, 2015).

Table 19 Biochemical composition of *S. Latissima* from literature

Macroalgae component	Average Percentage (%)
Laminarin	8.2%
Mannitol	18.6%
Alginate	28.5%
Cellulose	11%
Total carbohydrate content (all 4 above)	63%
Polyphenol	0.41%
Protein	7.1%
Overall Organic Carbon	26.6%
Total Nitrogen content	1.5%
Overall inorganic content	30%
Moisture	85%

Naturally, *S. Latissima* is found growing in littoral to sub littoral regions in the oceans where the area is characterised by continuous high and low tides and where the depths reach up to the continental shelf (Luning 1990). up to the continental shelf (Ltining). Out of the biomass components described above, alginate is a structural carbohydrate found in Kelps which helps the plant in adapting to survive the environmental conditions. Previous research by McHugh (2003) has found that alginate percentages varies between species and also between habitats with plants growing in more turbulent water containing more alginate than plants grown in calmer water (McHugh, 2003). Similarly, in this study, samples from Isle of Seil and Strangford Lough could also have had higher percentage of structural carbohydrates than in comparison to samples from Ventry Harbour owing to the rocky and higher hydrodynamic regions where they were cultivated. Of the two samples from Isle of Seil and Strangford Lough, the former being a part of this highly dissected coast compared to Strangford Lough which is landlocked would have been therefore subject to wide range of environmental conditions resulting in the different biomass characteristics. Visual observation of the samples (as sent) also supported this as Isle of Seil samples had more thickness and plasticity in comparison to Ventry Harbour.

The nutrients available for the biomass growth is dependent on the surrounding waters which in turn is dependent on the benthic conditions of the cultivation site. As benthic depth varies, the irradiation, salinity, nutrient availability etc. also varies affecting the composition in Kelps. Pollution activities can increase or decrease of the level of the mineral concentrations in the surrounding waters in addition to natural erosion of rocks in the marine benthos. In this study, we compared the cultivated biomass samples in contrast to wild stock samples. In the three different sites used for this study, level of pollution is recorded to be minimal except for Ventry Harbour where seasonal pollution could be a contributing factor. However, even though the depths at which the buoys of long lines deployed in the sea surface (1 – 2m) was similar in every location, the tides and currents at each location being different could have had an effect on the amount of sediments (nutrients) available throughout the Kelp’s life cycle. In addition, benthic conditions also were rockier in Isle of Seil in comparison to Ventry Harbour and Strangford Lough. This could mean that apart from any sedimentation available to the Kelp in three sites due to land- based activities or pollution, Isle of Seil samples could have had higher access to nutrients from the erosion of these rocks due to higher hydrodynamic activity in the region. This could also be a reason found reflecting on the solids and ash concentrations recorded in the three samples with highest of ash content in the Isle of Seil samples.

The main objective of this study was to characterise the biomass for its suitability for anaerobic digestion. As discussed in the literature review, anaerobic digestion can better utilise biomass with higher carbohydrates, and higher volatile solids for both easier disintegration during microbial reaction within the digester and also for higher gas production from the biomass. However, to minimise inhibition during AD, lower ash percentages i.e. lower inorganic content is also preferred (Chen *et al.*, 2008). *S. Latissima* has already been researched as a suitable biomass for anaerobic digestion with its higher carbohydrate and volatile solids content (Milledge *et al.*, 2014a). However, from the results observed in this study, it could be seen that to cultivate optimum *S. Latissima* biomass for AD, choice of cultivation site is important as localised environmental conditions in those sites are influential for the biomass characteristics. This study has only collated existing data on the environmental gradients in the three sites for the factors of temperature, tides and currents, benthic conditions, pollution and sedimentation. It is observed that environmental conditions are inter related and this has a combined effect on the biomass growth influencing its biochemical composition. From an anaerobic digestion perspective, the three sites evaluated in this study would be suitable in the site capacity and the adjoining factors of temperature, tides and currents, benthic conditions, pollution and sedimentation with Isle of Seil biomass showing the highest of solids content and ash content. Therefore, it is then possible to hypothesise that *S. Latissima* from Isle of Seil will provide the highest methane production during anaerobic digestion. However, this research has been limited as the measurement of concentrations of sugars (mannitol and laminarin) accumulated by the Kelps was outside of the scope of this study. Also, it is essential to collect data on other environmental gradients such as irradiance, salinity, CO_2 concentration, etc. into every stage of the cultivation process right from seeding to deployment until harvest. Therefore, more studies are required on kelp species based on their geographical location if we need to compare their energy potential and thereby for identifying the best sites for biomass utilisation for process like anaerobic digestion. This would also be a better way leading to the conservation of the species and prepare for any paradigm shift in the species characteristics affecting its production and processes.

4.7.2 Impact of harvest time on biomass characteristics

In this study, the wild *S. Latissima* from Strangford Lough was harvested at different points of the year starting in winter 2015, followed by spring, summer and winter 2016 to understand the characteristics of the biomass harvested at different times of the year. The wild harvest was collected from the same surroundings as the cultivation site. From the analysis of the proximate results, it was observed that the spring biomass consistently had higher volatile solids, total solids and ash percentages. In contrast, winter biomass had the lowest percentage of solids and ash concentration. The biomass in spring had 9% more than volatile solids in comparison to the winter biomass and 7% more total solids and 4% more ash concentration. Statistical tests also confirmed that all four samples harvested at these four different harvest times were significantly different to each other. In terms of elemental composition however summer harvest had the highest concentration of carbon. Summer biomass had 30% of carbon while winter biomass only had 24% of carbon. These results show that macroalgae biomass harvested at different points of the year will be significantly different in their characteristics. This result is also important for logistics as this can determine the best time of harvest for the macroalgae biomass intended for specific purposes including bioenergy and for the extraction of high value products.

In the literature, macroalgae is studied to evaluate eutrophication due to the nutrient enrichment in coastal waters (Korpinen *et al.*, 2007). In coastal waters two important elements in algal metabolism are nitrogen and phosphorus where nitrogen is more likely to be growth limiting for the biomass in open ocean conditions. The study by Sanders *et al.* (2008) showed that there will be seasonal variability in the nutrients (e.g. nitrogen limitation in the coastal waters of north-west coast of Scotland) available to support the growth of macroalgae species resulting in the variation in biochemical composition of the biomass (Sanderson *et al.*, 2008). However, another research showed that on oceanic shores, nutrient enrichment increased the abundance of the green algae species with little impact on red or brown algae. In the case of perennial species and those occupying the littoral zones nutrient availability and utilisation of these nutrients for their growth were a result of complex interactions between the abiotic (environmental conditions) and biotic (fouling, epibionts) factors (Korpinen *et al.*, 2007). Therefore, to utilise the maximum potential of perennial species such as *S. Latissima*, it will be important to target the growth phase of the biomass to identify the best harvest time with optimum biochemical composition.

S. Latissima is recorded to exhibit faster growth from late winter months till spring at a rate of 1.1 cm/day up to a higher rate of 4.8 cm/day. Growth phase starts to decline from June and may cease in late summer. The length to width ratio of the newly grown tissue of *S. Latissima* is also found highest during the periods from December to June (i.e. winter to summer). In summer however, the shift is found more towards increasing the width to possibly maximise the frond area for photosynthetic activity and thereby increasing the amount of storage carbohydrates for winter months (Sjøtun *et al.*, 1993). This was also confirmed by a recent study performed on *S. Latissima* by Schiener *et al.* (2015) which found that the storage carbohydrates of laminarin and mannitol were higher in summer to late autumn and lowest in winter months. The structural carbohydrate of alginate and cellulose in *S. Latissima* however had lesser variations at different times of the year. Therefore, in terms of total organic carbon and nitrogen present in the biomass, summer months showed higher C percentages and lower nitrogen percentages. The inorganic content however was higher in winter till spring months and lower in summer and autumn (Schiener *et al.*, 2015).

Similarly, in this study, the biomass showing higher inorganic content were observed for the spring biomass in 2016 with its higher ash percentages. A similar trend is also observed for the carbon percentages for *S. Latissima* in this study as summer harvest has the highest carbon percentages while winter has the lowest accumulation of solids and carbon. As discussed above, the biomass reached its mature growth in summer to late autumn which makes the concentrations of the solids higher in those months while the plant is at its early growth stages in winter. Results observed in this study are also comparable to studies in the literature for other brown algae species like *L. digitata* and *L. hyperborea*. Both species showed positive correlation with summer temperature but negative correlation to warmer winter temperatures which are associated with the various temperature requirements for initiation and development of different life history stages of the macroalgae (Araujo *et al.*, 2016).

Several studies have discussed and suggested optimum time to harvest *S. Latissima* (da Silva Marinho, 2016, Taelman *et al.*, 2015, Marinho *et al.*, 2016). *S. Latissima* is suggested to be harvested in September for the phycocolloid industry in Europe however for human consumption, it is suggested to be harvested at the end of spring season when it is not affected by fouling (Taelman *et al.*, 2015). The importance of harvest time (season) and cultivation period (age) was tested for *S. Latissima* for succinic acid production which found that both the factors has an impact on the concentration of fermentable sugars (glucose and mannitol). The biomass is suggested to be harvested from May to September as the best suitable feedstock for biorefinery purposes including succinic acid production as the high value product (Marinho *et al.*, 2016). Another study conducted by Marinho *et al.* (2015) studied the composition of cultivated biomass of *S. Latissima* in one site adjacent to integrated multitrophic aquaculture site (IMTA) in Danish waters. The samples were harvested bimonthly from different points of the cultivation site at different times of the year. The study highlighted that the lipid quantity and quality of *S. Latissima* was reliant on harvest time rather than on spatial location in agreement with the findings presented in this study (Marinho *et al.*, 2016).

In this study at three cultivation sites, the biomass seedlings were deployed on long lines in September 2015 and harvested in June 2016. The main intention to use the biomass was for bioenergy purposes, specifically for anaerobic digestion. As the results have shown higher concentrations of C and solids in summer it could be suggested that summer will be an ideal time to harvest *S. Latissima* biomass if intended for AD processes. However, this study was performed on wild samples and research should be extended to cultivated biomass to identify the optimum time of harvest in long line cultivation sites. An anomaly found in this study was that even though a higher ash and solids content are observed for the winter 2015 biomass the pattern is not repeated in the 2016 biomass where the biomass has shown least solids and ash concentration. This could be attributed to the age and maturity of the samples collected over the course of this study. *S. Latissima* being a perennial species having a lifespan up to 4 years although plants may occur as annuals. The species normally take 8 to 15 months to reach fertility or mature stage to produce spores and in British Isles, months of October till April was the most frequent period for spore production. Therefore, the wild samples obtained could have been from differently aged wild samples exhibiting different characteristics owing to their maturity as well as reproductive stages (White N. & Marshall, 2007).

From an anaerobic digestion perspective, harvest time is an important factor for the optimum concentration of the desirable characteristics for AD. Even when the components of interest in the biomass is higher in summer months, harvesting the biomass has to be planned carefully for logistics purposes. In most regions, where kelp is cultivated, the biomass is harvested in the late spring or early summer due to high water temperatures or biofouling problems during the summer. Hence landing of several tons of wet seaweed over a number of weeks requires careful planning for logistics regarding transportation, stabilisation, and storage of biomass, also for the scalability of use in continuous processes (Fernand *et al.*, 2017).

Another important factor to be considered for optimised AD processes is the carbon to nitrogen ratio of the intended biomass feedstock. From the literature, it is seen that the C/N ratio for optimal anaerobic digestion is in the range 20/1 to 30/1. These values show that biomass with higher C/N ratios are best suited for anaerobic digestion as feedstock with higher nitrogen values can cause inhibition during digestion from excess levels of ammonia (Allen *et al.*, 2015). If the ratios are less than 15:1, excess levels of ammonia can lead to unstable digestion. Kelps are lower in protein concentrations, but higher in carbohydrate levels in the form of polysaccharides (laminarin, mannitol and alginate) (Allen *et al.*, 2015). Hence they are expected to be with higher carbon and lower nitrogen concentrations. In this study the C/N ratio overall for the summer biomass was higher with a value of 25.2 whereas the winter biomass has a lower ratio in the range 10-11. On the contrary, nitrogen values were also found highest in spring biomass (2.5%) followed by winter (2.3%) and lowest in summer biomass (1.2%). Percentage of sulphur is in the range of 0.35-0.9%, lower in summer and higher in winter biomass. Hence on the basis of C:N ratio, summer months will be the ideal harvest time for *S. Latissima* for optimal anaerobic digestion performance with least inhibition from ammonia and higher concentrations of easily degradable carbon.

4.7.3 Impact of growth type on biomass characteristics

In this study, wild and cultivated biomass of *S. Latissima* from two different locations were compared for the differences in their characteristics. In this study it was observed that the wild and cultivated biomass obtained from both Isle of Seil and Strangford Lough sites have significant variations in their composition. In their proximate composition, it was observed that wild and cultivated biomass from both sites are highly variable. For the biomass, the wild growth showed higher solids, and ash values than the cultivated biomass for both sites. The volatile solids of Strangford Lough wild samples were 36% more than its cultivated samples and total solids of wild samples 34% more than the cultivated biomass. However, for Isle of Seil samples the variations were higher, total solids of the wild samples were 22% more than cultivated samples and volatile solids were 52% more for the wild samples. From the statistical analyses it was found that the differences in the wild and cultivated biomass were significant. The wild samples and cultivated samples were significantly different from each other from all locations.

The elemental composition data also showed variations within the wild and cultivated biomass from both sites. However, the carbon concentrations in the biomass were similar in all four samples. Samples from Strangford Lough (wild and cultivated samples) had 30% and 28% (C) respectively. As for the samples from Isle of Seil, the wild samples had 29.63 % carbon and cultivated had 29.27 % respectively. The wild samples from Strangford Lough had 1.5 % more carbon than its cultivated counterparts whereas the wild Isle of Seil samples only had 0.36% more carbon than cultivated biomass. The C/N ratios were higher for the wild biomass (25) and lower in cultivated biomass (22) for samples from Strangford Lough. On the contrary C/N ratio for cultivated biomass was higher (23) than the wild (22) biomass for samples from Isle of Seil. The results observed in this study is in agreement with research performed on *S. Latissima* by Manns *et al.* (2007) where cultivated biomass from different sites in Denmark and wild biomass in North Sea (Danish waters) were monitored for 1 year and 3 years respectively. Wild biomass showed higher solids content (22.6% DW) than its cultivated counterparts (Manns *et al.*, 2017).

For Isle of Seil and Strangford Lough, the cultivated biomass is produced from the sori obtained from wild sources in the same region. Therefore, genetically the wild and cultivated samples have similar characteristics. The wild biomass is also growing around 25m to 1km of the cultivation site. As reviewed in the literature, wild and cultivated biomass have varying morphological features owing to the environmental conditions in which they are grown. This is observed in the varying solids, ash concentrations and elemental composition. In addition, age of the wild and cultivated biomass samples also vary resulting in significant variations in the characteristics of the biomass. In this study no characterisation of the holdfasts was made and the characterisation was only performed on the fronds of the wild and cultivated biomass from both the sites. The biomass was harvested in summer which had fouling infestations on the samples obtained. However, the characterisation of the fouling agents was beyond the scope of the study.

From the perspective of AD, quality of the biomass i.e. biomass with higher volatiles is desirable as this impacts the methane production potential of the biomass. In this study, higher volatile solids and ash are observed for the wild biomass with similar carbon percentages in both wild and cultivated biomass at both sites. Thus, it can be seen that such comparisons are informative as to show how much cultivated species resemble their wild counterparts. This indicates that there is significant variation in the samples and growth type do play an important role in determining the characteristics of the seaweed collected in a particular month from the same location is important as to decide which biomass to be focused on and when to be harvested and also for any modification to be introduced into the cultivation techniques. In addition, the results in this study are based on the characteristics (volatile solids and C %) of fronds of wild and cultivated biomass. In the previous studies in the literature, where the whole parts of Kelp plant including holdfasts and stipe were used for AD, biogas production was limited due to the higher percentage of inorganic minerals found in the holdfasts and stipe. However, fouling on the fronds of the biomass was found to have little effect on methane production (Marinho *et al.*, 2015). However fouling on the fronds of the biomass was found to have little effect on methane production (da Silva Marinho, 2016). Therefore, for the purpose of AD, fronds of the Kelp can be more suitable than the other parts of the biomass.

For the practical purposes of AD, having a constant biomass supply is also equally important. Harvesting wild biomass is not considered sustainable as Kelp forests are one of the most ecologically dynamic and biologically diverse habitats in marine environment. The holdfast, stipe and fronds of the Kelp plants act as substrates for colonisation my marine flora and other invertebrates. It is a strong link in the marine food chain in its growing environment and even Kelp derived detritus on the coastal shores are consumed by invertebrates and bird species (Kelly, 2005). Therefore, it is self-evident that harvesting of wild biomass will have significant negative impacts on the natural ecological balance of the region. In Europe, Norway has a well regulated and sustainable method of harvesting wild biomass however experiments carried to study the reestablishment of macro-faunal community in these areas showed that they were not re-established fully and restoration of both kelp and kelp community was slower (Christie *et al.*, 1998). If licenses are issued for regular harvesting of wild biomass in Irish waters, utilising fronds for bioenergy production can also aid in maintaining wild Kelp forests in a more sustainable manner where holdfasts can still continue to survive maintaining ecological balance in their marine environments (Kelly, 2005). Sourcing cultivated biomass for bioenergy purposes also have to be planned carefully as over production of the macroalgae biomass will also affect the ecosystem negatively with ‘macroalgal blooms’ where macroalgae compete with other species for living space in an aquatic ecosystem thereby causing a sea grass decline (van Hal *et al.*, 2014).

4.7.4 Suitability of macroalgae for anaerobic digestion

In summary, from analysing the biomass characteristics of *S. Latissima* in this study it has been observed that geographical location had little effect on biomass characteristics however localised environmental conditions determined the biochemical composition of the biomass. Results also showed that macroalgae biomass harvested at different points of the year will be significantly different in their characteristics. Wild biomass from both studied sites had higher volatile solids in comparison to their cultivated counterparts. Economically brown algae are of interest as they are cultivated in large quantities for human nutrition and the species *S. Latissima* are biogeographically widespread occurring from the high Arctic to the cold temperate region of North Atlantic (Olischläger *et al.*, 2014). In recent years *S. Latissima* has received greater scientific interest as an AD feedstock due to its higher carbohydrate content and lower inhibitions from ammonia and sulphur (Allen *et al.*, 2016, Allen *et al.*, 2015). Biomethane potential of macroalgae biomass is dependent on its chemical composition, which varies with type, habitat, cultivation method and time of harvest. *S. Latissima* is one among the highest biomethane yielding species in the brown macroalgae biomass (Tabassum *et al.*, 2017). *S. Latissima* has a reported methane yield of 34.5 m^3 CH_4 per tonne wet weight. A yield of 30 tonne VS/ha/year for *S. Latissima* was reported. With the biochemical yields combined, the species has a potential methane yield of 10,250 m^3/ha/year which is in the upper range of the existing energy crops (Allen *et al.*, 2016). However, most of the current studies in the literature have focused only on the feasibility of the species for anaerobic digestion. Knowledge gaps still exist regarding the influence of physicochemical conditions of the cultivation site on the properties of the biomass which can induce the type of bioenergy conversion process (Kerrison *et al.*, 2015). Therefore, the next phase of this study will evaluate the biomethane potential of *S. Latissima* from different cultivation sites, harvested at different times of the year and different growth type of wild and cultivated biomass. The results will focus on the biomass characteristics favouring AD process with higher rates and yields of methane production from *S. Latissima* biomass. Results of biochemical methane potential tests of *S. Latissima* will be discussed in the next chapter.

5 Results – Biochemical Methane potential of *S. Latissima*

The AD process is fairly well understood and the suitability of a biomass for AD is determined according to its physical and biochemical characteristics. As an organic feedstock, macroalgae is not dissimilar to other biomass currently utilised for AD. However, in order to ensure the process of growing, harvesting and valorising macroalgae is technically and economically viable and environmentally sustainable it is important to gain a better understanding of how factors such as location, harvest time and growth conditions impact on biogas yield and rate. From the previous chapter it is suggested that localised geographical conditions, growth phase at the time of harvest and the growth conditions (i.e. artificial cultivation or wild harvest) of biomass can impact upon the characteristics and composition of the macroalgae. Therefore, the following phase of research involved an evaluation of how these factors influence biomethane potential. As described previously (Chapter 1) Biomethane Potential (BMP) provides an indication of a biomass suitability for AD under optimised conditions.

5.1 Sample collection

Samples were collected as described in (Methods section 3.1) and analysed according to (Methods section 3.4). Samples were prepared in triplicate and tests were performed over a 30-day period. No buffers, trace elements or nutrients were added to the samples for any of the BMP tests conducted. A total of 8 BMP tests were performed to assess the biomethane potential of macroalgae biomass as a mono-digestion feedstock. A substrate to inoculum ratio of 1/4 (I/S) was used for all the BMP tests. The inoculum was sourced from Severn Trent Wastewater Treatment Plant treating sewage sludge operating at mesophilic temperature (see Methods section 3.1.3).

5.2 Inoculum Characteristics

Inoculum essentially contains the microbiological consortium from a stable/ acclimated operational AD plant. The quality of the inoculum can have a significant impact on the analysis of BMP. If the inoculum is of poor quality (i.e. insufficient active microbial population different for AD stages) then this can have an adverse effect on the analysis and lead to inaccurate results. The general characteristics of the inoculum (in terms of solids content and ash) used in this study are given in Table 20 .

Table 20 General characteristics of inoculum used in this study

Total Solids (%WW)	3.31±0.03
Volatile Solids (%WW)	2.12±0.03
Ash (%WW)	1.18±0.01
VS (%TS)	63.94

It can be seen that the inoculum in isolation has a lower solids content (3%WW) which is typical of any wastewater sludge reported in the literature (Wang *et al.*, 2014). The VS (%TS) is higher (63%) indicating an active inoculum with enough bacterial population required for hydrolysis and production of methane showing suitability for biochemical methane potential assays. Lower ash yields also indicated potential lower inhibition from inorganic content in the inoculum. This means that digestate obtained from active digesters at a wastewater treatment plant can be a suitable inoculum for use in BMP trials.

As described previously in (Chapter 1) the BMP test provides data on both the methane production rate and on potential yield. The BMP results for the inoculum (in isolation) were evaluated. Again, this is to ensure the quality of the inoculum (and therefore the reliability of the tests) and also to determine the indigenous methane potential of the sample. To deplete the residual biodegradable organic material present in the inoculum, the inoculum was degassed for up to 48hrs prior to the start of the test. This is in accordance with the method developed by Raposo *et al.* (2011). For the BMP tests on the biomass samples a substrate to inoculum ratio of 1/4 (1 part substrate to 4 parts inoculum) was chosen for this study as this ratio is reported to provide the optimal rate for methane production (Angelidaki *et al.*, 2009).

The trends for biomethane production for cellulose and inoculum over the course of the experiments are illustrated in Figure 24.

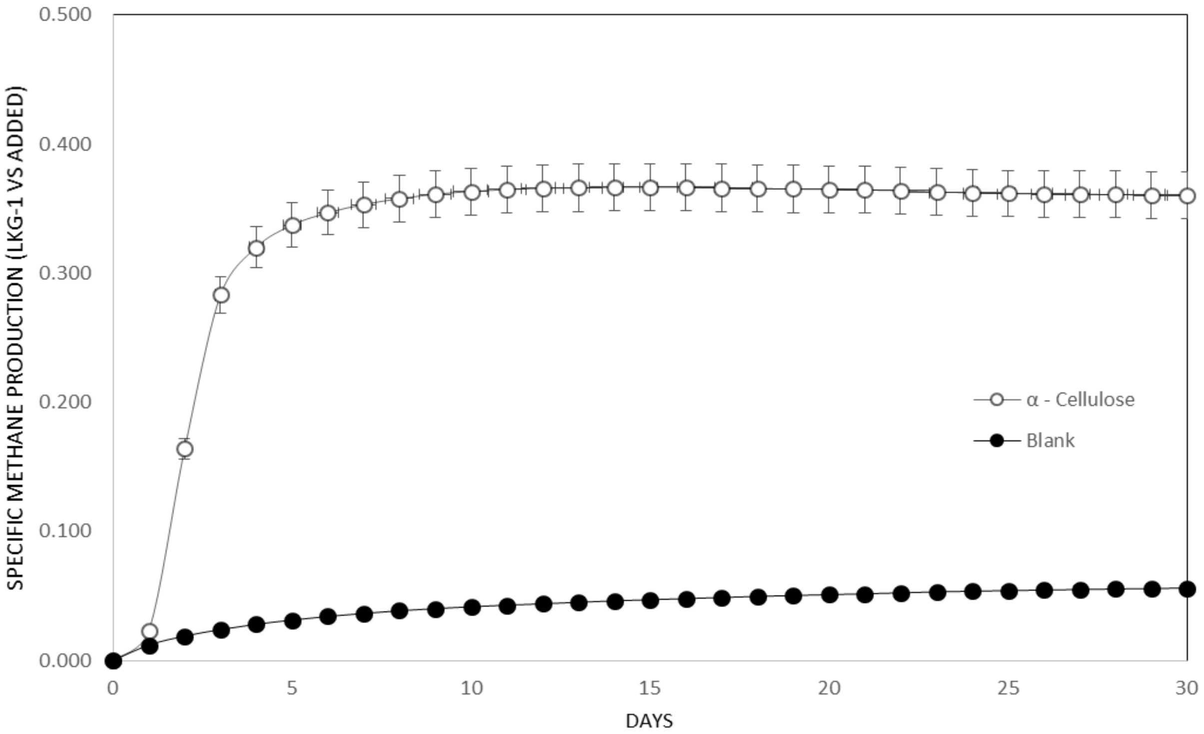

Figure 24 Trend of methane production for inoculum and cellulose

It can be seen that BMP curve represents an degradation curve where methane production from both the inoculum (blank) and cellulose have started within the first two days of the BMP test, i.e. there's no lag period in gas production. This shows that the methane forming bacteria in the inoculum were active right from Day 0. As it can be observed in the figure, the majority of methane production occurred during the first 5 days of the digestion. Both for cellulose and inoculum the pattern of methane production is similar. The flow rate of cellulose also spiked during the initial days (around 360 ml) and declined (to less than 2ml) towards the end of the test (Day 24). As expected, the inoculum has a lower methane production in comparison to the cellulose used for the trials. In a standard BMP test, the methane production from the inoculum is important as maximum methane production rates can be influenced by the viability and activity of the inoculum used (Elbeshbishy *et al.*, 2012). To ascertain this, the inoculum was used as the blank in accordance with the literature (Gunaseelan, 1997, Angelidaki *et al.*, 2009).

The average SMP for the inoculum used in this trial are given in Table 21.

Table 21 Average SMP from inoculum and cellulose used in this study

Average Specific Methane Production Inoculum ($L\ CH_4/kg\ VS\ added$)	0.054 ± 0.005
Average Specific Methane Production Cellulose ($L\ CH_4/kg\ VS\ added$)	0.367 ± 0.015
pH (Average)	7

From the table it can be seen that the average methane production from the inoculum was found to be $0.054\ \pm\ 0.005\ lCH_4/kg\ VS\ added$. The average was calculated from a total of 11 BMP tests performed as a part of this research. The pH of the inoculum was observed to be in the neutral range of 7 which is within the acceptable range for AD. Cellulose was used as a positive standard in this study. Cellulose is used as a positive control because it is an accepted standard to measure the inoculum activity or response towards standard substrates such as lignocellulosic materials (Angelidaki *et al.*, 2009) and the average specific methane production observed for cellulose was approximately $0.367\ lCH_4/kg\ VS\ added$ (see Figure 39). The average specific methane production observed for cellulose was approximately $0.367\ lCH_4/kg\ VS\ added$. The biodegradability of cellulose, 88 % (with the theoretical value of cellulose at $0.415\ lCH_4/kg\ VS\ added$) obtained in this study are in agreement with other reported values in the literature (Wang *et al.*, 2014). Methane production obtained in this study were also compared for significant variation using ANOVA tests. All the results were significant to the significant level 0.05.

The gas production from the blank from the inoculum only was subtracted from the gas production of the substrates prior to the determination of the methane yields. All methane yields are expressed as $L\ CH_4$ at standard temperature and pressure conditions per kg VS of the organic substrate added ($L\ CH_4/kg\ VS\ added$).

5.3 Impact of location

The impact of geographical location on the biomethane potential of macroalgae was assessed. The samples were taken from Strangford lough, Isle of Seil, and Ventry harbour. To ensure there was minimal influence from other external factors the samples were taken during summer 2016 season and from an artificial long line cultivation system.

Figure 25 shown below illustrates the specific methane production for the three chosen sites.

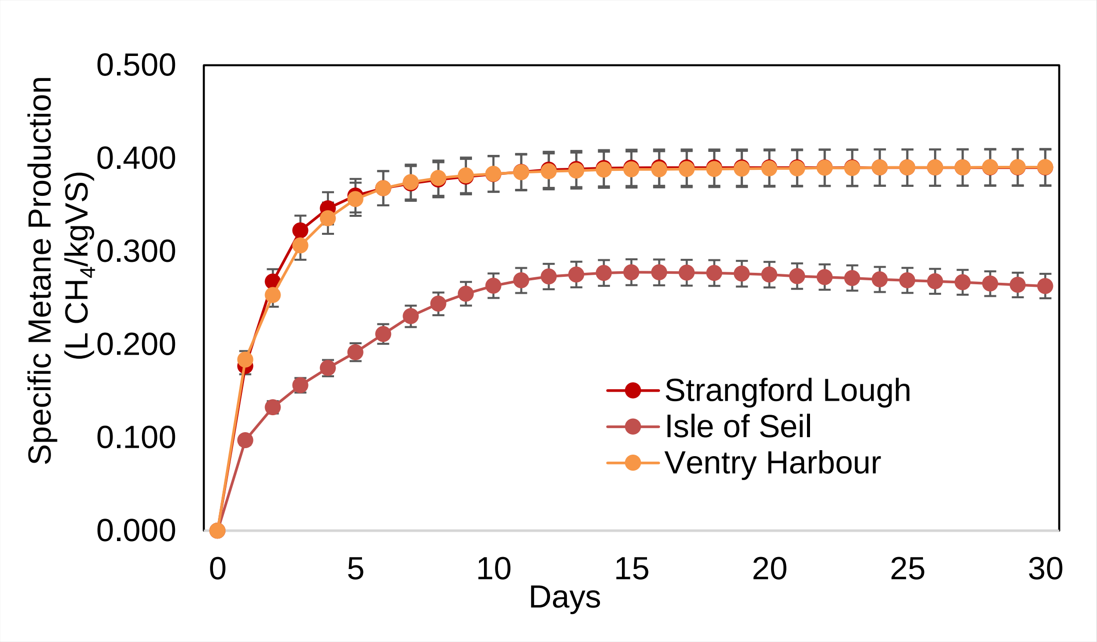

Figure 25 Specific methane production of *S. Latissima* from three locations

It can be seen that all three samples have degradation curves where samples from Strangford Lough and Ventry Harbour had very similar methane production pattern. The methane production for both these samples reached their maximum methane production values around day 10. Of particular note is the lower methane production for Isle of Seil which tends to attain maximum methane production later around day 13 in comparison to the other two samples.

To observe the rate of methane production, the initial methane production during the first 10 days of the BMP test were plotted for the three samples. The rate of methane production observed for the three samples are given in Figure 26.

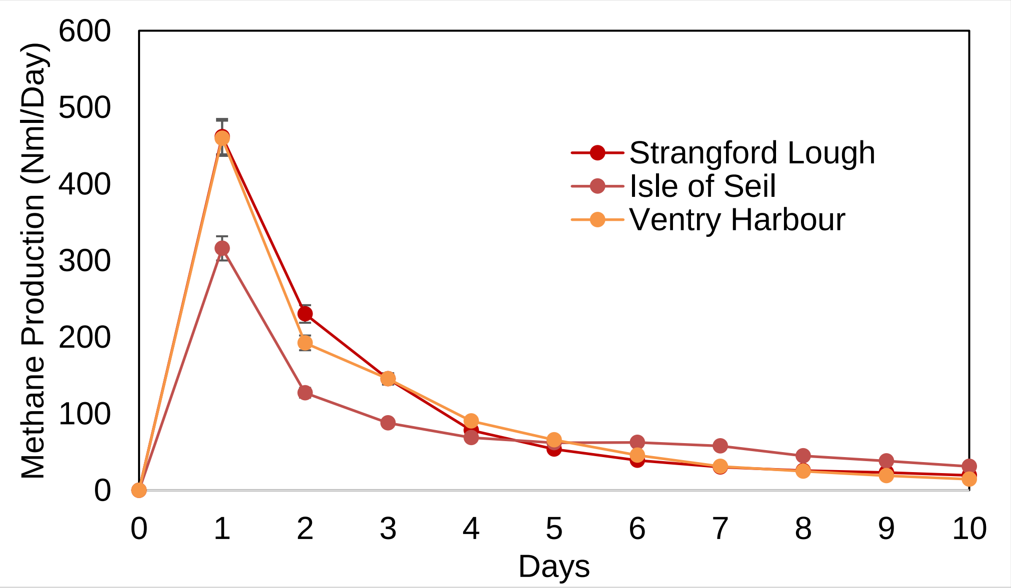

Figure 26 Rate of methane production from *S. Latissima* from three locations

It can be observed that the three samples have different rates of methane production. *S. Latissima* from Strangford Lough and Ventry Harbour have a similar methane production of 459 and 461Nml/day respectively while Isle of Seil sample only had a methane production of 315 Nml/day during the initial methane production. So the order of decreasing rate of methane production can be given as Ventry Harbour > Strangford Lough > Isle of Seil.

From the methane production observed during the 30 days of the BMP test, the SMP for the biomass from the three sites was calculated. The results are given in Table 22.

Table 22 Specific methane production of *S. Latissima* from three locations

Location	Strangford Lough	Isle of Seil	Ventry Harbour
SMP (L CH_4/kg VS $_{added}$)	0.393 ± 0.126	0.265 ± 0.012	0.391 ± 0.078
Initial VS (%WW)	11.29	18.84	10.60
Final VS (%WW)	1.65	1.68	1.74
pH (End of BMP)	7.51	7.41	7.63
Ash (%WW)	5.74	13.90	4.48

The specific methane production of biomass from Strangford Lough was found to be $0.393\ L\ CH_4/kg\ VS\ added$, while biomass from Ventry Harbour had a methane production of $0.391\ L\ CH_4/kg\ VS\ added$. The lowest of the specific methane production was shown for the biomass from Isle of Seil with a value of $0.265\ L\ CH_4/kg\ VS\ added$ (See Figure 40).

Interestingly, the methane production could be correlated to the characteristics observed for the biomass samples in terms of their volatile solids and ash percentages. The samples from Strangford Lough and Ventry Harbour is observed to have lower initial volatile percentages of 11.29% and 10.60% respectively in comparison to Isle of Seil. In addition, they also exhibit lower ash percentages in comparison to the sample from Isle of Seil.

The Volatile solids destruction was calculated for the samples from the initial and the final VS percentages recorded prior to the start and end of the BMP tests. The formula for calculating the VS destruction is given below.

$$\boldsymbol{Volatile\ solids\ destruction}\ \% = \frac{(\boldsymbol{Final\ VS} - \boldsymbol{Initial\ VS})}{(\boldsymbol{Initial\ VS})} * \mathbf{100\%}$$

Equation 7: Calculation for volatile solids destruction

For the sample from Strangford Lough and Ventry Harbour, VS destruction percentages were 85 and 83% respectively. The sample from Isle of Seil had a VS destruction percentage of 81%.

The SMP values observed in this study are higher than other studies reported in the literature such as Gurung *et al.* (2012) who observed a SMP for brown algae $0.166 \pm 0.026\ L\ CH_4/kg\ VS\ added$, for Irish *S. Latissima* by Allen *et al.* (2015) $0.342\ L\ CH_4/kg\ VS\ added,$ and another study by Jard *et al.* (2013) which observed a value of $0.246\ L\ CH_4/kg\ VS\ added$ (Jard *et al.*, 2013, Allen *et al.*, 2015, Gurung *et al.*, 2012).

5.4 Impact of harvest time

From Chapter 4 it was found that harvest time (or potentially growth phase/ plant age) has an impact on the characteristics of the macroalgae with spring harvested samples exhibiting higher TS, VS and Ash concentrations. In addition, the C content of samples were found to be highest in Summer 16 followed by Winter 16> Winter 15> Spring 16. The BMP's for the samples are provided in Table 22. These samples were taken from Strangford Loch and from wild harvests.

Firstly, the biomethane production curves for the 4 harvest times were compared. The results are provided in Figure 27 below.

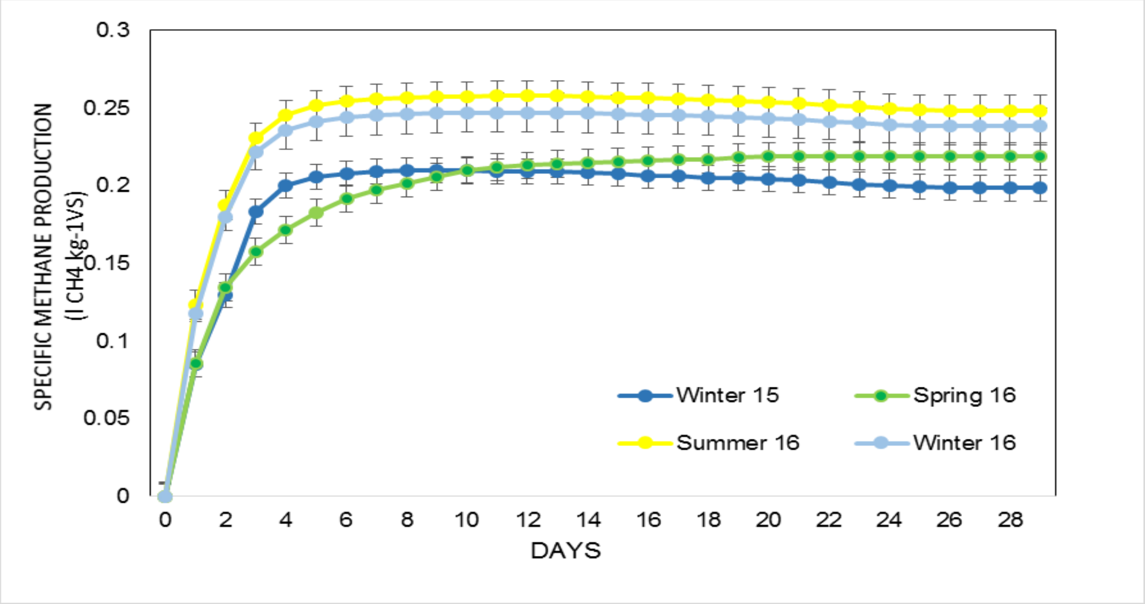

Figure 27 Specific Methane production for *S. Latissima* from 4 different harvest times

It can be seen that all four samples have shown degradation curves where highest methane production is exhibited by the summer 2016 samples. The lowest methane production among the four tested samples is observed for winter 2015 samples. To observe the rate of methane production, the initial methane production for the four samples are plotted as shown in Figure 28 below.

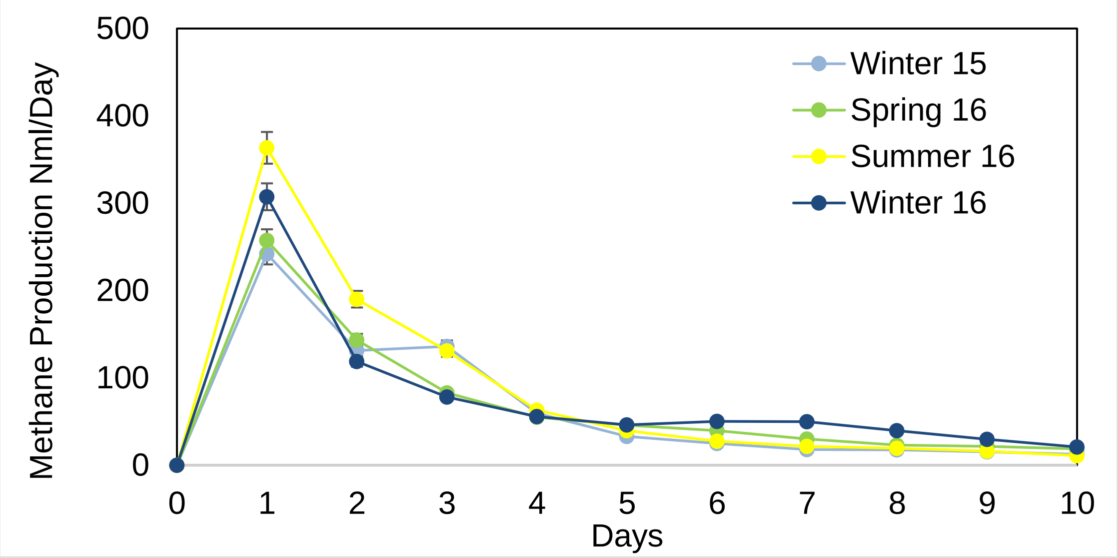

Figure 28 Rate of methane production of *S. Latissima* from 4 different harvest times

As observed summer samples has the highest methane production of 363 Nml/day followed by winter 2016 samples with 307 Nml/day. Winter 2015 samples had the lowest methane production of 242 Nml/day while spring samples had a relatively higher methane production of 257 Nml/day. The maximum methane production of all four samples is observed to occur within the first five days of the test

From this data the SMP values were calculated and the results are presented in Table 23.

Table 23 Specifc methane production characteristics of *S. Latissima* from 4 different harvest times

Season	**Winter 2015**	**Spring 2016**	**Summer 2016**	**Winter 2016**
SMP (L CH_4/kg VS $_{added}$)	0.198 ± 0.016	0.219 ± 0.018	0.249 ± 0.012	0.313± 0.015
Initial VS (%WW)	33.67	18.11	17.83	09.12
Final VS (%WW)	1.65	1.67	1.74	1.80
pH (End of BMP)	7.51	7.41	7.63	7.51
Ash (%WW)	19.28	10.39	08.13	06.17

The specific methane production from the four samples can be given in the decreasing order as winter 2016 > summer 2016 > spring 2016 > winter 2015.

Relating the methane production to the characteristics for the biomass samples, it can be seen that ash and volatile solids are the important factors that can be related to the methane production potential of the samples harvested at different times of the year. The trend in the VS percentages can be shown as winter 2016 > summer 2016 > spring 2016 > winter 2015. On the other hand, the trend in the ash percentages is observed as winter 2015 > spring 2016> summer 2016 > winter 2016.

The pH at the end of the four BMP tests were observed in the neutral range of 7 indicating favourable conditions for methane production in all of the digesters.

VS destruction is also calculated for the samples tested which showed 95% destruction for winter 2015, 90.7% for spring 2016, 90.2% for summer 2016, however only 80% for winter 2016 samples.

The SMP yields obtained in our study are similar to those found in the literature for *S. Latissima* (Jard *et al.*, 2013). The study found that the SMP yields increased from their harvest periods in May till August. In their study Jard *et al.* (2013) found the SMP values increasing from $0.204 - 256\ L\ CH_4/kg\ VS\ added$ from May to August. In our study as well, where the seasonality was studied in marked intervals of winter, spring, summer and winter, the SMP values also increased from 0.219 to $0.313\ L\ CH_4/kg\ VS\ added$.

5.5 Impact of growth type

From chapter 4 it was found that growth conditions (i.e. whether macroalgae was artificially cultivated or harvested from wild sources) may also have an impact on the characteristics of the macroalgae. It was found that wild samples exhibited higher TS, VS, Ash and C content. The same wild and cultivated samples from Strangford Lough and Isle of Seil (summer 2016) were analysed for their biomethane potential.

The results for specific biomethane production are presented in Figure 29.

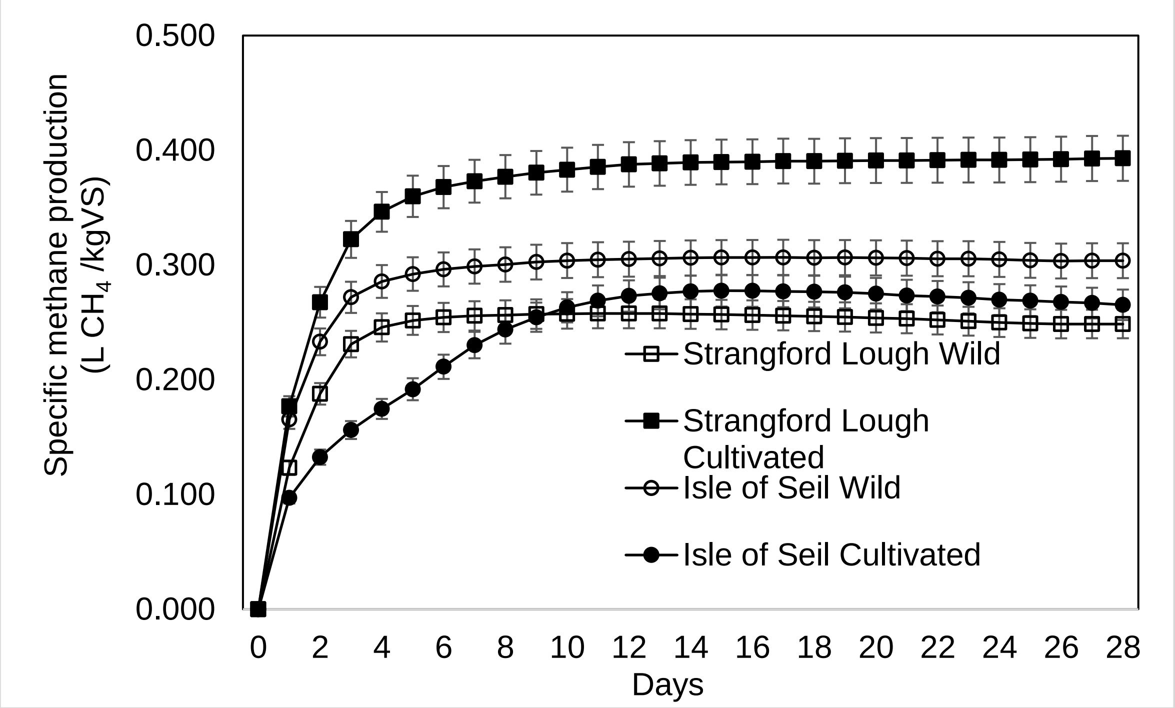

Figure 29 SMP of wild and cultivated samples from Strangford Lough and Isle of Seil

From the specific methane production curves it could be seen that the wild and cultivated samples from different locations showed different patterns. The methane degradation curve is without any lag phase in any of the four samples. To understand the methane production better, the initial methane production curves were plotted. The rate of methane production for wild and cultivated samples from Strangford Lough and Isle of Seil are shown in Figure 30.

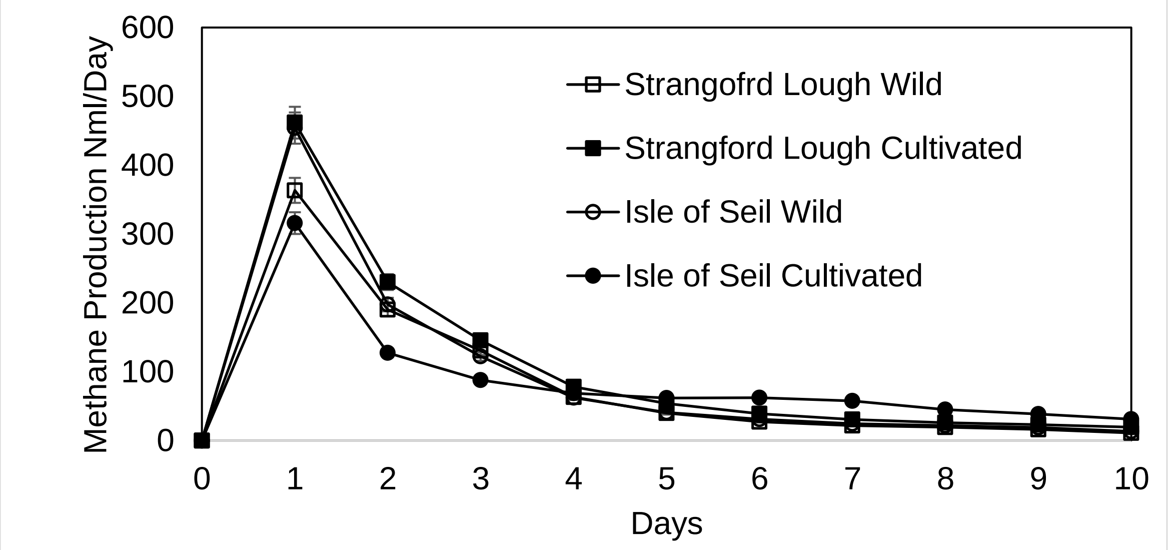

Figure 30 Rate of methane production of wild and cultivated samples from Strangford Lough and Isle

It can be seen that the maximum methane production for all four samples occurred within the first five days of the BMP test. The wild sample had consistently lower methane production when compared to the cultivated sample in Strangford Lough while it showed the opposite trend among the samples obtained from Isle of Seil.

Table 24 presents the overall results for SMP for the samples tested.

Table 24 Overall results for SMP of wild and cultivated samples from Strangford Lough and Isle of Seil

Location	**Season (2016)**	**Growth type**	**SMP (L CH_4/kgVS_{added})**	**VS %WW (%ww)**	**Ash (%WW)**
Strangford Lough	Summer	Wild	0.249±0.012	11.29	5.74
		Cultivated	0.393±0.126	17.84	8.13
Isle of Seil	Summer	Wild	0.304±0.015	18.84	13.90
		Cultivated	0.265±0.012	39.25	15.72

Firstly, it was noted that the trends for the two sites are different. For Strangford Lough the highest specific methane production was observed for the cultivated biomass ($0.393\ \pm 0.126\ L\ CH_4/kg\ VS\ added$) and the lowest for the wild biomass with a specific methane production of $0.249\ \pm 0.0.012\ L\ CH_4/kg\ VS\ adde$d). In the case of Isle of Seil biomass, the highest specific methane production is observed for wild harvest in summer ($0.304\ \pm\ 0.015\ L\ CH_4/kg\ VS\ added$) and the lowest specific methane production is observed for the cultivated biomass ($0.265\ L\ CH_4/kg\ VS\ added$).

Comparing the biomass characteristics however showed that the cultivated samples from Strangford Lough and Isle of Seil had higher volatile solids percentages as well as higher ash percentages in comparison to their wild counterparts.

5.6 Discussion

The following sections will discuss the biochemical methane potential of *S. Latissima* based on the following research question.

- To what extent are the differences in the biomethane potential of *S. Latissima* related to its characteristics varying with environmental conditions, harvest times and growth type

5.6.1 Methane production of inoculum and cellulose

As reviewed in Section 1.12, BMP tests are batch tests used to study the methane production potential of organic feedstock. The inoculum source and characteristics are important for the BMP studies as different inoculum would result in different performance during operation. The performance of the inoculum is controlled by the overall microbial community structures formed in the reactors over time (Wang *et al.*, 2014). However, the amount of gas produced per kg of material varies with the type of material used. The quality of biogas is also dependent on the C/N ratio of the material digested (Okonkwo *et al.*, 2016).

As BMP tests were performed in the AMPTS ii system, it is assumed that there would be limited oxygen in the reactors as they were purged with N_2 before the digestion. The acid forming bacteria in the inoculum will become active after all the oxygen in the reactors is used up, and then methane production begins. This is reflected in the specific methane production curve for inoculum and cellulose as methane production is exponential without any lag phase. Gas production is observed to be stable around the period of peak production until it started declining gradually. During the initial days when gas production begins to rise the bacteria acts on the substrate and starts biogas production, however the gas concentration will contain more carbon dioxide. As the days progress, the organic material is acted upon by the increasing number of anaerobic bacteria. When the gas production rises and reaches its peak, bacteria will be acting on the maximum substrate possible. As the gas production declines, it could be as a result of decreased availability of the substrate as a decrease in either C or N available for use. This decline continues however the anaerobic environment leads to a higher concentration of methane in the gas produced. When the substrate becomes exhausted the gas production stops (Okonkwo *et al.*, 2016).

Previous studies on AD of macroalgae biomass have reported that the use of sewage sludge as an inoculum for digestion is beneficial as it can actively contribute to the digestion stability and control the reduction of pH by VFAs accumulation (Tedesco and Stokes, 2017). This is also favourable for reducing the requirement for pH buffers during the digestion process. No pH buffers were added to the reactors during this study. The pH during the end of the BMP studies were also observed in the neutral range of 7 after every digestion trial which also showed an indication of balanced chemical reactions within the digesters.

AMPTSii is the second-generation automatic methane potential tests system specifically designed for BMP analysis and it has been cited by an increasing number of publications (Wang *et al.*, 2014, Xu *et al.*, 2016, Lüdtke *et al.*, 2017). All the BMP tests were performed in triplicates including the blanks and the cellulose. The methane yields achieved in the AMPTSii process showed a low standard deviation which demonstrates higher precision and lower random error for the automated apparatus. Therefore, the trends in overall methane production from inoculum and cellulose showed that the BMP tests were reliable and reproducible and a good indicator for measuring the methane production of the macroalgae biomass.

5.6.2 Biochemical methane potential of *S. Latissima*

The biomethane potential of *S. Latissima* was evaluated according to three criteria of location, harvest times and growth type according to the samples collected in this study.

5.6.2.1 Impact of location and environmental conditions

Three samples of *S. Latissima* collected from Strangford Loch, Isle of Seil and Ventry Harbour long line cultivation sites in summer 2016 were analysed for their methane production potential in this study. The decreasing order of specific methane production observed in the study can be given as Strangford Lough > Ventry Harbour > Isle of Seil. The samples from Isle of Seil also showed the lowest rate of methane production in comparison to samples from Strangford Lough and Ventry Harbour. From the statistical analysis performed, location is found to be a significant factor influencing the biochemical methane potential of *S. Latissima*. There was significant difference between the SMP values for Isle of Seil and the other two sites however there was no significant difference between Ventry Harbour and Strangford Lough. This could suggest that environmental conditions affecting the biomass characteristics also influence the biochemical potential of the biomass harvested from that geographical location.

In this study, significant differences were observed in the characteristics of the biomass derived from the three different locations. For the selection of optimum site for biomass production, environmental factors such as pH, sea water temperature, benthic conditions etc. were evaluated for the three locations and were found to be contributing to the variation observed in the characteristics of the biomass. This was reflected in the concentration of total and volatile solids, ash, and elemental composition of the biomass. Out of the three biomass samples, the biomass from Isle of Seil showed highest concentrations of ash (13.90%WW), total solids (32.74%WW), and volatile solids (18.84%WW). Even though the biomass from Isle of Seil had the highest concentration of volatile solids, the SMP was the lowest with a value of $0.265\ L\ CH_4/kg\ VS_{added}$. This suggests that there is an inverse relationship between the volatile solids concentration and biomethane produced from the biomass.

Locational or spatial variation in the biochemical composition of Kelp is less investigated in the literature as biomass can vary considerably across environmental gradients (Nielsen *et al.*, 2016). The spatial variation affecting the biochemical methane potential of Kelp has also been limited in the literature. However, this is of great importance from a bio-refinery perspective for selecting the optimum site location for the species selected for bioenergy production. As environmental conditions are significant factors for macroalgal growth, it becomes quite clear that the concentrations of the constituents such as laminarin, mannitol and alginate will also be governed by the conditions under which the biomass grows over time. In this study, on analysis of the *S. Latissima* characteristics, Isle of Seil samples were found with higher volatile solids content hence expecting the highest methane potential from the same biomass. However, in contrast *S. Latissima* with higher methane potentials were from Southern Ireland Dingle Bay (Ventry Harbour samples) with warmer temperatures than other locations. This could be because Isle of Seil samples will have produced more alginate (structural carbohydrate) in its composition than storage carbohydrates of laminarin and mannitol to survive higher hydrodynamic activities in the region. Higher concentrations of alginate will not increase methane production as alginate has is reported to be more difficult to digest by microorganisms in anaerobic digestion (Adams *et al.*, 2011, Gunaseelan, 1997, Black, 1950, Briand and Morand, 1997).

The findings of this study are also in agreement with research conducted on *S. Latissima* analysed for their spatial variability in Danish waters by Nielsen *et al.*, (2016). The authors of that study found that areas suitable for high biomass production are not necessarily optimal for producing a specific biomass quality such as high carbohydrate concentration for bioenergy production. The same study also showed that spatial differences in composition happen to reflect phenotypic plasticity and/or genotypic adaptations to local conditions and thus are relevant when selecting a particular species for cultivation and/or when selecting a site for cultivation (Nielsen *et al.*, 2016). This is a challenge not only for cultivation practices, but also for choosing the cultivation sites specifically for bioenergy purposes. This also confirms that the large variability shown by the species needs to be investigated further in relation to their environmental gradients to specifically analyse the effect on biomass and their constituents sampled at specific locations across macroalgae cultivation farms in different regions on kelp species for energy production.

5.6.2.2 Impact of growth cycle and harvest times

In this study wild samples of *S. Latissima* from Strangford Lough were studied for any seasonal significance on methane production. From an AD perspective, different harvest times is an important consideration to obtain the most suitable biomass for easier disintegration and higher biogas production. In this study, the highest specific methane potential is shown by the winter harvest (2016) and the lowest by winter harvest (2015). In the SMP graphs, there is no lag observed for methane production in all four biomass and they reach the maximum methane production within the first five days of digestion. However, the rates of methane production for the samples from different harvest times showed highest for the summer biomass, followed by the winter 16, spring 16 and the least by winter 15 biomass. On statistical analysis, the biomethane production of the samples from different harvest times were found to be statistically different from each other. On pairwise comparison, high significance was observed between summer and winter samples and spring harvest was found insignificant with the other samples. Therefore, in the order of decreasing SMP values, the specific methane production observed can be represented as Winter 16 > Summer 16 > Spring 16 > Winter 15. The volatile solids of these samples are found to be in the increasing order as Winter 15 > Spring 16 > Summer 16 > Winter 16. In addition, the ash percentages of these samples are also found to be increasing in the order of winter 15 > spring 16 > summer 16 > winter 16. This could suggest that as volatile solids increased there is a trend for increased specific methane production, however, after a certain point the inorganic content in the biomass becomes inhibitive for any further methane production from the biomass.

In the literature, seasonal variability in kelp species has been investigated since Black (1950) for the seasonal variation in the chemical composition of European macroalgae biomass. Research has continued to study for seasonal impact on anaerobic digestion of brown species including recent researches by Tabassum *et al.* (2016) on *Ascophyllum nodusum*, and another study on *Laminaria digitata* (Tabassum *et al.*, 2017). Seasonal variability of *S. Latissima* in North West Europe was first studied by (Black, 1950), and more recently by (Vilg *et al.*, 2015, Schiener, 2014, Schiener *et al.*, 2015, Marinho *et al.*, 2016, Manns *et al.*, 2017).

The implications of these studies has had great significance on utilisation of *S. Latissima* either as a feedstock for bio-refinery purposes serving high value compound extraction or for bioenergy production like anaerobic digestion and bioethanol production. All these reported studies were also performed on the wild sourced biomass of the respective species.

Methane yield is related to the level of storage sugars in macroalgae biomass (Hughes *et al.*, 2012). Therefore, from an AD perspective it is important to harvest the biomass with higher storage sugars. The higher SMP yields obtained in the winter months can be explained because of the increased level of storage sugars in the winter months for *S. Latissima* (Østgaard *et al.*, 1993). Ostgaard *et al.* (1993) observed that the batch experiments of *S. Latissima* produced a methane production in the range of $0.22 - 0.271\, lCH_4/kg\, VS_{added}$ with increasing methane yield obtained from the species harvested in autumn/winter (Østgaard *et al.*, 1993). A similar pattern with increasing methane production is observed in this study from winter harvest 2015 with SMP yields from $0.198\, to\, 0.313\, lC/kg$ in winter 2016. However, the winter SMP yields are not replicated over the yearly samples. This could be related to the age of the wild biomass plant which was beyond the scope of this study. As the samples were obtained from the wild samples, samples would have been obtained from plants of different ages having different maturity and reproductive stages which could have resulted in the difference in methane yields.

From the literature, furthermore differences are found to occur between macroalgae harvested at different periods of the year than between different species. Also, the structure of the storage products in the macroalgae depends on the phase of life cycle when harvested and on environmental conditions (Jard *et al.*, 2013). From a biochemical composition point of view, *S. Latissima* seems to show good digestibility with high SMP yields. One of the limitations of this study is that the seasonal variation is only monitored in the wild biomass. In future studies, seasonal profiling should be extended to the cultivated macroalgae biomass. Hence biomass intended for bioenergy production through anaerobic digestion should be given longer harvest time for allowing maximum carbohydrates concentration and higher biomass growth for higher gas yields provided similar pattern is observed for the cultivated biomass.

5.6.2.3 Impact of growth type on biochemical methane potential of macroalgae biomass

In this study, the impact of growth type was observed on the BMP yields of the wild and cultivated biomass. The specific methane production from Strangford Lough showed the highest specific methane production for the cultivated biomass and the lowest for the wild biomass. In the case of Isle of Seil biomass, highest specific methane production is observed for wild harvest and the lowest specific methane production is observed for the cultivated biomass. The rate of the methane production between wild and cultivated biomass from Strangford Lough and Isle of Seil were compared for observing variation in methane production. For Strangford Lough biomass, cultivated biomass showed higher flow rate in comparison to the wild biomass. For Isle of Seil biomass, wild biomass has shown a higher methane production rate than cultivated biomass.

Statistical analyses were also performed to analyse any significant difference in the biochemical methane production of the *S. Latissima* obtained from the wild and cultivated samples collected from Strangford Lough and Isle of Seil sites during summer 2016. Based on statistical analysis, comparing the wild and cultivated samples used in the study, significant difference was only found between the wild samples from both the locations. Interestingly no significant difference was found in the methane potential of cultivated biomass from either of the locations. This could be explained by the age of the plant i.e. biomass harvested. The wild populations will be older when compared to the cultivated biomass as for the wilder biomass new growths are produced from the already existing holdfasts of the biomass. While for the cultivated biomass, as the biomass is cultivated using long lines, the maximum age of the plant is only up to a year. This would have greatly contributed to the significant difference between the wild samples. Also, the environmental gradients would contribute to the growth of wild samples in different locations which again is shown significant to the variation of biomass characteristics in this study. Therefore, it is not advised to harvest wild sources as it is both unsustainable and highly variable.

In addition, based on statistical analyses, pairwise comparison of the wild and the cultivated biomass obtained from both the locations was found insignificant. This could be because they are genetically similar as the gametes for the long line cultivation are obtained from the wild sources. Therefore, when sourcing for anaerobic digestion, the biomethane yields from wild and cultivated samples are insignificant. The finding is quite relevant for bioenergy production cultivated biomass from Ireland and Scotland have been found suitable for anaerobic digestion with high SMP yields. However, wild biomass can have significant variation depending on the location of the wild population. This result is also important as it shows the potential for cultivated macroalgae biomass to contribute towards sustainable bioenergy production in the UK.

5.6.3 Factors influencing methane production of macroalgae biomass

As BMP tests can be used as good indicators for AD, the implications observed in this study are discussed in terms of factors influencing the methane potential of macroalgae biomass.

From an AD perspective methane yields are important as biomass with higher methane yields are preferred for bioenergy production. Research into the characteristics of macroalgae biomass in the literature have shown that brown algae biomass has higher productivity. Brown algae biomass is reported to be more easily biodegradable in comparison to other macroalgae species as the major constituents are carbohydrates and sugars (laminarin, mannitol and alginate) and therefore has the potential for higher methane production (Jung *et al.*, 2013). The specific methane production values observed in this study were found to be comparable to other studies conducted on *S. Latissima* biomass, for example, the study by Vanegas and Bartlett (2013) who observed a SMP of $0.335\, L\, CH_4/kg\, VS\, added$ on the biomass obtained from Irish Coast. The results observed in this study were also higher than yields observed when biomass was treated with steam explosion pre-treatment ($0.22, 0.26\, L\, CH_4/kg\, VS\, added$) (Vivekanand *et al.*, 2012, Vanegas and Bartlett, 2013). The main factors influencing methane production from macroalgae biomass were volatile solids, inorganic content (ash percentages) and carbon to nitrogen ratio.

5.6.3.1 Ash and volatile solids

In this study, the SMP values for the biomass from Strangford Lough and Ventry Harbour were found to be similar with the value 0.393 and $0.391\, L\, CH_4/kg\, VS\, added$. The lowest SMP was found for the Isle of Seil sample with the value of $0.265\, L\, CH_4/kg\, VS\, added$. Analysing the characteristics of *S. Latissima* from the perspective of AD, volatile solids and ash percentages were found to be the important factors for higher methane potential from macroalgae biomass. However, an inverse relationship was found for ash and volatile solids with biomethane production from *S. Latissima* in this study. This is in agreement with the study that reviewed the biogas potential from macroalgae by Hughes *et al.*, (2012) where the authors reported that gas yield from macroalgae biomass is related to both ash content and inversely related to volatile solids (Hughes *et al.*, 2012). Regression analysis was performed to analyse the relationship between the SMP yields, ash and volatile solids. The relationship was found to be curvilinear with a best fit of polynomial curve of order 2 (R^2 value = 0.7). This showed that both volatile solids and inorganic concentrations influenced the biomethane production in macroalgae biomass to a certain extent after which it becomes prohibitive to any further increase in methane yields.

From an AD perspective, concentration of ash could also be an indicator for the presence of any inhibitor for the observed difference in the methane production from *S. Latissima*. Common inhibitors reported in the literature for *S. Latissima* include the presence of salts, Na and K ions (Jard *et al.*, 2012). Isle of Seil had the highest ash concentration of 13.90 (%WW) while biomass from Ventry Harbour only had 4.48 (%WW) and Strangford Lough samples had 5.74 (%WW). In seasonal profiling of *S. Latissima* obtained from Isle of Seil, Schiener *et al.* (2015) confirmed that metal concentrations in the biomass were strongly related to the ash content of the biomass. Ash content consisted of micro as well as macro nutrients including Na, K, Ca, Mg, Sr, Al, As, Zn, Ti and Fe. However, K and Al were the only significant ions which were again found was significantly lower in months of June and July. The concentration of K was found to be around 4000 mg/kg while Al was found to be around 200 mg/kg (Schiener *et al.*, 2015). On the other hand, polyphenol content in *S. Latissima* reached its maximum during the summer months (around 0.4%WW). In the study by Jard *et al.,* (2012), the polyphenol content concentration was not quantified and the concentration of K was found higher than the inhibiting range of 0.25-12g/l (Jard *et al.*, 2012).

For the biomass obtained from Isle of Seil similar concentrations described by Schiener *et al.* can be assumed. Therefore, the higher concentration of K+ ion of 0.4 g/l could be the inhibitor yielding the lowest SMP of all three locations. Similarly, for wild samples used in this study, highest SMP yielding biomass of the winter16 has lowest ash yields (6%) whereas the lowest SMP yield biomass WD15 has the highest ash yield (19%). Again, this could be related to the presence of K+ ions in the ash content of *S. Latissima* as discussed earlier. The summer16 harvest is found with an ash content of 6%.

Our study has been limited to assess and quantify the environmental conditions for the biomass. In addition, the concentration of the constituents of the biomass was also not quantified including the concentration of carbohydrates (storage and structural), inorganic components and lignin. These factors can be quantified in future researches to specifically understand which factor contributes the most towards biomethane production from *S. Latissima*.

5.6.3.2 Carbon to nitrogen ratio

Carbon to nitrogen ratios are another indicator for better choice of biomass to be used in AD. The carbon to nitrogen for *S. Latissima* is reported to be lower around 7 during spring and high during summer at around 21 in cultivated biomass (Handå *et al.*, 2013). In agreement with literature, in this study, the C/N ratios for cultivated biomass was found to be higher with values of 23 (Isle of Seil), 17 (Ventry Harbour) and 22 (Strangford Lough). For wild biomass harvested at different harvest times C/N ratio was higher for summer biomass with a value of 25.2 while lower for winter biomass with a value of around 10 (winter 15), 11 (winter 16) and 8.5 (spring 16). Hence on the basis of C/N ratio, summer months would be ideal harvest time for *S. Latissima* for optimal AD performance with least inhibition from factors such as ammonia.

5.6.4 Suitability of macroalgae biomass for continuous digestion operations

In summary, *S. Latissima* is found to be feasible for anaerobic digestion with highest specific methane potential obtained from Ventry Harbour samples and lowest from Isle of Seil samples. Environmental conditions are found significant for selecting cultivation sites for anaerobic digestion. Methane potential of *S. Latissima* harvested from different harvest times were found significantly different from each other. Comparing the methane production from the wild and cultivated samples used in the study, significant difference was only found between the wild samples from both the locations. Interestingly no significant difference was found in the methane potential of cultivated biomass from either of the locations. In addition, pairwise comparison within the wild and the cultivated biomass obtained from both the locations was found insignificant. Therefore, when sourcing for anaerobic digestion, the biomethane yields from wild and cultivated samples are insignificant.

BMP tests being performed as batch studies can only function as indicators for methane production from macroalgae biomass whereas semi continuous digestion trials can offer more insight into suitability of *S. Latissima* for large scale AD operations. *S. Latissima* from Ventry Harbour samples showed higher specific methane production, and was obtained from a seaweed cultivation farm. Therefore, to analyse the influence of different parameters on continuous operations of AD it was chosen for the next phase of the study.

6 Results – Semi Continuous digestion of *S. Latissima*

Continuously Stirred Tank Reactors are a preferred method for the evaluation of biogas production from biomass as they offer a more dynamic and long-term assessment of performance. They help in developing real time profiles for AD process variables. They also provide a greater understanding of the process and particularly issues relating to scalability. A review of different continuous studies reported for *S. Latissima* has shown that there are very few continuous studies performed on the species. Therefore, this study explores the effect of process parameters of temperature, pH, alkalinity, gas production, volatile solids destruction, chemical oxygen demand, and trace element addition on AD of macroalgae biomass. The evaluation is divided into three main areas as follows:

- Mesophilic semi-continuous digestion of *S. Latissima*
- Process optimisation using trace element addition
- Process optimisation using thermophilic temperature

The digesters used in this study and their operating conditions are given in Table 25.

Table 25 Experimental conditions for semi continuous study

Digesters	Operating conditions	Optimisation
R1, R2	Mesophilic	None
R3, R4	Mesophilic	Trace element addition
R5, R6	Thermophilic	Higher temperatures

The biochemical methane potential (BMP) tests were conducted in the previous chapter (Bell and Redpath Museum) for *S. Latissima* harvested during varying season, location and for wild and cultivated biomass. The summer harvest from Dingle Bay Seaweed Ltd., Ventry harbour location exhibited the highest methane yield. This feedstock was therefore used for the ongoing semi continuous digestion trials. The substrate characteristics and inoculum characteristics are shown below.

6.1 Feedstock characteristics

The characteristics of *S. Latissima* used for semi continuous digestion sourced from Dingle Bay seaweed Ltd. is given in Table 26. The biomass had total solids content of 15(%WW) and an ash content of 4(%WW). The elemental composition analysis highlighted that the sample was comprised 29% carbon, 1.7% nitrogen and 0.4%

sulphur (as a %TS). The C/N ratio of the biomass was found to be 17.1. The BMP of the biomass was found to be $0.391\ L\ CH_4\ kg/VS\ added$.

Table 26 Characterisitics of *S. Latissima* used for semi continuous digestion

Seaweed ID	Total Solids (%WW)	Volatile Solids (%WW)	VS (%TS)	Ash (%WW)	Moisture (%WW)	Calorific Values (MJ/kg)	SMP (L CH_4/ kg VS $_{added}$)
Ventry Harbour	15.08	10.60	70.30	4.48	84.92	07.30	0.391

6.2 Inoculum characteristics

The inoculum was sourced from Severn Trent Wastewater sewage treatment as described previously (Section 3.1.3). This inoculum was used for both mesophilic and thermophilic experiments. No buffers were added to the inoculum to maintain the pH. The inoculum characteristics and the standard deviations are given in Table 27. The experiments were performed in duplicates for the semi-continuous digestion run.

Table 27 Characteristics of inoculum used for semi continous digestion

Inoculum source	Severn Trent Wastewater Treatment Plant
Inoculum source temperature	Mesophilic (37°C)
Total Solids, TS (% WW)	3.10 ± 0.01
Volatile Solids, VS (%WW)	2.01 ± 0.01
Ash (%WW)	1.07 ± 0.01
Moisture (%WW)	96.94 ± 0.24
VS (%TS)	64.84
pH	7.4± 0.04
BMP (L CH_4 /kg VS $_{added}$)	0.056

6.3 Experimental conditions

Semi continuous digestion experiments were performed on *S. Latissima* for three hydraulic retention times (HRT), a total of 105 days. The digestion was performed under mesophilic conditions of 37°C. The mesophilic digesters were for labelled reactors R1, and R2. The digesters had a working volume of 2L and fed with an organic loading rate of 3gVS/L/day with a daily feed of 56.6g for 3 HRT. The biomass was macerated (<1cm) before it was fed to the digesters. The effluent from the digesters were analysed for pH, conductivity, dewaterability- (via capillary suction time or CST), chemical oxygen demand (COD) (total and soluble), alkalinity, and %VS destruction. The gas produced was collected using Tedlar bags and gas composition was performed on a weekly basis for all the digesters. The temperature and gas volumes were measured daily for mesophilic digesters.

The results are discussed in the sections below.

6.4 Mesophilic digestion

6.4.1 Temperature

The daily temperature for the mesophilic digesters is shown in Figure 31. Variations in the temperature recorded for the mesophilic digesters was considered acceptable with no extreme deviation in temperature over the period of the trials. Mild fluctuation in the temperature was noted during the first hydraulic retention time (SD±0.8). On day 24, a temperature of 40.9°C was noticed due to a functional complication of the PID system and it was rectified from Day 25. However, temperatures were stable for the second (SD ± 0.5) and third retention times (SD±0.5). The average temperature recorded for the mesophilic digesters were 36.35 ± 0.50.

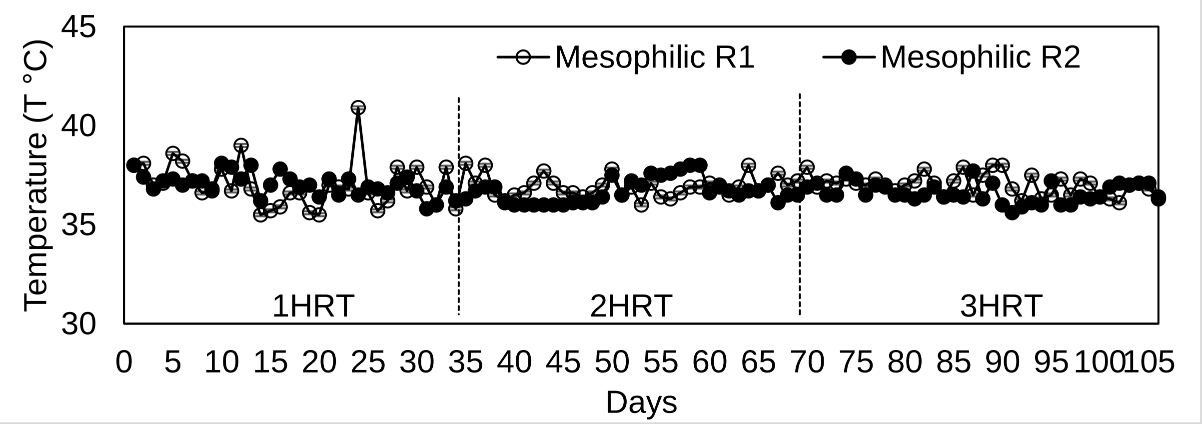

Figure 31 Temperature for mesophilic digesters

6.4.2 pH

pH in the mesophilic digesters ranged between 7.48 – 6.36. The pH was similar in both the digesters. The initial pH for the mesophilic digesters was 7.48, however an increase in the pH was observed in the second HRT to 7.85. pH was considerably stable for the mesophilic digesters and had been ideal for the methanogenesis for gas production for the mesophilic digestion. During the third HRT, a decrease in the pH was observed in the digesters to 6.36. The pH changes observed in mesophilic digesters is shown in Figure 32.

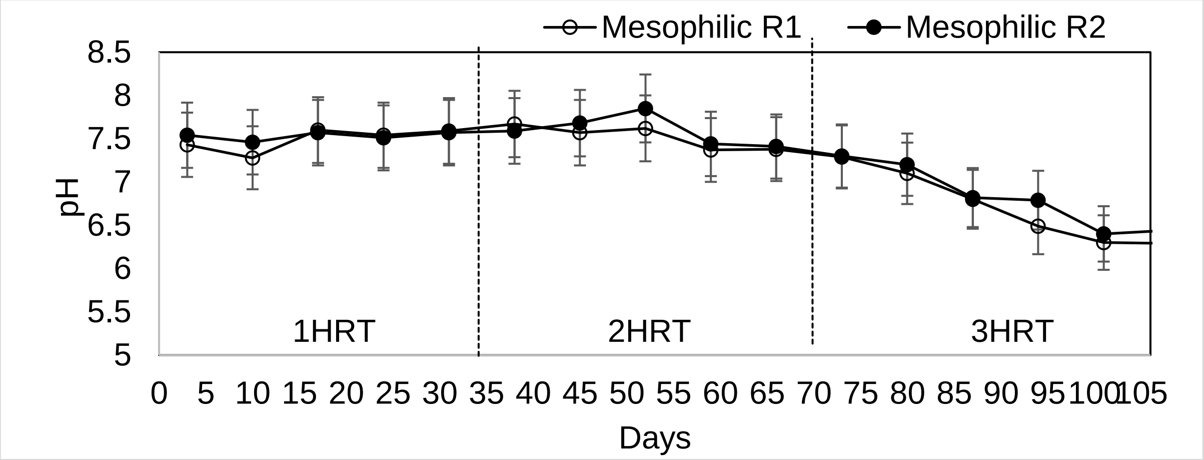

Figure 32 pH for mesophilic digesters

6.4.3 Conductivity

Conductivity measurements observed for R1, R2, mesophilic digesters were in the average range of 2.8 – 4.85 m S/cm. As expected, the conductivity measurements increased from 2.63 to 4.88 m S/cm in digester R1 and from 2.98 to 4.82 m S/cm in digester R2. The increase in conductivity is expected because of the variation in the ionic content in the digester such as Hydrogen H+, hydroxide OH- and nutrients such as nitrate, phosphate and other inorganic metallic ions (Levlin, 2010). Therefore, as digestion of macroalgae biomass occurs, the presence of these ions also increase with the dissociation of the biomass increasing the conductivity inside the digesters.

6.4.4 Alkalinity

Alkalinity measurements were taken and intermediate, partial and total alkalinity was calculated for the mesophilic digesters. The alkalinity observed for the mesophilic digesters is shown in Table 28. In general alkalinity measurements show the ability of the solution to neutralise acids and in AD processes it is strongly influenced by the presence of carbonate and bicarbonate, ammonia, phosphate, and volatile fatty acids. As noticed in the table below, the values are observed high for anaerobic digestion. Total alkalinity can be divided into partial and intermediate where partial alkalinity is caused particularly by the presence of OH-, NH3, CO3- and HCO3-. However the intermediate alkalinity is related to VFA presence (Bolzonella, 2011). Therefore, it can be said that VFA production was occurring highly in mesophilic digesters resulting in higher intermediate alkalinity values.

Table 28 Alkalinity for mesophilic digesters

Mesophilic digesters	**Alkalinity (mg/LCaCO$_3$)**		
Retention Time	**Partial**	**Intermediate**	**Total**
First	895	1243.5	2117.9
Second	1213.0	1636.4	2863.0
Third	428.0	1463.3	1866.6

6.4.5 CST

Capillary suction time (CST) is used as a simple technique to measure the sludge disintegration or dewaterability for the digestate from anaerobic digesters. CST can also act as a supportive indicator for the soluble COD release within the digesters (Apul *et al.*, 2009). Dewaterability of the digestate (effluent) was measured using capillary suction time (CST) measurements (Section 3.4.10). The CST observed for the mesophilic digesters increased from 624.2s to1632.9 in the 1st HRT and was still found increasing during the 2nd HRT to 1813.1s. However, by the third HRT, the CST values were lowered and were recorded in the range 858.9 – 347.0s. The increase in CST values during first and second HRT indicate the requirement of longer time duration for the settling of solids in the digestate however the decrease in the values in the third HRT indicate that with longer retention times, digestate from mesophilic digestion would require lesser time to separate between the solid and liquid fractions.

6.4.6 Chemical Oxygen Demand (COD)

Total and Soluble COD can be indicators of organic degradation within the digesters (Abbasi *et al.*, 2012). Samples from the digesters were taken weekly and analysed for Total and soluble Chemical oxygen demand. Total COD was in the range of 35000-40000mg/l to values of 35133mg/l in R1 and 35161 mg/l in R2 digesters respectively. However, soluble COD values were found to gradually increase in both the digesters over the period of the trial (from 2000mg/l during 1st HRT to approx16000mg/l during the 2nd HRT). During the transition from second to third HRT, the values increased to 30000 mg/l. The changes in total and soluble COD for the mesophilic digesters is given in Figure 33.

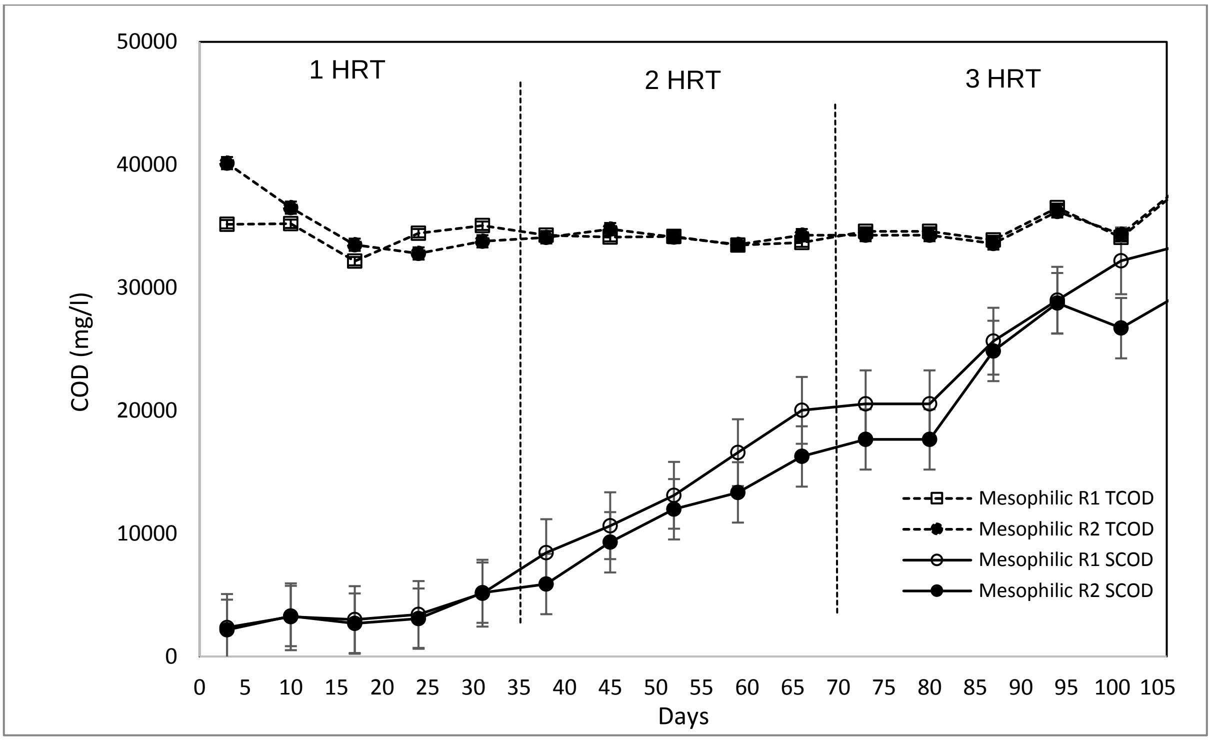

Figure 33 Total and Soluble COD for mesophilic digesters over three HRT

6.4.7 Volatile Solids degradation

The degradation of organic matter in the digesters is measured using volatile solids analysis. In principle, as organic material is broken down the organic material is converted into VS. The trend for volatile solids is shown in Figure 34.

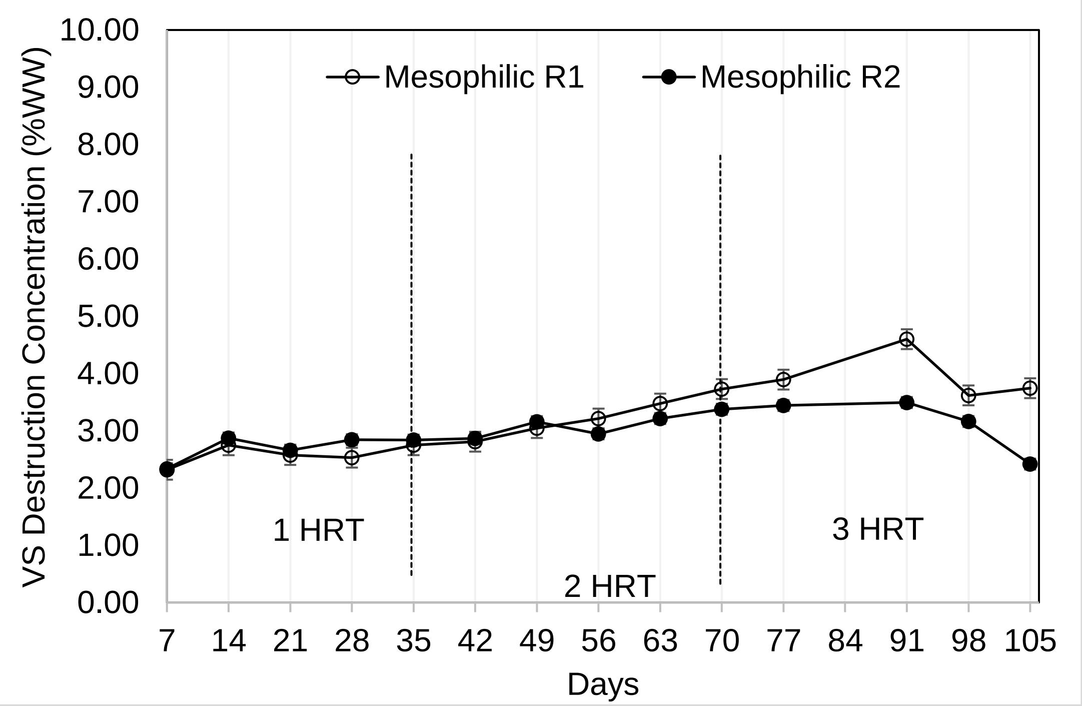

Figure 34 Final volatile solids concentration for mesophilic digesters over three HRT

The volatile solids concentration increased from 2%WW to 4%WW over the three HRTs. The ash percentage also was found to increase from 1.5% to around 5% wet weight. The digestate was found to get progressively thicker over the course of the trial (from the watery consistency in the first HRT with a moisture content of 96% to approximately 90%WW by the end of the trial). The initial and final solids, ash and moisture concentrations of the mesophilic digesters are given in Table 29.

Table 29 Initial and final solids, ash and moisture for mesophilic digesters

Digester	**Total solids (%WW)**		**Volatile solids (%WW)**		**Ash (%WW)**		**Moisture (%WW)**	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
R1	3.91	9.57	2.32	4.50	1.59	5.07	96.09	90.43
R2	4.04	8.52	2.34	3.85	1.71	4.68	95.96	91.48

6.4.8 Gas production

The biogas and methane production of mesophilic digesters is shown in Figure 35. Gas produced within the digesters was collected in Tedlar bags and measured using a standard displacement method. The gas production for digester R1 was measured directly and the gas produced in the digester R2 was passed through 3M $NaOH$ solution to obtain the biomethane production. The biogas production ranged from 2.75 l/day (at STP) in the first HRT to 2l/day in the second HRT. The gas production started to decrease in the 2nd HRT to almost 0.50l/day. The lowest gas production values were observed in the third HRT. The gas production in the 3rd HRT was typically in the range 0.15-0.2 l/day.

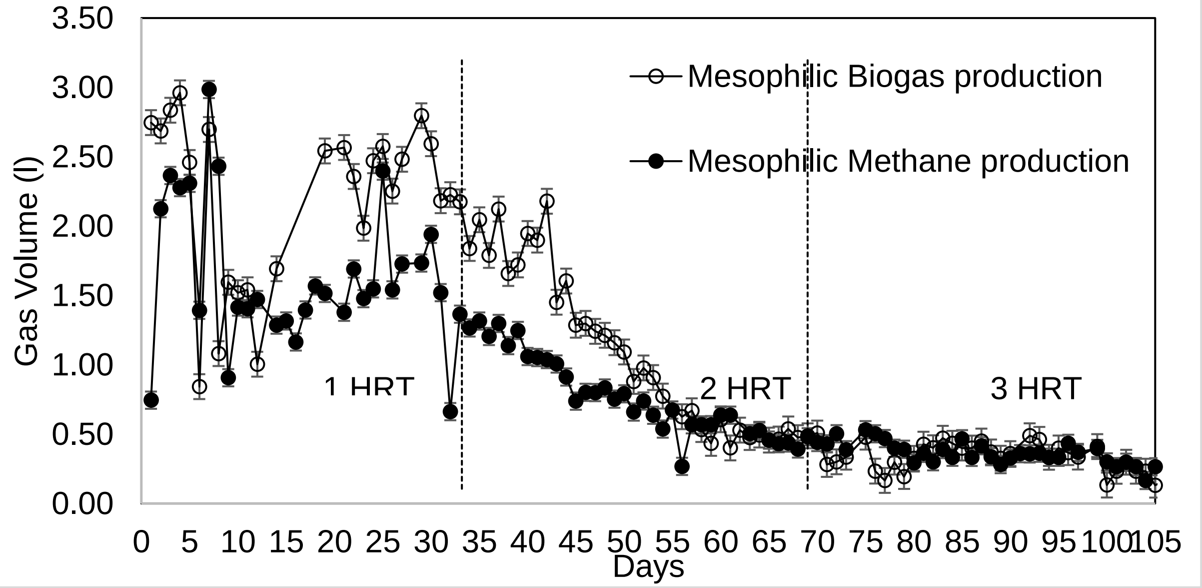

Figure 35 Gas production for mesophilic digesters

For digesters R1 and R2, it can be seen from Figure 22 that methane concentrations were notably higher in the first and second HRT, however lower in the 3rd HRT. Methane concentrations varied between (50-60%) in the first and second HRT, however from week 8, methane concentration decreased to between 10-30% lower than the values obtained in the second HRT.

6.5 Impact of trace element addition

From the gas production observed in mesophilic digesters, the gas volumes are found to decrease from the second HRT to lower values in the third HRT. This could be cause of the digester instability caused by variation in the C/N ratios. As discussed in literature review, during semi continuous digestion of brown macroalgae species, the digester balance was found to be unstable due to the decrease in C/N/P ratios and a trace element addition was found to aid digestion of the biomass with higher methane production (Hinks *et al.*, 2013). Therefore, in this study optimisation of mesophilic digestion was performed by the addition of trace element solution to the digesters. Trace element solution was prepared according to (Suhartini, 2014). The composition of the trace element solution used for this study where trace elements as 'compounds used', 'element concentration' and 'compound concentration in stock solution' is given in Table 30.

Table 30 Composition of trace element solution used in this tudy

Trace element	Compound used	Element concentration after diluted by 1000 times (mg/l)	Compound concentration in stock solution (g/l)
Aluminium (Al)	$AlCl_3.6H_2O$	0.1	0.895
Boron (B)	H_3BO_3	0.1	0.572
Cobalt (Co)	$CoCl_2.6H_2O$	1.0	4.038
Copper (Cu)	$CuCl_2.2H_2O$	0.1	0.268
Iron (Fe)	$FeCl_2.4H_2O$	10.0	35.597
Manganese (Mn)	$MnCl_2.4H_2O$	1.0	3.602
Nickel (Ni)	$NiCl_2.6H_2O$	1.0	4.050
Zinc (Zn)	$ZnCl_2$	1.0	2.084
Molybdenum (Mo)	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.1	0.184
Selenium (Se)	Na_2SeO_3	0.1	0.219
Tungsten (W)	$Na_2WO_4.2H_2O$	0.1	0.179

The solution was added on a daily basis at a rate of 1 ml of for every 1L of digestate removed to maintain the initial concentration (Yirong *et al.*, 2015). R3 and R4 were the trace element added digesters. The performance of the mesophilic digesters was optimised by the addition of trace element solution for three hydraulic retention times for 105 days. The performance of trace element added digesters were compared against the previous results obtained from the mesophilic digesters R1 and R2.

This optimisation experiment was designed to answer the following research questions specifically: -

- To what extend does process optimisation (in the form of trace element addition) impact on overall gas yields from the digesters?
- Was there any significant difference in methane concentrations between those digesters with and without trace element addition?
- Was there any significant increase in volatile solids destruction between those digesters with and without trace element addition?

Source: (Suhartini, 2014)

The various process parameters such as temperature, pH, CST, COD, solids, volatile solids degradation, and gas production was observed for the trace element added reactors R3 and R4.

6.5.1 Temperature

The temperature of trace element added digesters (R3 and R4) are shown in Figure 36.

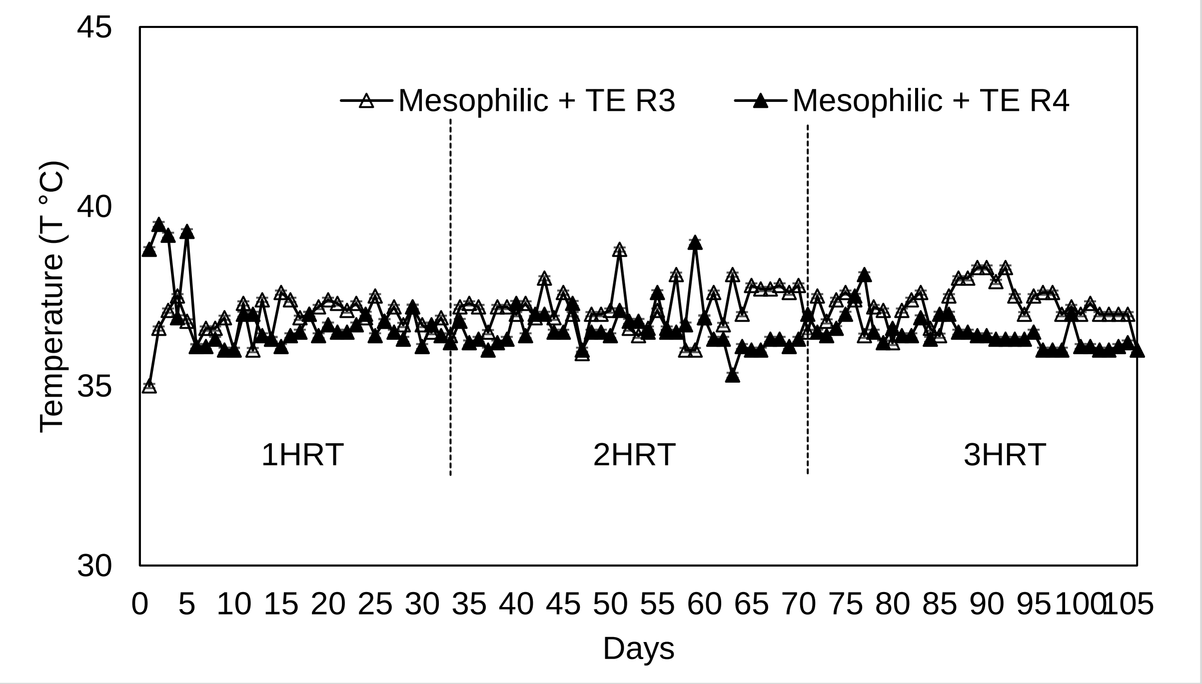

Figure 36 Temperature for trace element added digesters

The temperature variation in R3 was higher in comparison to R4. This could be attributed to the heating coils used for heating the digesters. In addition, it could also be possibly associated with the defect in the PID system used to record the temperatures in the study. However, the changes in the temperature for trace element added digesters were within standard deviation limits. The average temperature recorded for the trace element added digesters were 36.0 ± 0.4

6.5.2 pH, Alkalinity, VFA

The pH of the digesters were recorded and results are presented in Figure 37. In the first HRT it can be observed that the pH was similar for the four digesters until the end of second HRT. However, it was noted that from day 55, variation in pH was observed between the digesters with trace element addition compared to those without. In the digesters which had trace element added (digesters R3 and R4) an increase in pH towards alkaline values were observed. The pH increased from 7.75 to 8.06 in these digesters compared to R1 and R2 digesters where no trace elements were added.

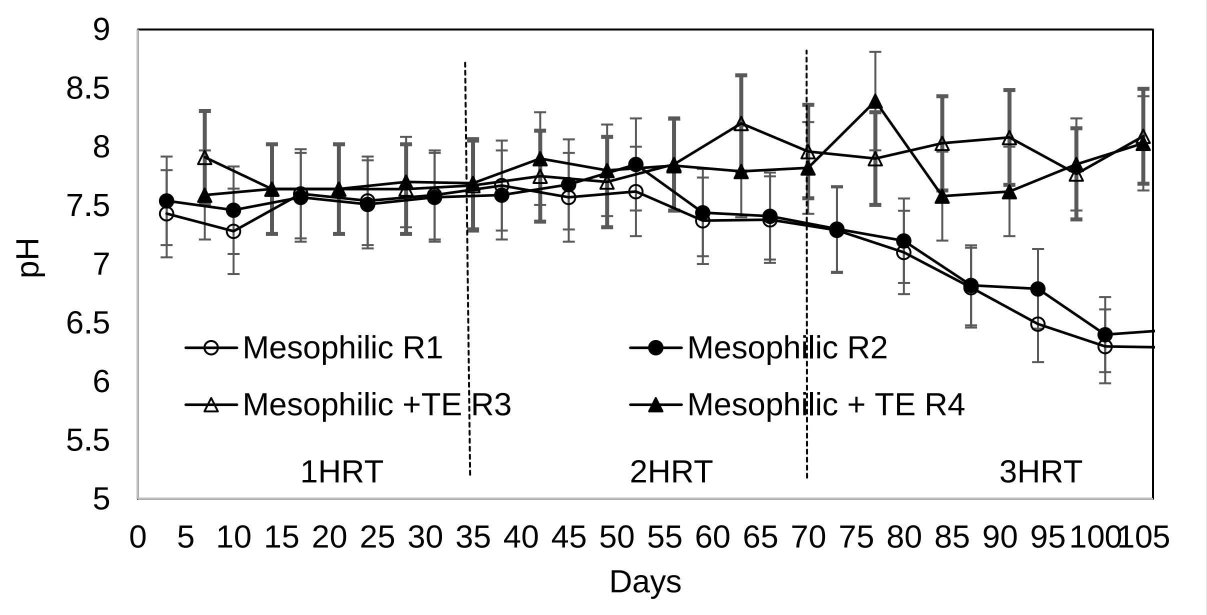

Figure 37 pH for trace element added digesters

Table 31 Alkalinity for trace element added digesters

Digesters R3, R4	**Alkalinity (mg/LCaCO$_3$)**		
Retention Time	**Partial**	**Intermediate**	**Total**
First	816.6	1116.8	1933.3
Second	1134.2	1470.8	2605.0
Third	1235.3	1299.0	2895.9

The alkalinity for digesters with trace element addition is given in Table 31. In the case of partial and intermediate alkalinity, the trace element added digesters had the values continuously increasing throughout the digestion. The total alkalinity for the trace element added digesters, total alkalinity ranged between 1900 – 2900 mg/l. In comparison to digesters R1 and R2, total alkalinity continuously increased from first to third HRT in R3 and R4. However, the partial alkalinity values are noticeably higher in R3 and R4 indicating the formation of bicarbonates and carbonates equally with VFA production in these digesters maintaining the balance within the digesters.

6.5.3 Conductivity, CST and COD

The trend for soluble COD for mesophilic digesters with trace element addition is shown in Figure 38.

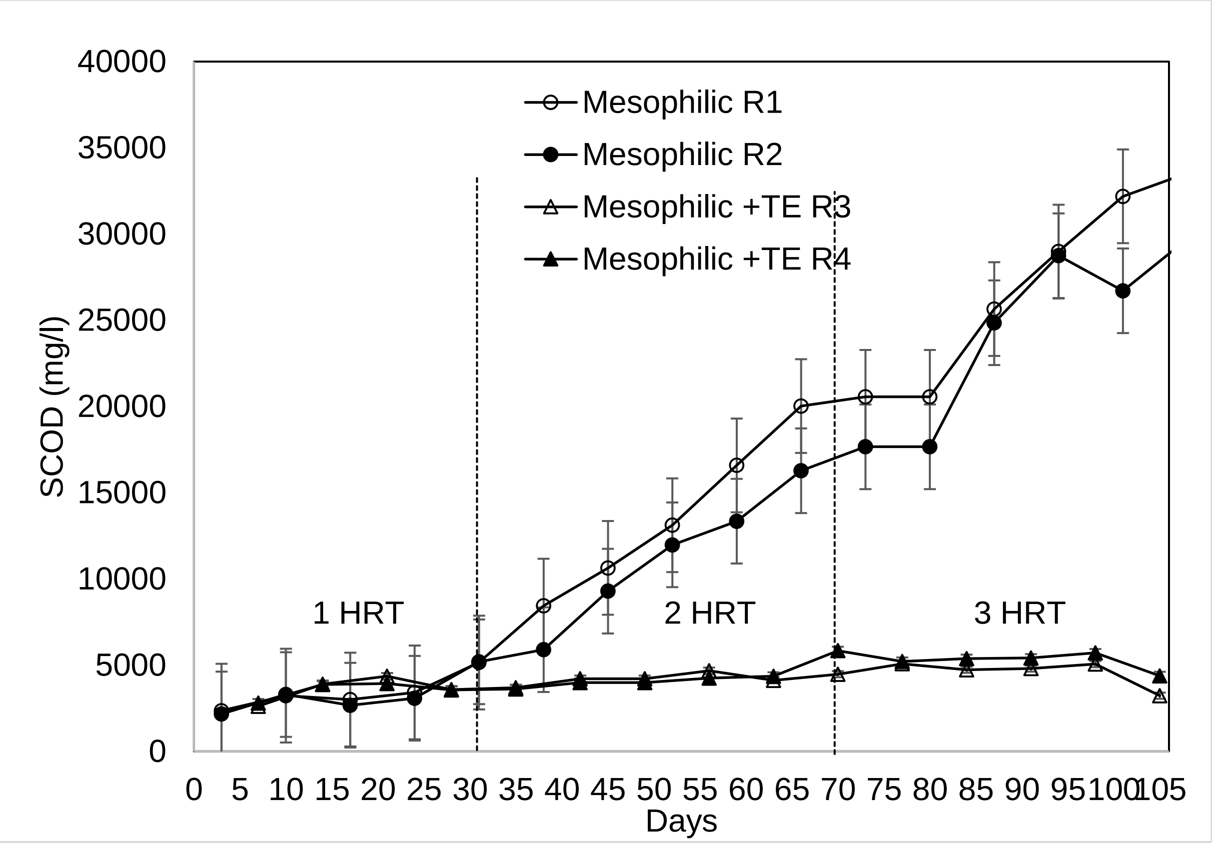

Figure 38 Soluble COD for trace element added digesters

The conductivity, CST and COD values for the four digesters are shown in Table 32.

Table 32 Conductivity, CST and COD for trace element added digesters

Mesophilic			**Mesophilic + Trace element**		
Total COD (Average R1, R2) mg/l	37000		**Total COD (Average, R3, R4) mg/l**	37000	
Soluble COD (Initial – Final) mg/l	2200	32000	**Soluble COD (Initial – Final) mg/l**	2700	3800
CST(Initial-Final) s	624	347	**CST(Initial-Final) s**	576	640
Conductivity m S/cm	2.8	4.8	**Conductivity m S/cm**	3.3	6.8

Trace element added digesters R3 and R4 were observed to have higher conductivity values and CST values. However, the soluble COD values were considerably lower when compared to the R1 and R2 mesophilic digesters. Increased CST values indicated that the dewaterability of the sludge was poorer in trace element added digesters. This showed that the disintegration of biomass in the trace element digesters was slower and increased conductivity values were due to the presence of the ions in the trace elements added to the digesters rather than from the dissociation of biomass. This could also be the reason of very low soluble COD values in the digesters R3 and R4.

6.5.4 Gas Production

The gas production trends for the digesters with and without trace element addition were compared in Figure 39. All four digesters had higher variability in gas production during the first HRT. This could be attributed to the acclimation period of the inoculum with the macroalgae biomass. In comparison, the digesters with trace element addition showed a comparatively higher biogas production than the digesters that had none. The gas production rate was relatively consistent for the digesters with no trace element addition, however digesters with trace element addition exhibited a variations of ±5 l/day. For these digesters gas production started decreasing from day 70 until the end of the trial.

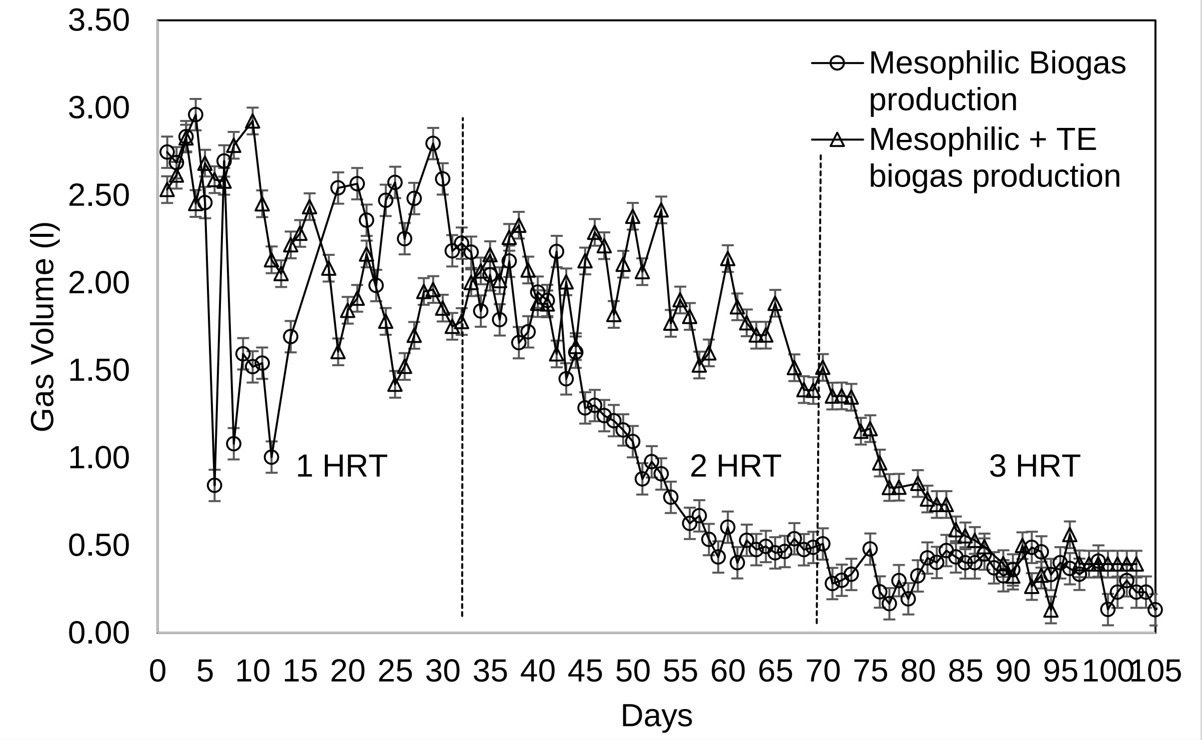

Figure 39 Gas production for trace element added digesters

The gas compositions for the digesters are shown in Table 33.

Table 33 Gas composition for mesophilic and trace element added digesters

Mode of operation	**Mesophilic**						**Mesophilic + Trace element Addition**					
Digester	**R1**			**R2**			**R3**			**R4**		
$NaOH$ Scrubber (3M)	No			Yes			No			Yes		
HRT	1	2	3	1	2	3	1	2	3	1	2	3
Avg. gas per HRT vol (L)	2.12	0.68	0.34	1.53	0.58	0.32	2.16	1.75	0.51	1.25	1.22	0.28
CH_4 (%)	48	31	<10	54	68	11	41	45	25	47	51	20
CO_2 (%)	34	28	22	<1	<1	<1	33	24	25	<1	<1	<1

The $NaOH$ scrubber has lowered the percentage of carbon dioxide to less than 1% in the trace element added digester R4 as well. Observing the gas composition data, methane percentages were higher in all 4 digesters but comparatively higher in R3 and R4. This was expected with trace element addition, however the percentage of methane concentration has remained within 40 – 50% in the first and second HRT however lower in the third HRT. The methane percentages are comparable to results observed in other studies involving *S. Latissima* where methane production is not found to be stable in semi continuous operations where a higher organic loading rate is used similar to this study (Jard *et al.*, 2012).

6.5.5 Volatile Solids disintegration

In comparison to R1 and R2, the trend for volatile solids disintegration was lower with trace element addition in R3 and R4 digesters. It appears that the solids content was higher in R3 and R4. This could be due to the trace elements added to the digester for stability of the digesters forming a deposition on the inside of the digesters increasing the total solids of the digesters. At the start of third HRT however the volatile solids disintegration seems to increase but further decrease until the end of the 3rd HRT. The volatile solids destruction were compared for the digesters with and without trace element addition Figure 40.

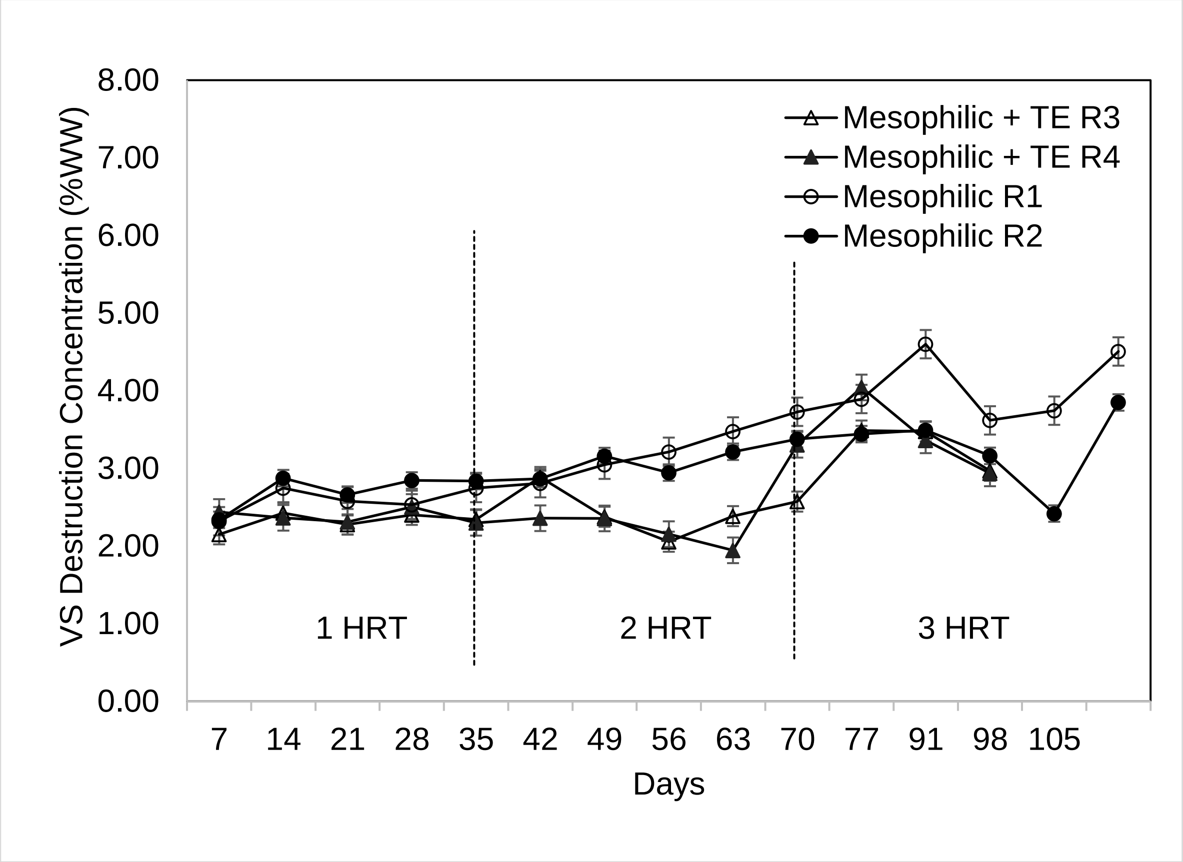

Figure 40 Final volatile solids concentration for trace element added digesters

6.6 Impact of thermophilic temperature

The next stage of research involved an evaluation of the impact of temperature on the digestion of macroalgae biomass. This experiment involved increasing the temperature of digestion to 55°C. As with previous experiments the semi continuous method was adopted and the digestion was monitored for 3 HRTs (for 105 days). Process variables such as pH, temperature, CST, COD, solids, VS degradation and gas production were recorded on a weekly basis. The results for thermophilic digestion were compared to mesophilic digestion. For the purpose of this trial the reactors were numbered R5 and R6.

6.6.1 Temperature

Thermophilic digestion is generally considered to take place at a temperature range between 50 and 60°C. The daily temperature for the thermophilic digesters is shown in Figure 41.

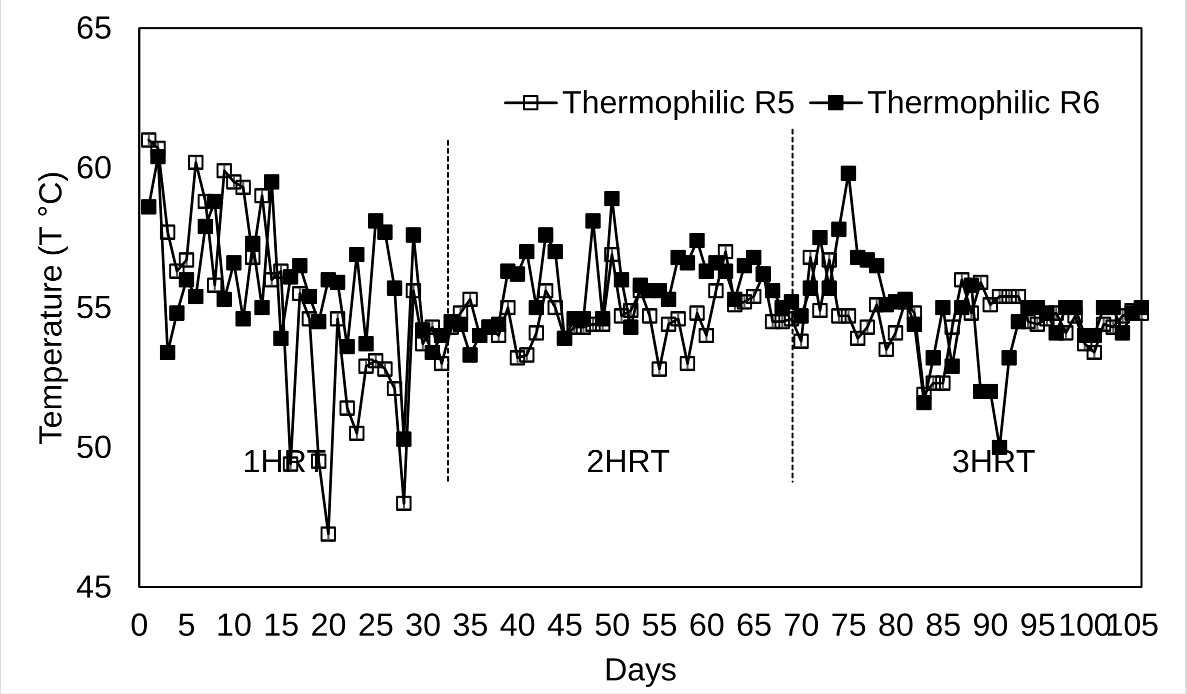

Figure 41 Temperature for thermophilic digesters

Fluctuations were observed for the thermophilic digesters during first hydraulic retention time. Temperatures were stable during second HRT, however fluctuation in the temperature was again observed during the third hydraulic retention time. The average temperature recorded for the thermophilic digesters were 54.9 ± 1.62. Temperature fluctuation was observed in both the digesters equally in digester R5 and R6.

6.6.2 pH

The pH for thermophilic digesters is shown in Figure 42.

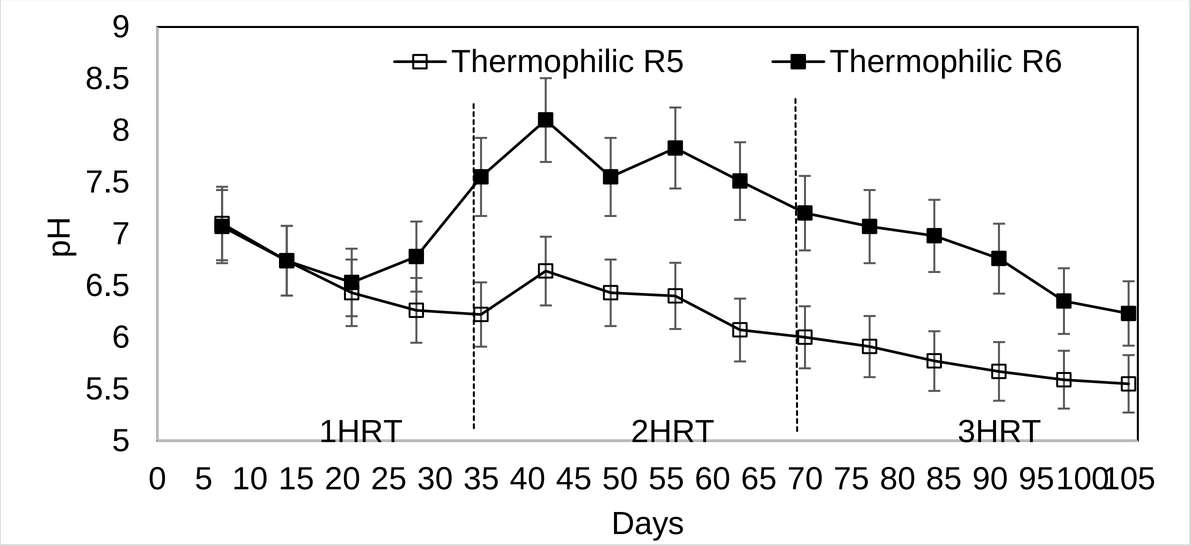

Figure 42 pH for thermophilic digesters

The average pH in the thermophilic digesters ranged between 5.89 – 7.07. The initial pH for digester R5 was 7.1 and for R6 was 7.07. It can be seen for both digesters there was a decline in pH up to day 20 in the first HRT. In the second HRT, in both digesters there was an increase and peak in maximum pH at day 42 and then a general decrease in pH from day 45 to day 105 is observed. It is also observed that despite similar starting pHs there is a difference between the pH of the duplicate digesters over the course of the experiment. The final pH within the reactors at day 105 was 5.5 in R5 and 5.9 in R6.

6.6.3 Conductivity

Conductivity measurements observed for the thermophilic digesters (R5&R6) were in the range 3.24 – 5.24 mS/cm. As expected, the conductivity measurements increased from 3.23 to 5.23 mS/cm in digester R5 and from 3.26 to 5.25 mS/cm in digester R6 over the three hydraulic retention times. In comparison to the mesophilic digesters, conductivity was higher in thermophilic digesters.

6.6.4 Alkalinity

The alkalinity for thermophilic digesters is given in Table 34.

Table 34 Alkalinity for thermophilic digesters

Thermophilic digesters			
Hydraulic Retention Time (Average mg/l)	**Partial**	**Intermediate**	**Total**
First	309	1025	1252
Second	332	1301	1269
Third	155	1159	956

The average alkalinity concentrations for the thermophilic digesters in the first, second and third HRT are given in the table below. Intermediate alkalinity was found to increase over the first and second retention times. In comparison to the mesophilic digesters alkalinity values were lower in thermophilic digesters. Of particular note is the lower partial alkalinity values which was the lowest observed in this study. The lower partial and intermediate values could indicate lower disintegration of biomass in thermophilic temperature in comparison to mesophilic temperatures.

6.6.5 CST

Dewaterability of the digestate (effluent) for the thermophilic digesters was also measured using capillary suction time (CST) The CST observed for the thermophilic digesters decreased from 889.3s to 614.6s in the 1st HRT and was found to decrease during the 2nd HRT to 1813.1s. However, by the third HRT, the CST values were lowered and were recorded as 174.5s. The lowest CST values were observed for the thermophilic digesters indicating better separation of solid and liquid fractions in the digestate at higher temperature.

6.6.6 COD

Samples were taken weekly from the digesters and analysed for total and soluble COD. The changes in SCOD concentrations within the digesters over the course of the trial are shown in Figure 43.

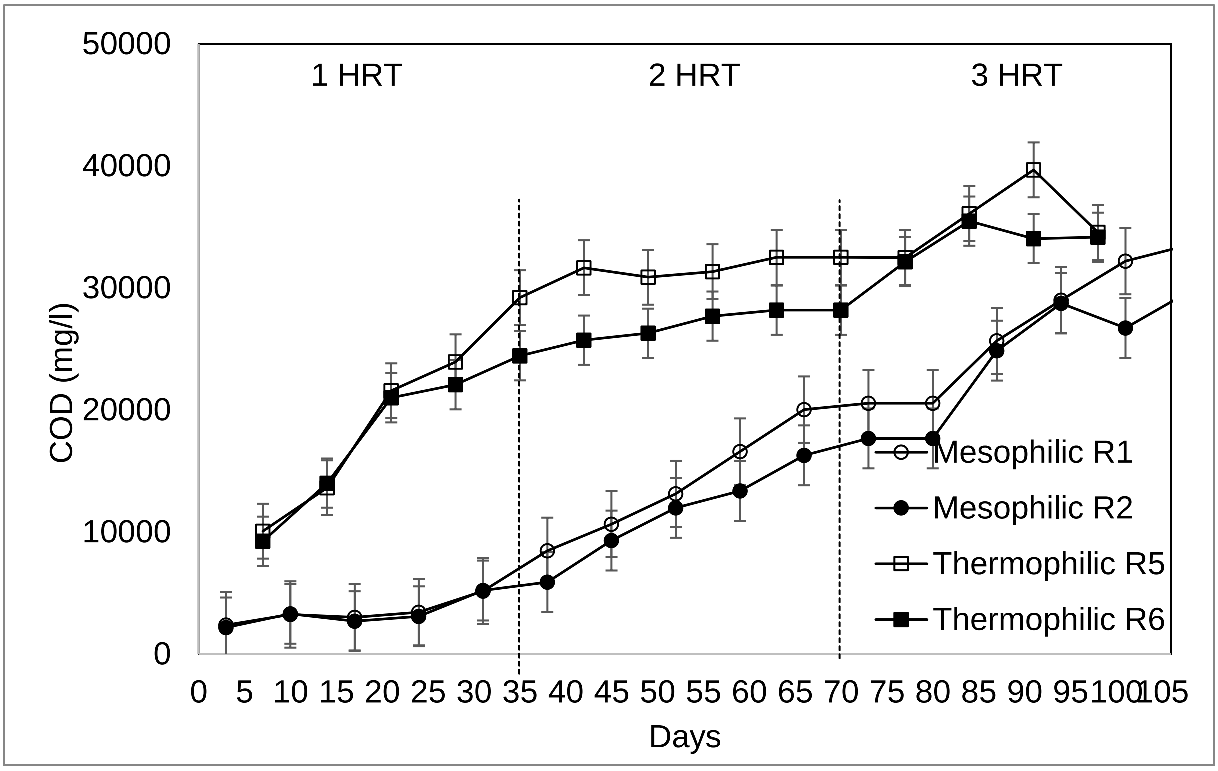

Figure 43 Soluble COD for thermophilic digesters

Soluble COD values were observed increasing for the thermophilic digesters from 1st HRT to beginning of 3rd HRT. However, towards the end of 3rd HRT, SCOD values are found to decrease. In comparison to mesophilic digesters it could be observed that the soluble COD was higher in thermophilic digesters.

6.6.7 VS destruction

Volatile solids destruction observed for thermophilic digesters are shown in Figure 44.

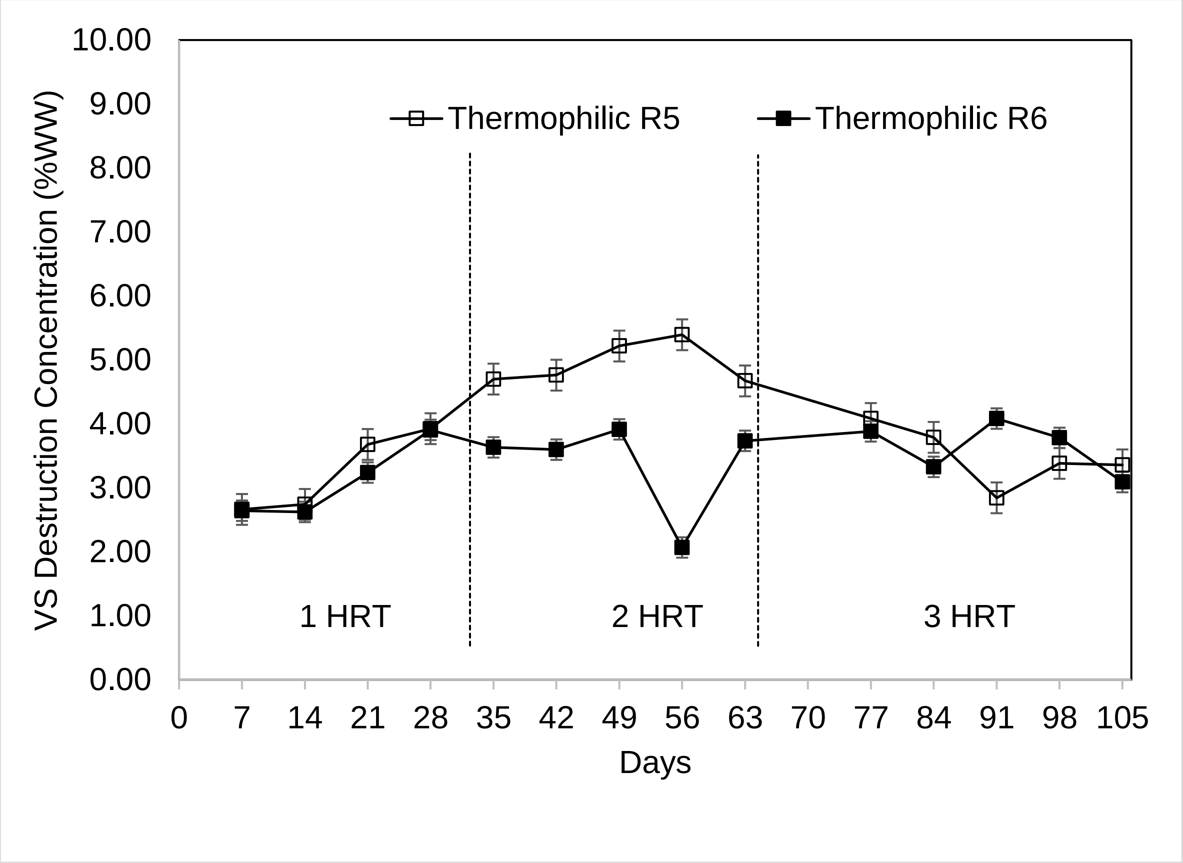

Figure 44 Volatile solids destruction for thermophilic digesters

As observed in the figure R5 and R6 digesters showed different trends in volatile destruction. Volatile solids concentrations were found to be lower in the thermophilic digesters than in mesophilic digesters.

In comparison to mesophilic digesters, the total solids, volatile solids and ash percentages were lower for thermophilic digesters. This could indicate that thermophilic digestion was not effective for macroalgae biomass with lower VS destruction resulting in lower gas production.

The initial and final values observed for the total, volatile and ash percentages are given in Table 35.

Table 35 Initial and final solids, ash, and moisture for thermophilic digesters

Digester	Total solids (%WW)		Volatile solids (%WW)		Ash (%WW)		Moisture (%WW)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
R5	4.63	7.63	2.66	3.36	1.98	4.27	95.37	92.37
R6	4.72	7.46	2.64	3.09	2.04	4.36	95.28	92.54

6.6.8 Gas production

Gas production observed for thermophilic digesters is shown in Figure 45.

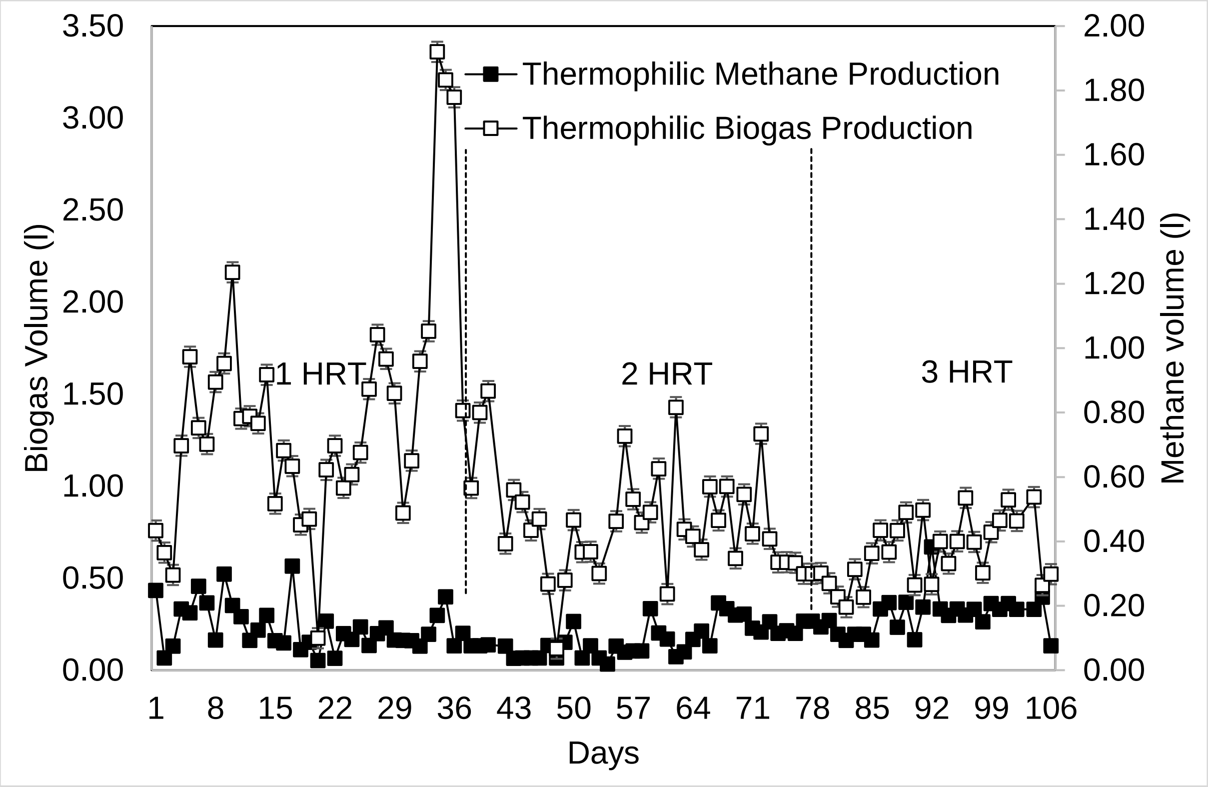

Figure 45 Gas production from thermophilic digesters

Biogas production was observed in digester R6 and bio-methane production in digester R5 by passing the gas produced through sodium hydroxide solution before it was collected in Tedlar bags. Biogas volumes were 0.51 – 2.22l/day in the first HRT. However, biogas production continuously decreased in the second and third HRT. The biogas volumes decreased to less than 0.35l/day in the third HRT. Methane concentrations in the analysed samples were noted very low in thermophilic digesters. The concentrations ranged from higher concentrations of 5.2% in the 1st HRT to continue decrease to less than <1% in the 3rd HRT. This could indicate the possibility of inhibition of methanogenesis at higher thermophilic digesters that resulted in the lower production of biogas production in both the digesters.

6.7 Discussion

As previously discussed in Section 2.3, there have been a few studies in the literature for the semi continuous digestion of macroalgae. Experimentation times are longer than the batch experiments to allow the system to acclimatise to the conditions and provide a more accurate indication of scaled AD processes under dynamic conditions.

The experiments included investigations on a high loading rate, for a longer retention time, and corresponding changes in gas yield, total solids, and volatile solids.

In this study, semi continuous digestions were performed for *S. Latissima* from Ventry Harbour based on the findings from batch BMP tests. The following sections will discuss the semi-continuous digestion performance of *S. Latissima* based on the following research question.

- To what extent are the differences in digestion performance of *S. Latissima* reflected in gas production, volatile solids destruction, and sludge characteristics?

Impact of higher temperatures and trace elements in macroalgae digestion was also investigated in this study in two ways by: -

- The addition of trace element solution under mesophilic conditions, and
- Using thermophilic temperatures

The following sections will discuss the findings of these semi-continuous digestion studies in. In terms of overall digester stability and performance, gas production and biodegradability.

6.7.1 Semi continuous digestion of *S. Latissima*

In this study, continuously stirred tank reactors (CSTRs) were used for the semi continuous digestion experiments. A higher organic loading rate of 56.6 gVS/l/d was applied to the digester daily for a total of 105 days this is a reduced experimental time compared to Troiano *et al.* (1976) at 330 days but higher than Hanssen *et al.* (1987) at 56 days. Hydraulic retention times (HRTs) are indicative of the time required by the bacterial community to break down the biomass and it cannot be too short or too long as the digester can wash out of the nutrients and the bacterial community could starve (McKennedy and Sherlock, 2015). This study recorded the digestion performance over 3 HRT which is considered sufficient to ensure acclimation and stable digestion conditions. Reactors R1 and R2 were the mesophilic digesters in this study.

6.7.1.1 Temperature

In our semi-continuous digestion trials, the mesophilic temperatures for both the digesters (R1 and R2) were found to be 36.35 ± 0.50°C. This is considered acceptable for mesophilic digestions which occurs between 35-42°C. Moderate fluctuations in temperature were observed in the mesophilic digesters during the first retention time. However, the fluctuations were not higher or lower than ±3°C. Mesophilic bacteria are reported to tolerate temperature differences of ±3°C without any significant reductions in methane production (Weiland, 2010). In addition, no operational issues were observed for the mesophilic digesters. The inoculum used in the study was sourced from a mesophilic source. Therefore, acclimation of the inoculum and the micro-organisms for the mesophilic digestion would have been faster and more favourable for higher methane production in these digesters.

6.7.1.2 pH, Alkalinity and VS destruction

In this study, the pH of the mesophilic systems was recorded in the range 7.1 – 7.4 and were continuously found stable throughout the 3HRT's. However, a lower value of 6.8-6.3 was observed in the 3rd HRT towards the end of the digestion. Methane formation as a result of methanogenesis proceeds in the pH range of 6.5 to 8.5, and in an optimum range between 7.0 to 8.0. The process will be severely inhibited if the pH decreases below 6.0 or rises above 8.5 (Weiland, 2010). In this study, in the early stages of digestion there is only a slight reduction of the pH, however none of the digesters exhibited pH below 5 which indicated that the digesters were stable throughout the longer retention time. The lower pH values towards the end of the 3rd HRT could have been due to the lowering of the buffering capacity of inoculum.

In terms of alkalinity, total, partial and intermediate alkalinity were calculated for mesophilic digesters. Total alkalinity concentrations were lower in the initial days of digestion in mesophilic digesters but increased as the digestion progressed. Alkalinity measures refers to the buffering capacity of the inoculum and it could be strongly influenced by the presence of the carbonate and bicarbonate salts, ammonia, phosphate, and volatile fatty acids. Total alkalinity measures the whole range of pH from higher values 8 to lower values of 4.3. However, partial alkalinity specifically corresponds to the pH of 5.7 which can be due to the presence of OH-, free ammonia, or carbonates and bicarbonates. The difference between the total and the partial alkalinity, known as intermediate alkalinity could be a better indicator for the presence of VFA in the digesters (Bolzonella, 2011). Alkalinity concentrations observed for the mesophilic digesters evaluated in this study consistently over three HRTs exhibited lower total alkalinity values than reported for *S. Latissima* in semi continuous studies. In a study by Ramirez (2015) who observed alkalinity values of 68-72 mg/l initially and increased concentrations of 12,570 mg/l towards the end of digestion for *S. Latissima* in semi continuous digestion and $NaOH$ was added to balance the alkalinity levels for digestion of the biomass (Ramírez, 2015). Intermediate alkalinity values were also observed higher in comparison to partial alkalinity in mesophilic digesters which could suggest that there was active VFA production in the mesophilic digesters aiding biogas production in the digesters.

In this study, for mesophilic digesters, pH values have been found in the neutral range in the first HRT and a slight increase over the second HRT followed by a decrease in the third HRT. The same pattern was observed for the alkalinity levels for the mesophilic digesters evaluated in this study. Usually, a drop in the pH is observed in the first retention time due to hydrolysis of organic material and the production of higher concentrations of volatile fatty acids. Volatile Fatty Acids is a key intermediate in the digestion process and are capable of inhibiting methanogenesis if produced in high concentrations. Acetic acid, propionic and butyric acid are the key VFAs that are formed during the acidogenesis stage. Propionic and butyric acid, even though present in smaller amounts can inhibit the methanogenesis in digesters and are associated with their un-dissociated form (Weiland, 2010). A concentration higher than 13mM of acetate and a concentration of 0.06mM – 0.17mM of iso butyrate and iso valeric have been found to indicate imbalance in AD systems (Horan *et al.*, 2011). However, in this study, the pH begins to rise in the digester as VFAs are consumed by methanogens and easily converted to methane. This is also evident in the gas productions for mesophilic digesters, as gas production was found higher in 1st HRT at 2.75 l/day to 2l/day in the 2nd HRT and gradually decreasing to around 0.2 – 0.15 l/day (3 HRT).

As organic material is degraded VS concentrations increases and which is utilised by the methanogens to produce methane. Volatile solids destruction is used to determine the extent of hydrolysis and solubilisation of biodegradable material available during AD (Ramírez, 2015). However, comparing the initial and final volatile solids concentration for mesophilic digesters it was found to increase from 2%WW in the first HRT to 4%WW in the third HRT indicating degradability of the biomass in the first HRT and gradually accumulating in the digesters towards the 3rd HRT. This could also mean that higher organic loading rates (3g/L used in the study) would have initially resulted in higher methane production in the first HRT however lead to accumulation in the digesters with lowered methane production towards the end of digestion.

6.7.1.3 COD, CST and Conductivity

Digesters were fed with the macroalgae biomass on a daily basis. Total COD values is an indication of the amount of organic matter available for methane production in the digesters. Total COD values were in the range of 35000 mg/l and soluble COD was in the range 16000 mg/l. Biodegradability is affected by the nature of the COD fractions and the COD availability within the biomass. COD can vary with the variations in the biochemical composition of *S. Latissima*. As discussed in the BMP of *S. Latissima*, the easily degradable carbohydrate fraction would have been easily converted to methane, however the inorganic content, and the other recalcitrant fractions such as phenolic content could have reduced the metabolic activity hence reducing the conversion of all available organic matter into methane but possibly into other gases. This is in agreement with other studies utilising *S. Latissima* for semi continuous studies inhibition due to ash, lipid and mineral content of the species resulted in accumulation and lower methane production and inhibition from H2S (Ramírez, 2015).

In our study, CST values were recorded for the mesophilic digesters. CST for mesophilic digesters were found higher (624s) during the initial HRT, however values were found lower during the end of digestion (347s). CST values provided an indication of the dewaterability of digestate digested in mesophilic methods. Generally, with relation to digestate, dewatering is required as it can reduce the subsequent treatment and therefore disposal costs of the overall operations. In addition, dewatered sludge is much easier to handle, however sludge dewatering remains most expensive and poorly understood wastewater treatment process. The lower CST values obtained in this study for the digestate can be an indicator for good dewaterability of the macroalgae digestate produced in mesophilic digestion which can offer better handling opportunities for either nutrient recovery options or storage and transport for macroalgae digestate having a positive impact on the overall economics of the digestion process.

Conductivity measurements were also recorded for mesophilic digesters. In this case, conductivity measurements were reported to be increasing from 2.4 to 4.8 mS/cm from 1st HRT to 3rd HRT. Conductivity values indicate the presence of metal ions in the digesters as electrical conductivity is the ability of a solution to conduct electrical current and it is directly proportional to the ion concentration. Studies in the literature have shown that conductivity measurements are affected by both VFA and bicarbonate concentrations. Conductivity measures the dissociated ions in the digesters occurring due to the degradation of the biomass alongside metallic ions in the biomass which are not digested however present in the digester increasing the measured values (Levlin, 2010). A strong correlation has been reported in the literature between electrical conductivity and bicarbonate measurements and methane concentrations in the start-up of the AD processes (Jimenez *et al.*, 2015). Therefore, increasing conductivity measurements together with increased alkalinity measurements indicate that dissociation of ions i.e. biodegradation of organic material was occurring in the mesophilic digesters. However, as no quantification of the VFAs or the metals in the digester was performed in the study, electrical conductivity measurements alone were not a good indicator for higher biodegradability in the digesters.

6.7.1.4 Gas production

Gas production over the 3 HRTs was measured in R1 and R2 respectively for mesophilic digestion. A solution of $NaOH$ (3M) was used as a gas scrubber for removal of carbon dioxide from the biogas. The digesters with the NaOH scrubbers were used to evaluate methane production. $NaOH$ scrubbing of the produced gas was only performed with R2. Therefore, R1 showed the total biogas production from the digestion while R2 demonstrated the methane production from the digestion of *S. Latissima*.

It was observed that there was a steady gas production during the first towards second HRT in mesophilic digesters. The maximum methane production occurred in the first HRT with a volume of 2.75l/day. Gas production has been higher during the first and second HRTs, however lower towards the third HRT. Gas production was reduced by 50% by Day 45, and continued to decrease until cessation towards the end of digestion (day 105). Methane production had started in the first week of digestion and continued till the end of digestion for both the digesters.

Methane percentages were found higher in the first HRT (50%) and lowering towards the end of digestion (10%). This could be due to the rapid conversion of the components of *S. Latissima* (Laminarin and mannitol) during the first days of digestion. As discussed in the characteristics of the biomass, methane yield is related to the level of storage sugars, ash and volatile solids in the macroalgae biomass (Hughes *et al.*, 2012). Easily degradable components in the biochemical composition of macroalgae such as laminarin, mannitol would have been easily converted to methane however the alginate, polyphenol and cellulose would have been recalcitrant towards digestion and become inhibitors for higher methane production as they are reported to be difficult to be digested by microorganisms (Jard *et al.*, 2012, Adams *et al.*, 2011, Gunaseelan, 1997, Black, 1950, Briand and Morand *et al.*, 1997). Inorganic content of various elements especially the K and Al concentrations in the biomass also could have resulted in the inhibition during semi continuous digestion of *S. Latissima* (Schiener *et al.*, 2015). In this study, higher methane percentages were observed in R2 which had

$NaOH$ scrubber, where the percentage of carbon dioxide is less than 1% continuously in all three HRTs. This showed that 3M $NaOH$ is effective as a scrubber to remove carbon dioxide from biogas.

6.7.2 Impact of trace element addition on digestion of *S. Latissima*

Brown algae biomass was found to be balanced for their C/N ratio aiding higher methane production during semi continuous digestion studies performed by Hinks *et al.* (2013). In this study, a trace element solution comprising of aluminium, boron, cobalt, copper, iron, manganese, nickel, zinc, molybdenum, selenium and tungsten prepared according to Suhartini (2014) was added to digesters R3 and R4 to study the impacts on digester performance. The digester characteristics in terms of pH, alkalinity, stability, gas production, and biodegradability are compared to mesophilic digestion within R1 and R2 discussed previously.

6.7.2.1 Temperature, pH, Alkalinity and VS destruction

In terms of temperature, similar to the mesophilic digesters, (R1 and R2) the temperature in the trace element added digesters were not found to fluctuate ± 3°C during the 3 hydraulic retention times confirming the functional stability of the digesters. In addition, no operational issues (gas or liquid leaks, failures) were reported during this trial. With respect to pH digesters R3 and R4 did exhibit an increase in pH towards alkaline values towards the end of second HRT till end of digestion in contrast to previous mesophilic trials (R1 and R2) which exhibited a decrease in pH.

In terms of volatile solids destruction, VS degradation was similar for the four digesters in the 1st HRT, however VS degradation started decreasing in the 2nd HRT whereas for the mesophilic digesters had higher VS degradation until the end of digestion. For digester R3 the total solids content increased from 3.99-5.81%WW and for digester R4 total solids increased from 4.50 – 7.34%WW. In comparison to R1 and R2, it appears that the total solids content was higher in R3 and R4. For the decrease in percentage of volatile solids disintegration observed for trace element added digesters, this could mean that the metal content in the trace element solution was forming sediments in the digesters which increased the total solids content in the digesters but not the volatile solids content to increase gas production as they are inorganic in nature. The presence of metals in the biomass such as higher arsenic content in the seaweed could also be increasing the solids content in the digesters. When sludge wastewaters have been used for digestion, it was found that arsenic, even after microbial transformation (i.e. methylation of arsenic) a fraction of arsenic still remained in the sludge as a stable fraction (Wang *et al.*, 2005). As brown algae is high in arsenic concentrations, even after microbial transformation a fraction of arsenic would have still remained in the sludge inside the digester increasing the solids content in the digesters. Also, in our study, we had not identified the trace elements in the inoculum which could have also had an interaction with the trace element added reactors.

6.7.2.2 CST, COD and conductivity

Digesters with trace element addition consistently had increasing conductivity values from 1st HRT to third HRT. This was expected as conductivity measures the ionic concentrations in the digester and due to the presence of various metals in the added trace element solution, it could be assumed that the added elements were also forming their carbonates increasing the conductivity measurements.

In terms of CST, in contrast to R1 and R2 values increased from 1st HRT to 3rd HRT. This could also be due to the presence of the metals in the trace element solution requiring more time to settle and separate from the liquid fraction of the digestate.

With respect to COD, values recorded for the digesters with trace element addition were very low in comparison to R1 and R2. The SCOD values were similar for all 4 digesters in the first HRT, however continuously decreasing for the digesters in the second and third HRT. This could suggest that trace element addition was causing nutrient overload in the digesters preventing the degradation of the organic matter by microorganisms. It could also be suspected that some of the elements added were toxic for the stability of the digesters.

6.7.2.3 Gas production

In terms of gas production, the gas production has been consistent for mesophilic digesters however biogas production for the digesters R3 and R4 had variations of ±5 l/day. Gas production started decreasing from day 70 until the end of digestion. Observing the gas composition data, methane percentages were higher in all 4 digesters but comparatively higher in R3 and R4. The gas volumes were significantly higher for R3 and R4 during the second HRT when compared to R1 and R2. $NaOH$ scrubber has also lowered the percentage of carbon dioxide to less than 1% in the trace element added digester R4 as well. Overall, the percentage of increase in the gas production is not enhanced by the addition of trace elements. However, percentage of methane was higher in the trace element added digesters. This could mean that trace element addition can improve the quality of the biogas produced from *S. Latissima*. Therefore, it could be summarised that contrary to the enhancement of digestion with higher gas yields and better biodegradability, in the case of *S. Latissima* macroalgae biomass, addition of trace element solution only enhanced the methane concentrations in the biogas production.

Depending on the substrate digested and digester type and digestion procedure, trace elements requirements also are varied (Demirel and Scherer, 2011). In the case of micro nutrients, a balanced composition of metal ions are also essential for optimising the digester performance (Alaswad *et al.*, 2015). However, macroalgae biomass also contains high concentrations of alkali and alkaline earth metals. *S. Latissima* is rich in nutrients such as Na, K, Ca, Mg, P and Si along with trace elements such as Fe, Zn, Mn, Al, and Cu. When the biomass is digested these elements in the biomass present in the aqueous digestate act as a nutrient medium for the microorganisms for the digesters (Anastasakis and Ross, 2011). Since macroalgae biomass already has higher metal concentrations, it is important to understand which trace element can enhance the gas production as presence of one metal can be toxic for another as it can allow formations of inhibitory compounds.

Trace element solution addition has shown to enhance anaerobic digestion of different substrates including food waste (Banks *et al.*, 2012), agricultural feedstock such as maize silage (Pobeheim *et al.*, 2010) and microalgae (Yen and Brune, 2007). The trace element solution used in our study was made as per Suhartini, (2014). The trace element solution has been used for studies with food waste, sugar beet residues etc. Structurally, macroalgae biomass has polysaccharides proteins on their cell walls (carboxyl, sulphate and phosphate groups) which are excellent binding sites for metal retention, hence they are used as biosorbents for removal of excessive heavy metals from wastewaters or the environment they grow. Elements such as arsenic, cadmium and lead can be present in macroalgae due to contamination and elements such as copper, chromium, molybdenum, nickel and selenium are retained by the cell walls. Hence trace element addition should enhance digestion of the biomass and the undigested cell walls retaining the nutrients can increase the nutrient value of the digestate as fertiliser (De La Rocha *et al.*, 2009). However, in this study, we haven't analysed the inhibitory effect of individual trace elements on gas production. Therefore, use of advanced molecular biology techniques would be required to understand the response of methanogenic archaea to trace element concentrations in the digester to actually determine the demand of methanogens classes for these micro-nutrients (Demirel and Scherer, 2011).

This study has used combinations of cobalt, nickel, and iron, with molybdenum, tungsten and selenium. Combinations of several trace elements can have either synergistic or antagonistic effects. Combination of Ni and Co have shown to produce more acetate when compared to being added separately. Molybdenum has been shown to enhance digester performance only in combination with cobalt and nickel. Furthermore, metals such as iron, cobalt and nickel are the most widely studied trace elements whereas research on selenium and tungsten are scarce (Feng *et al.*, 2010).

In summary, this study, while providing a useful insight into the impacts of specific trace elements on digester performance and biogas production from macroalgae, has also highlighted that further work is required to ascertain the interrelationship between specific elements and their impact on the microorganisms aiding AD processes. Given the findings here, together with the review of literature it would be recommended that Ni, Co, Cu, Mo, Se, W and heavy metals may be of interest to explore due to both their biosorbent potential in the macroalgae biomass and added benefits to the nutritional value for the digestate as fertiliser.

6.7.3 Impact of thermophilic temperatures on digestion of *S. Latissima*

In this study, thermophilic temperatures were applied to evaluate the impact of higher temperature on digester performance and biogas production. As observed in the results, digester R5 and R6 were different in their digester characteristics. The digester performance and biogas production were compared to those observed for mesophilic digesters.

6.7.3.1 Temperature, pH, Alkalinity and VS destruction

In terms of temperature variability, the mesophilic digesters were relatively stable around ±3°C. Variability was much greater for the thermophilic digesters where temperatures recorded in R5 were >±5°C and for R6 were stable around < ±3°C. The fluctuations were more prevalent in the first HRT however more stable in the second HRT for both the digesters. The temperature fluctuations were not observed in the second HRT and some fluctuations (lower than ±3°C) were observed in the third HRT for thermophilic digesters. This may be attributed to operational defect associated with maintenance of thermophilic temperatures. The heating coils used for maintaining the temperature may have been effective for mesophilic range but not very effective for higher temperature ranges.

In terms of pH, variations were observed in both the digesters. The digesters started off in a neutral pH of 7.01 (R5) and 7.07 (R6) respectively. As digestion progressed, a reduction in pH was observed in both the digesters from day 5 till day 25, around 6. A change in pH was observed in R6 towards the end of 1st HRT. From day 35, digester R6 showed an increase in pH values to 7.5 and continued until the 80 days. A reduction in pH was again noted from day 87 until the end of digestion. In the case of digester R5, a reduction in pH continued until the end of digestion with a final reduced pH value of 5.55. In terms of alkalinity, thermophilic digesters had lower alkalinity values than mesophilic digesters. This was noticed for partial, intermediate and total alkalinity concentrations in both the thermophilic digesters. However similar to mesophilic digesters, intermediate alkalinity was higher than partial alkalinity indicating VFA production in the digesters.

Accumulation of VFAs in the digester is characterised by lower pH values. The drop in pH for thermophilic digestion was also noticed by Vanegas and Bartlett (2013) for *Laminaria digitata* species. The pH imbalance was attributed to the accumulation of VFAs due to the high hydrolysis rates during the initial rates of AD. The pH imbalance is shown to affect the activity of methane producing bacteria and ultimately the collapse of the digester (Vanegas and Bartlett, 2013). In this study, digester R5 started off with neutral pH however reduction of pH which continued from the end of the first HRT till the end of digestion indicate higher hydrolysis rate in the digester leading to VFA accumulation and finally failure of the system. Digester R6 seem to regain the buffering capacity by converting the accumulated VFAs to methane towards the end of first HRT to improve the digestion performance of the system. This could be linked to the buffering capacity of the inoculum stabilised by the buffering capacity of the feedstock (Weiland, 2010).

In terms of volatile solids degradation, concentrations for thermophilic digesters were higher than mesophilic digesters. This shows that for macroalgae feedstock, thermophilic digestion aided in a greater degree of solids destruction compared to its mesophilic counterpart. This is in agreement with literature as temperature increases, the microbial growth rate also increases, leading to higher solids destruction by microorganisms during the AD processes (Huang *et al.*, 2011).

6.7.3.2 COD, CST and conductivity

In terms of digestate dewaterability, CST values were found lower than the mesophilic digesters indicating a higher dewaterability of digestate produced in the thermophilic digesters. *S. Latissima* fed daily into the digester had almost 70-80% moisture (water content) in the biomass. At higher temperatures, the water of hydration and vicinal water attached to the biomass could potentially be reduced faster than at lower temperatures hence decreasing the floc formation by the degraded biomass in the digester. Thus, digestate can separate into liquid and solid fractions faster reflected in lower CST values (Yin *et al.*, 2004). Thus, digestate can separate into liquid and solid fractions quicker reflecting in lower CST values. The viscosity of thermophilic digestate was also found lower in comparison to mesophilic conditions. This is in agreement with literature where digestate formed from *S. Latissima* digestion under thermophilic conditions had decreasing viscosities with higher loading rates which enhanced mixing properties in conventional AD systems (Ometto *et al.*, 2017). Conductivity measurements were also recorded for thermophilic digesters. In comparison to mesophilic digesters, conductivity was higher in thermophilic digesters. As indicated by the drops in pH, higher conductivity could also be an indication of high concentrations of VFAs in the thermophilic digesters.

6.7.3.3 Gas production

Gas production obtained in this study were compared for thermophilic and mesophilic digesters respectively. The cumulative volumes of biogas and methane percentages are compared for both mesophilic and thermophilic digesters. Digester R5 had a $NaOH$ scrubber thus recording methane production and R6 recording biogas production. Gas productions were higher in the first HRT however decreased sharply from the second HRT till the end of digestion. From the overall gas production results, it could be seen that thermophilic digesters produced lower volumes (almost one third volume) of biogas than produced by the mesophilic digesters for *S. Latissima*. The methane concentrations were also very low in thermophilic digesters (almost one tenth) compared to methane concentrations observed for mesophilic digesters. The higher biogas production for thermophilic digesters, observed during the first HRT could be attributed to the high hydrolysis rates at the start of the digestion with neutral pH however as VFA's accumulate (indicated by a lowering of pH) in second and third HRTs, gas volumes also decreased simultaneously. This is also in agreement with other studies in literature by Hansson *et al.* (1983) for green macroalgae Ulva and

Vanegas and Bartlett (2013) for brown algae *Laminaria digitata*. These studies suggested that different microbial communities were developed in the mesophilic and thermophilic reactors as these microorganisms being complex in their characteristics exhibited diversity and different levels of activity under different temperatures (Hansson, 1983, Vanegas and Bartlett, 2013). Hence, it is clear that the choice of temperature for AD operation is very important and from the results observed in this study, thermophilic temperatures would not be recommended for scaled up digestion operations for *S. Latissima*. For the digester R5, that included $NaOH$ scrubbers, the percentage of carbon dioxide was found to be less than 1%. This showed that 3M NaOH is effective as a scrubber to remove carbon dioxide from biogas in both mesophilic and thermophilic digesters.

In summary, thermophilic digesters in this study have shown less stability than mesophilic digesters with lower overall gas production. In general, thermophilic digestion is preferred over mesophilic digestion as it is reported to result in higher gas production and improved solids destruction. This is because growth of methanogenic bacteria is higher at thermophilic temperatures and thus increases rate and efficiency (Ge *et al.*, 2011). This is because growth of methanogenic bacteria is higher at thermophilic temperatures and thus making the process faster and more efficient. In addition, the effect of temperature on the hydrolysis step is very important as thermophilic digesters provide five or six times higher efficiency in hydrolysing the biomass when compared to mesophilic systems (Bouallagui *et al.*, 2005). However, the duration of the start-up phase or the acclimation stage in the first HRT is influenced, to some extent, by the seed inoculum which also plays an important role in biogas production rate during hydrolysis. In this study, the inoculum was obtained from a mesophilic source and no incubation at thermophilic temperatures was performed prior to inoculating the thermophilic digesters. This could have been one of the factors impacting the activity of the microbial community at higher temperatures resulting lower biogas production. VFA accumulation as indicated by lowered pH values in this study could have been because of the higher loading rate of 3gVS/l/day. This also could have resulted in lower gas production because at thermophilic temperatures VFA degradation tendency is to firstly convert acetate and butyrate and later convert propionate to methane. Literature regarding the kinetics of semi continuous studies have observed that if the concentrations were concentrations of propionate were higher than 400mg/l, inhibitions occurred and when levels were 800 mg/l, gas production completely stopped (Kim and H. Hyun, 2004). With lower gas production, instability and higher energy requirements thermophilic digestion, cannot be recommended for large scale AD applications utilising *S. Latissima*. Therefore, further work is required to ascertain the optimal balance of nutrients in the digester for stable AD processes such as addition of buffering agents like $NaOH$, or trace elements or co-digesting macroalgae biomass with other organic feedstock. Results for co-digestion of *S. Latissima* with different organic feedstock will be discussed in the next chapter.

7 Results – *S. Latissima* as a co-digestion feedstock

While co-digestion using macroalgae biomass is a developing area of research there remains limited studies on the Kelp species, *S. Latissima*. Co-digestion has been suggested as an approach to optimise the C/N ratio and thereby enhance methane production and digestibility of the composite feedstock. Therefore, in this study, the feasibility of utilising traditional AD feedstock of agricultural feedstock and animal manure and brewery spent grain were assessed in combination with macroalgae *S. Latissima*. This chapter will describe the results for the digestion of organic feedstock selected for this study – i.e. agricultural crop residues, pig manure and brewery spent grain and the effect of co-digesting these organic feedstock with macroalgae biomass. Previously in this study, *S. Latissima* was assessed for its methane potential and the impact of location, harvest times and growth type on methane production. It was observed that methane potential varied with location, season and growth type. Therefore, in this chapter the sample with the highest methane potential from the BMP tests (Chapter 4 Section 4.1) was chosen for this co-digestion feasibility assessment. This was the biomass from Ventry Harbour obtained in summer 2016. The co-digestion feasibility study was carried out using biochemical methane potential batch tests (reference methods). The specific methane production and C/N ratio of the feedstock selected was used to justify the choice of feedstock for subsequent co-digestion with *S. Latissima*. The study will evaluate the performance of *S. Latissima* as a co-digestion feedstock and can inform the best way of utilising seasonal feedstock of macroalgae biomass. The results would also better inform waste management practices for feedstock such as crop wastes, manures, and brewery wastes like spent grain. The results observed from the characterisation of the feedstock, and their biochemical methane potential tests will be discussed in the following sections.

The samples were characterised for solids (Total and Volatile), ash, and moisture content. Samples were also analysed for their elemental composition.

7.1 Sample collection and location

The organic feedstock selected for this study were from three main sources.

- Agricultural crop waste residues -wheat, maize, grass, sugar beet – vegetable mix (SBV mix)
- Manure -pelletised pig manure
- Brewery waste - brewery spent grain (BSG)

This is described more fully in the methodology chapter 3 section 3.1.2.

S. Latissima obtained from Ventry Harbour was selected for the assessment of co-digestion potential of macroalgae biomass as this biomass showed the highest biochemical methane potential during the BMP tests.

7.2 Organic Feedstock characterisation

The organic feedstock was tested on arrival at BCU prior to sample preservation. Samples were analysed for total and volatile solids, ash and moisture content. The feedstock was characterised using standard methods (as described in Methods Chapter Section 5) and all the results were tested for their significance using ANOVA tests.

7.2.1 Total, volatile solids and ash

As described previously these parameters are important when considering a feedstock for AD. With TS providing an indication of the overall solids and moisture content and VS providing an indication of the readily biodegradable fraction of the material. Ash content is an indication of the inorganic content of the feedstock.

The organic feedstock characteristics are presented in Table 36 and Figure 46.

Table 36 Characteristics of organic feedstock used in this study

Feedstock	Moisture (%WW)	Total solids (%WW)	Volatile Solids (%WW)	Volatile solids (%TS)	Ash (%WW)
Wheat	13.6	86.4	84.6	98.0	1.8
Maize	65.9	34.7	32.9	94.9	1.8
Grass	62.0	38.1	34.1	89.5	4.0
SBV Mix	74.1	25.9	22.5	87.0	3.4
Pig manure	10.0	90.0	58.8	65.3	31.3
BSG	74.1	25.9	25.1	96.9	0.8
S. Latissima	84.9	15.1	10.6	70.3	4.5

As shown the table above and figure below, of the feedstock tested, pig manure demonstrated the highest total solids content at 90%WW followed by wheat at 86%. Sugar beet - vegetable mix exhibited the lowest total solids content at 22%. However, the highest volatile solids concentration was found in the sample of wheat at 84%, followed by pig manure, grass, maize, BSG and finally SBV mix at (58%<34%<32%<25%<22% respectively). Pig manure exhibited the highest ash content at 31% and BSG exhibited the lowest ash content at 0.8%. Ash content is an important factor to be considered for AD as it can potentially impose inhibitions on the biochemical processes limiting biogas production. It can be seen that the organic feedstock selected generally exhibited lower moisture contents than *S. Latissima* and significantly higher VS content.

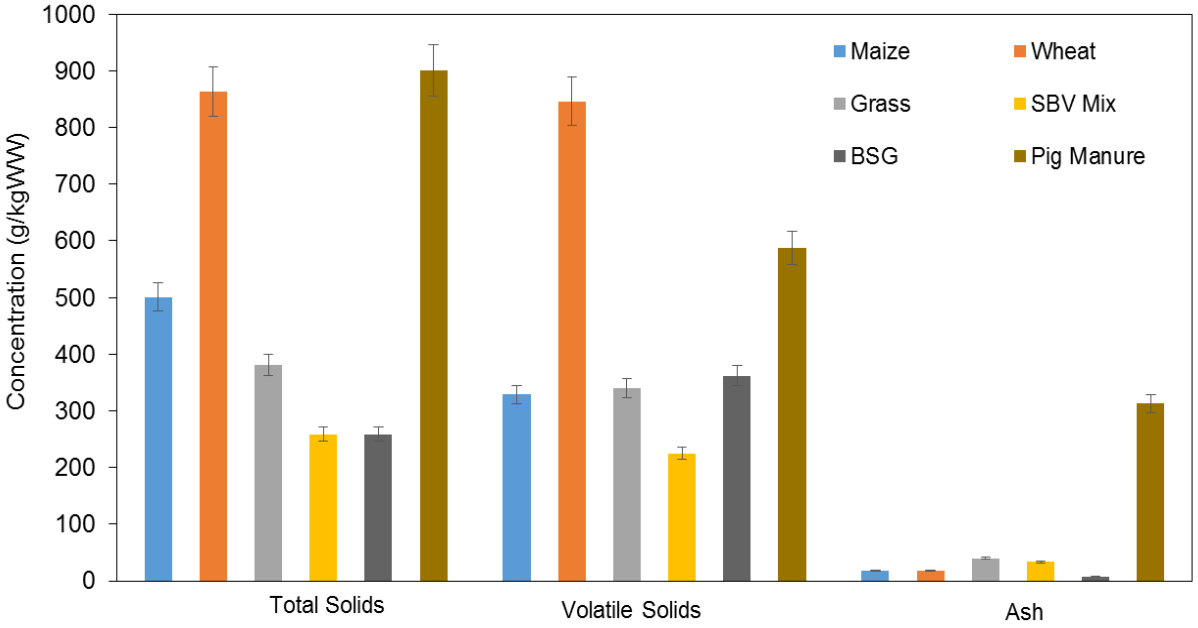

Figure 46 Characteristics of organic feedstock used in this study

7.2.2 Elemental composition

The elemental composition of the organic feedstock was also analysed and the results are given in Table 37.

Table 37 Elemental composition of organic feedstock used in this study

Feedstock	C	H	O	N	S	Other
Wheat	40	5	45	2	0.14	7.86
Maize	43	5	40	2	0.07	9.93
Grass	42	5	38	1	0.13	13.87
SBV mix	35	4	40	1	0.07	19.93
BSG	35	4	39	1	0.2	20.80
Pig manure	29	2	27	3	1	38.00

From the table it can be seen that, as expected the carbon and oxygen were the predominant elements present in all feedstock followed by hydrogen, nitrogen, sulphur and then trace elements. In terms of their suitability as a feedstock for AD carbon and nitrogen content are important elements as they are the main or macro nutrients present in the biomass forming the plant tissues, cells and cell membranes. It can be seen that, the crop residues (wheat, maize and grass) had relatively higher carbon content in the order maize, grass, wheat (43%>42%>40% respectively).

The carbon content of the sugar beet-veg mix and brewery spent grain were similar (at 35%). Of the samples tested, pig manure had the lowest carbon content at 29%. With regards to nitrogen content there was little difference between the samples tested with the percentages in the overall biomass ranging between 1 and 2%. It was noted that pig manure had a slightly higher N content than the other samples tested. With regards to Sulphur content, pig manure had the highest percentage among all of the feedstock while the others having less than 1%. If we take the sum % of the major elements and assume that the remaining elements are ‘trace’ and/or ‘other’ (these were not analysed) it can be seen that wheat had the lowest % trace elements. For SBV, BSG and Pig manure it can be seen that there remains a high % of other/ trace elements making up the overall composition of the feedstock.

As described previously (Section 1.8.9) the C/N ratio of a feedstock is an important consideration for AD as it ensures balance within the digester and prevents over accumulation of volatile compounds or inhibition from ammonia. The C/N ratio of the feedstock used in this trial are shown in Figure 47.

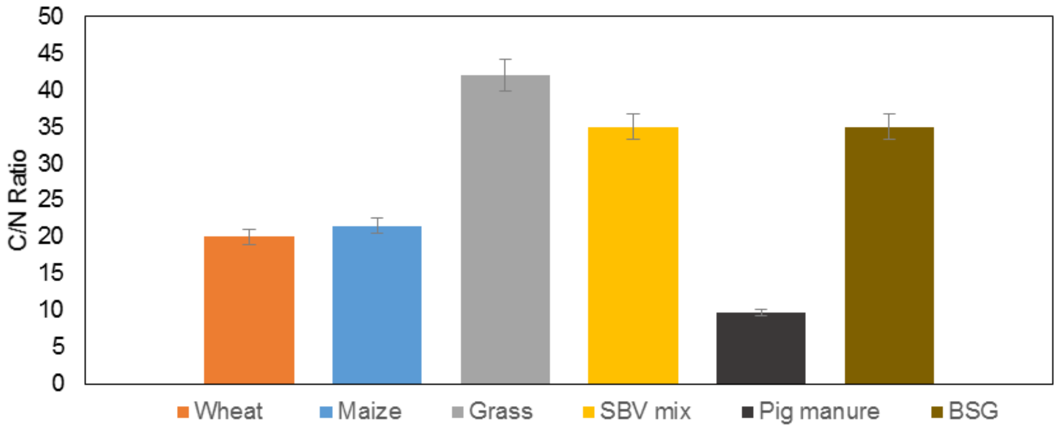

Figure 47 Carbon to nitrogen ratio of organic feedstock used in this study

The carbon to nitrogen ratio was found to be higher in the agricultural crop based residues than in pig manure. Grass exhibited the highest C/N ratio of 42 followed by the SBV mix, BSG, maize, and wheat (35, 35, 22, 20, 10 respectively).

It is important to have a good understanding of the nature and composition of different feedstock to ensure the correct balance of TS/VS, C/N and trace elements is achieved through co-digestion The characteristics of the feedstock will influence the rate and yield of methane production.

7.2.3 Bio Methane Potential (BMP) of organic feedstock in Isolation.

As described previously (Chapter 1, Section 1.10) this test provides a range of data which can be used to evaluate the biomethane production potential of a feedstock under carefully controlled and optimised conditions. The test can provide an insight into the dynamics of biogas production over time (in this case 30 days of the test or until methane production has plateaued). The specific methane potentials for the organic feedstock tested can be seen in Figure 48 below.

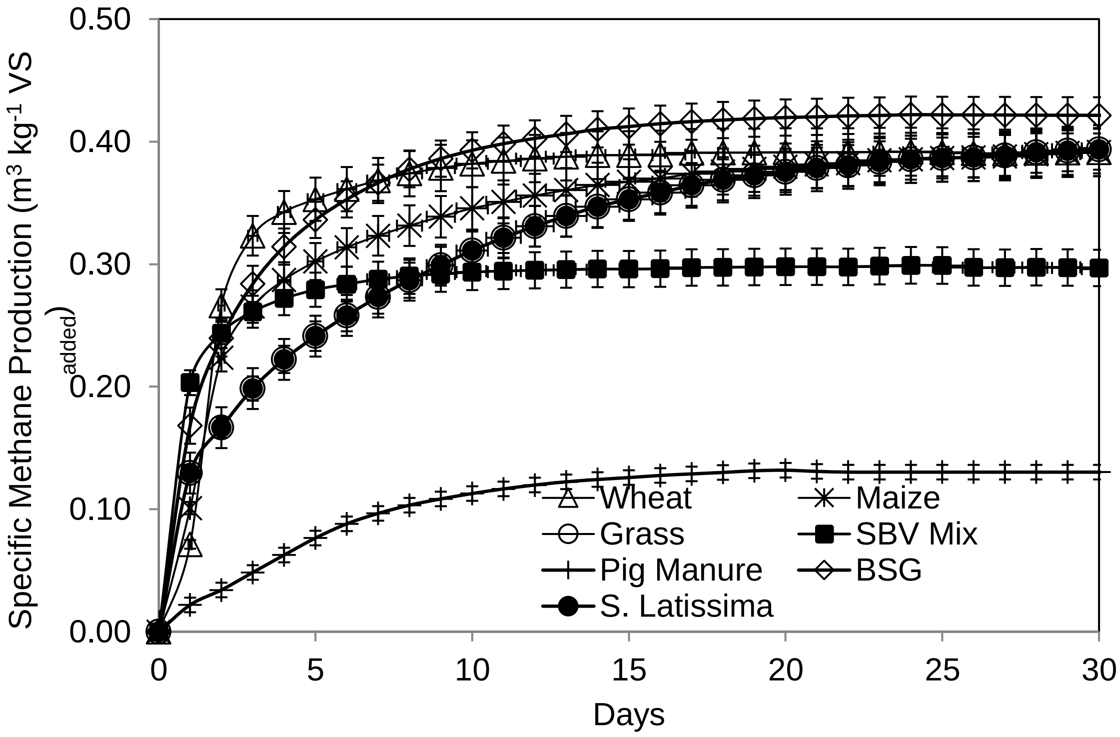

Figure 48 Specific methane production of organic feedstock

Firstly, it can be seen that all of the samples tested exhibited a typical trend in terms of biogas production with exponential methane production during the first 3-5 days of the BMP test and all samples reached plateau by the end of the test.

However, it is interesting to note that there are some differences in the trends between the feedstock tested. For example, it can be seen that SBV mix and BSG demonstrated a sharp increase in the first 1-2 days. Thereafter SBV mix plateaus quickly at day 3 while BSG plateaus at day 5. In contrast pig manure exhibited a much slower rate of production and overall had the lowest methane production among the tested feedstock. This trend is demonstrated more clearly in the daily methane production for the first 10 days of the trial. The rate of methane production for the organic feedstock is shown in Figure 49.

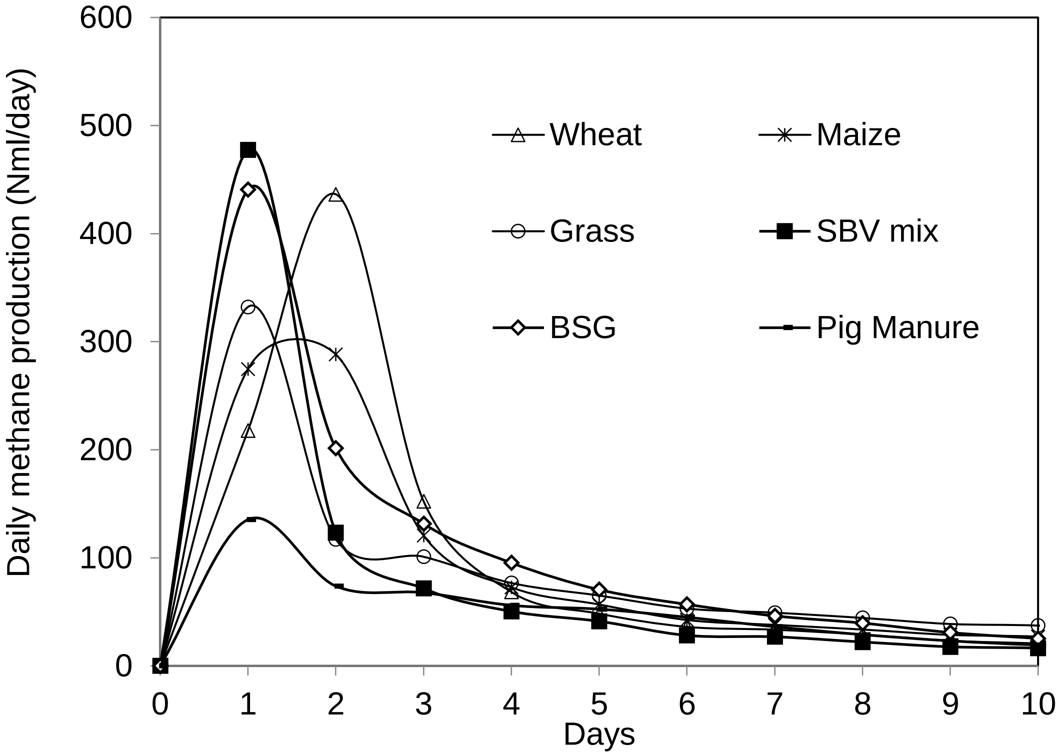

Figure 49 Rate of methane production from organic feedstock

From the figure it is clear that all feedstock tested reach maximum methane production in the first 3-5 days. However, it was noted that SBVmix and BSG exhibit the maximum methane production in day 1 of the test. Wheat and Maize appear to reach maximum production later at day 2-3. It can also be seen that Pig Manure has the lowest methane production within the first 3 days.

As described previously the SMP is a measure of the cumulative methane production of a feedstock based on optimised conditions.

The specific methane production for the organic feedstock tested and corresponding C/N ratios is given in Table 38.

Table 38 Specific methane production and C/N ratio for organic feedstock

Feedstock	Specific methane production ($L\ CH_4/\ kgVS_{added}$)	Std dev	C/N
Wheat	0.393	± 0.037	20
Maize	0.391	± 0.013	22
Grass	0.395	± 0.011	42
SBV mix	0.292	± 0.037	35
Pig Manure	0.130	± 0.007	10
BSG	0.456	± 0.004	35

It can be seen from the table above that the SMP for Wheat, Maize and Grass were very similar ($0.391 - 0.396 l\ CH_4\ /kg\ VSadded$). Of the Feedstock tested BSG exhibited the highest overall SMP at $0.456\ CH4\ /kg\ VSadded$. Pig Manure had the lowest SMP of all the feedstock tested at $0.130\ CH4\ /kg\ VSadded$. It is clear that the rate of and yield of methane production within the first 5 days has a significant impact on the overall methane yield. It can be seen that for Wheat, Maize and Grass while they have a similar SMP their C/N is significantly different. For pig manure it can be seen it had the lowest SMP and also lowest C/N. It can be seen that SBV mix and BSG have the same C/N ratios however demonstrate significantly different SMPs.

Having gained a clear picture of the characteristics and BMP of a variety of organic feedstock the next phase of investigation involved an assessment of the impact of *S. Latissima* addition (as a co-digestion feedstock) on biogas production (yield and rate).

7.2.4 BMP of macroalgae biomass as a co-digestion feedstock

For this trial the organic feedstock was mixed in a 70:30 (wet weight) ratio (whereby the organic feedstock made up 70%WW and the macroalgae biomass made up 30%WW). All of the feedstock were milled (to <1mm) and fed into the digester bottles. The inoculum for the BMP tests was sourced from Severn Trent WWTP, Minworth. The method was in accordance with that described in the methodology chapter section 3.1.3. For this trial the inoculum had a volatile solids concentration of 20.283 g VS/kg WW. Cellulose was used as the positive standard.

The rate of methane production for the organic feedstock co-digested with *S. Latissima* is given in the figure below.

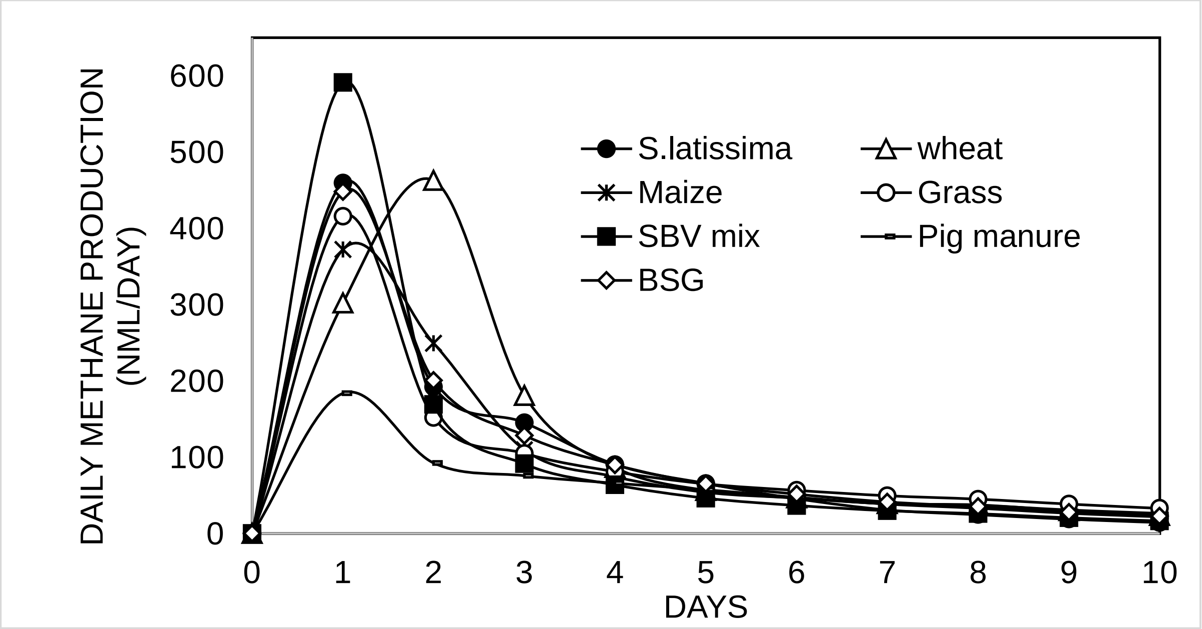

Figure 50 Rate of methane production in co-digestion

As shown in the figure above, it was found that the trends in biomethane production were similar during co-digestion as for mono digestion. In terms of the rate of production across the first 5 days it can be seen that all feedstock tested reach maximum methane production in the first 3-5 days. However, it was noted that for SBVmix+*S. Latissima* biogas production rate increased from 477ml/day to 591ml/day. For BSG+*S. Latissima* it was observed that biogas production rate at day 1 remained relatively similar to mono digestion at 440ml/day while in co-digestion production was observed at 448 ml/day. Wheat and Maize co-digested with *S. Latissima* appear to reach maximum production later at day 2 – 3 as in mono-digestion conditions although it can be observed that the production rate declines faster for Maize under co-digestion conditions. It can also be seen that Pig Manure + *S. Latissima* has the lowest methane production within the first 3 days.

The rate of methane production for the feedstock in isolation and in co-digestion with *S. Latissima* are compared in Table 39.

Table 39 Comparison of rate of methane production for monodigestion and co-digestion

Feedstock	Monodigestion					Co-digestion				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
S. Latissima	459.6	192.5	145.4	90.5	65.7	-	-	-	-	-
Wheat	217.8	436.4	152.4	68.4	48.3	302.1	462.8	181.0	86.7	56.5
Maize	274.8	288.3	120.6	72.8	56.8	372.2	249.4	109.8	74.5	54.1
Grass	332.1	117.2	101.0	76.7	65.1	415.9	152.1	105.1	81.1	65.1
SBV mix	477.3	123.3	71.8	50.4	41.2	591.0	169.0	91.4	63.8	46.4
BSG	440.6	201.4	131.6	95.3	70.5	448.2	200.7	128.5	90.0	65.3
Pig Manure	135.3	73.8	67.7	56.0	52.5	183.8	92.5	75.8	66.0	57.4

From the table above, results showed that addition of macroalgae resulted in both increased as well as decreased methane production over the period of 30 days. Therefore, it becomes essential to calculate the percentage of increase or decrease in specific methane production of each feedstock.

The percentage increase or decrease in measured specific methane production is calculated as per the equation below.

$$\frac{(\boldsymbol{SMP\ in\ codigestion - SMP\ in\ monodigestion})}{(\boldsymbol{SMP\ in\ monodigestion})} * \mathbf{100}$$

Equation 8: Calculation for percentage increase or decrease in SMP

The specific methane production of various organic feedstock co-digested with *S. Latissima* and the percentage increase for the measured SMP values are given in Table 40.

Table 40 Percentage increase for specific methane production in co-digestion

	Specific Methane Production (SMP) as L CH$_4$/kgVS					
Feedstock	**Wheat**	**Maize**	**Grass**	**SBV mix**	**Pig Manure**	**BSG**
Mono- Digestion	0.393	0.391	0.395	0.292	0.130	0.421
Co-Digestion	0.472	0.397	0.395	0.373	0.172	0.404
% difference	21.9	1.01	0	27.7	32.3	-4.03

*SMP of *S. Latissima* in isolation $0.391\ L\ CH_4/\ kgVS\ added$

It can be seen from the table above that with the exception of BSG and Grass the SMP increased as a result of co-digesting *S. Latissima* with the organic feedstock. The highest % increase was observed for pig manure where a 32.3% increase was observed.

Although the results of co-digestion suggest an increase in measured biogas yield the feedstock were mixed at a ratio of 70:30. According to Labatut *et al.* (2011) the increase or decrease in methane production as a result of co-digestion can be calculated by determining the difference between the measured SMP of the co-digested feedstock and the estimated SMP for co-digestion based on the ratios of mixed feedstock (Labatut *et al.*, 2011). In this study, this calculation will only consider the specific methane production in co-digestion and will not be considering the biodegradability of the co-digested feedstock as to observe whether the co-digestion improved the digestion of either of the feedstock.

The estimated SMP for the co-digestion is calculated using the formula below.

$$(\boldsymbol{0.7 * SMP\ organic\ feedstock\ in\ monodigestion + 0.3 * SMP\ macroalgae\ biomass\ in\ monodigestion})$$

Equation 9: Calculation of the estimated SMP for co-digestion

From this calculation, the net percentage increase and decrease was calculated as follows.

$$\boldsymbol{\frac{(Measured\ SMP\ in\ codigestion - Estimated\ SMP\ in\ codigestion)}{(Estimated\ SMP\ in\ codigestion)} * 100}$$

Equation 10: Calculation of net percentage increase or decrease in SMP

These calculation was applied to the data and the results for net percentage increase or decrease in co-digestion are presented in Table 41.

Table 41 Net percentage increase or decrease for codigestion

Feedstock	Measured SMP on co-digestion ($L\ CH_4/kgVS$)	Estimated SMP (based on 30:70 ratio) ($L\ CH_4/kgVS$)	Net Percentage increase or decrease (%)
S. Latissima	-	-	-
Wheat	0.472	0.388	21.59
Maize	0.397	0.392	1.17
Grass	0.395	0.394	0.30
SBV mix	0.373	0.322	15.95
Pig Manure	0.172	0.208	-17.43
Brewery spent grain	0.404	0.412	-1.94

From the table, it can be seen that *S. Latissima* when added as a co-digestion feedstock to Wheat resulted in the greatest percentage increase in methane yield 21.59% based on estimated yields. In contrast the co digestion of *S. Latissima* with pig manure resulted in a decrease of 17% in methane based on estimated yields.

7.2.5 Carbon to nitrogen ratio optimisation.

As described previously, co-digestion is commonly adopted by operators to optimise the C/N ratio within an AD plant. This is particularly important when considering feedstock such as agricultural crop wastes as they can have high carbon content in their structure leading to an imbalance in the digester with accumulation of volatile solids or in manures with a high nitrogen content where ammonia inhibition can lead to digester imbalance.

The carbon to nitrogen ratio of a feedstock in monodigestion can be calculated as the equation below:

$$\frac{C}{N} = \frac{Percentage\ of\ Carbon}{Percentage\ of\ Nitrogen}$$

Equation 11: Calculation of C/N ratio for monodigestion

Where C is the Carbon and N is the Nitrogen.

If there are more than one feedstock involved such as in co-digestion, then the carbon to nitrogen ratio can be calculated by the formula below:

$$\frac{C}{N} = \frac{Q1\ (C1 * (100 - M1) + Q2\ (C2 * (100 - M2)}{Q1(N1 * (100 - M1) + Q2\ (N2 * (100 - M2)}$$

Equation 12: Calculation of C/N ratio for co-digestion

Where, Q1 is the quantity of the macroalgae feedstock (30% or 0.3), Q2 is the quantity of the organic feedstock (70% or 0.7), C1 is the carbon percentage of macroalgae biomass, C2 is the carbon percentage of organic feedstock, M1 and M2 are the moisture content (%) of the macroalgae and organic feedstock respectively and finally N1 and N2 are the nitrogen percentages of the macroalgae and organic feedstock.

The carbon to nitrogen ratios observed in isolation and estimated carbon to nitrogen ratio for the 30:70 co-digestion ratio used in this study are compared in Table 42.

Table 42 Comparison of C/N ratios in isolation and estimated for codigestion

Feedstock	**C/N (Single feedstock)**	**Estimated C/N (Based on 70:30 ratio)**
S. Latissima	**17.19**	-
Wheat	20.00	19.83
Maize	21.50	20.91
Grass	42.00	36.44
SBV mix	35.00	29.69
Pig Manure	9.67	9.96
Brewery spent grain	35.00	29.70

When carbon to nitrogen ratios were estimated for the co-digestion trials based on the measured carbon and nitrogen percentages for individual feedstock, it was found that wheat was estimated to have a C/N of 19.83 while for maize, grass, SBV mix, and BSG the C/N ratio was reduced as a result of *S. Latissima* addition. For pig manure the C/N ratio increased slightly from 9.67 to 9.96 as a result of *S. Latissima* addition.

The data was plotted so as to observe the relationship between the SMPs derived from co-digestion trials and the estimated C/N ratio of the mixes used in this study in Figure 51. The obtained trend line is a polynomial trend line with an R^2 value of 0.7741. The relationship of C/N and measured SMP is not observed to be linear.

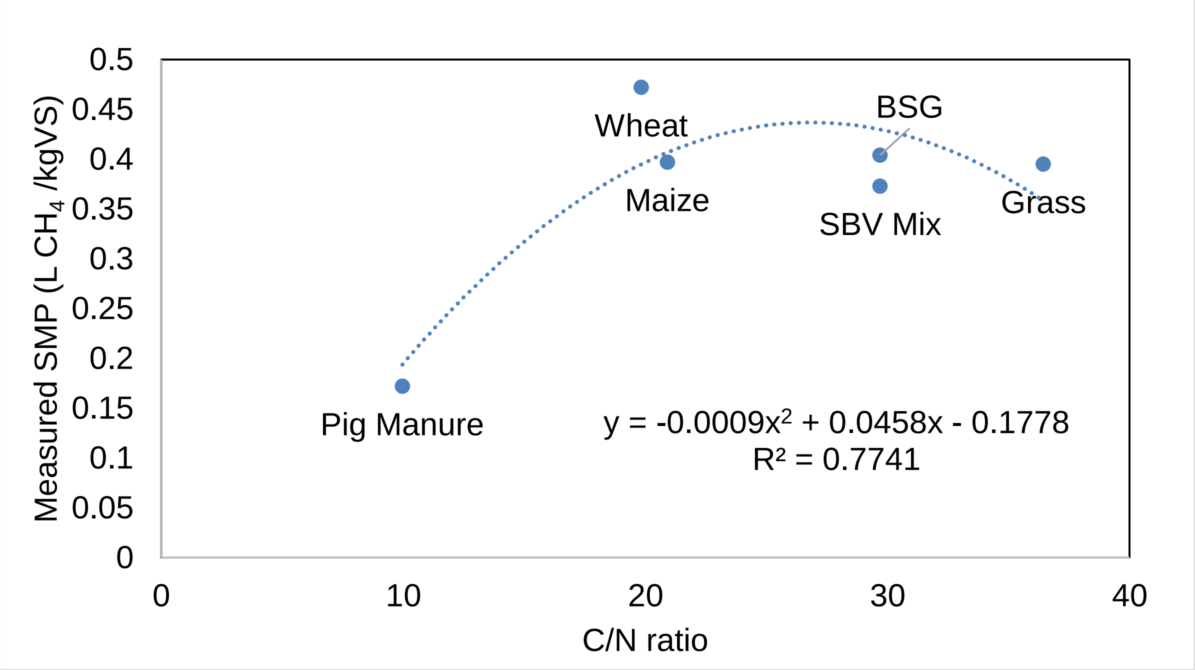

Figure 51 Relationship between specific methane prodcution and C/N ratio

When measured methane production is plotted against the C/N ratios, the trend shows that higher methane production is found to be occurring between the C/N ratios of 20 and 30 and tends to decrease after 35. SMP also tend to decrease below C/N ratio of 20. However, wheat is an outlier with the highest SMP and a border line C/N of 19.83. From the literature it is seen that macroalgae biomass is rich in different kinds of metals and micronutrients (Schiener *et al.*, 2015). Therefore, it could also be suggested that the micronutrients in the macroalgae also balances the feedstock in co-digestion. However, in this study no finite analysis was performed on the co-digested samples hence it cannot be said for certainty which element in the macroalgae aided for high methane production.

7.3 Discussion

The following sections will discuss the co-digestion potential of *S. Latissima* based on the following research question.

- To what extent does the characteristics of *S. Latissima* favour co-digestion with other organic feedstock?
- To what extent does carbon to nitrogen ratio influence the methane yields of the co-digestion mix?

7.3.1 Organic feedstock characteristics

Co-digestion, as an approach, is commonly used more frequently to overcome challenges surrounding biomass consistency and supply. The following sections will discuss the nature of the organic feedstock chosen and the impact of macroalgae (*S. Latissima*) addition on biomethane production.

7.3.1.1 Solids and Ash

Of the organic feedstock chosen for this study, pig manure demonstrated highest total solids content while sugar beet vegetable mix had the lowest solids content. In terms of volatile solids, wheat had the highest concentration while sugar beet-vegetable mix had the lowest concentration. With ash content, pig manure exhibited the highest and brewery spent grain had the lowest concentration. In comparison to macroalgae biomass, all feedstock had higher solids and ash content however lower moisture content.

As discussed previously in Section 1.20.1, agricultural crop waste residues are included in the renewable biomass sources of second generation bioenergy producers.

Utilising the residues for anaerobic digestion offers benefits such as extraction of methane releases from the degradation of these wastes for energy purposes and prevention of their release into the atmosphere (Kim and Dale, 2004) (Chandra *et al.*, 2012). In terms of availability, in the EU, 1500 million tonnes of biomass could be used for AD from agricultural sector each year (Scarlat *et al.*, 2010). Pig manure is another feedstock readily available as a result of in the EU due to intensified animal production activities in Europe, with increased size of the animal production units. This also increases the amount of animal manure and its disposal can have detrimental impacts on causing considerable pollution threat for the environment in these areas. For Therefore for optimised nutrient capture and recycle and manure management, AD is generally the preferred sustainable route (Holm-Nielsen *et al.*, 2009). Brewer's spent grain (BSG) represents around 85% of the by-products produced from brewing industry. BSG has received little attention as a marketable product and its disposal is an environmental problem. Currently the main market for BSG is dairy cattle feed but as it provides fibre, protein and energy, its consumption has also been trialled tried for poultry, pigs and fish. Biogas production from BSG has been evaluated and recommended as way to energy sustainability for breweries. However more studies are required to fully evaluate the potential of BSG for anaerobic digestion (Mussatto *et al.*, 2006).

The characteristics observed for these organic feedstock applied in this study is comparable to those observed in the literature. The characteristics from the literature for these organic feedstock is given in the table below.

Table 43 Characteristics of organic feedstock from literature

Feedstock	**Composition**	**TS (%)**	**VS (%TS)**	**C:N**	**Biogas yield (m^3/kgVS)**	**CH_4 (%)**	**Inhibiting substance**	**Reference**
Wheat	Carbohydrates Sugars, Starch, Cellulose	-	80-90	115	0.290	60-78	Fibre content, pesticides	(Chandra *et al.*, 2012, Wang *et al.*, 2012, Amon *et al.*, 2007)
Maize		-	75-90	73	0.338	55-62		
Sugar beet		24.2	93.2	24	0.5-0.6	80	Pesticides	(Suhartini *et al.*, 2014)
Grass silage		15-25	90	10-25	0.56	34-53	Pesticides	(Steffen *et al.*, 1998, Lehtomäki *et al.*, 2008)

Pig slurry	Trace organic compounds Proteins	3-8	70-80	3-10	0.25-0.50	70-80	Antibiotics and disinfectant	(Steffen *et al.*, 1998)
S. Latissima (fresh)	Carbohydrates Cellulose Hemi cellulose Lignin	10	54	8.8	0.279	31-52	Salinity	(Jard *et al.*, 2012, Vivekanand *et al.*, 2012)
Brewery Waste (Spent grain)	Sugars Proteins Fibres	24	-	25	0.35	60-70	High nutrient content, moisture	(Thomas and Rahman, 2006, Mussatto *et al.*, 2006)

As observed in the table, the selected organic feedstock does represent higher solids content than *S. Latissima* and high potential to methane production with their high volatile solids (%TS) concentration. The potential inhibiting substances for AD process present in the ash content of the biomass include pesticides, antibiotics and disinfectant which can be either in the form of inorganic minerals or other recalcitrant chemicals. It can also be noted that the composition of the feedstock also varies due to varied elemental composition of the feedstock.

7.3.1.2 Elemental composition

In this study, crop waste residues showed a relatively high carbon content in the order maize > grass > wheat. The carbon content of the sugar beet mix and brewery spent grain were found similar. Of the organic feedstock selected in this study, pig manure had the lowest carbon content. In terms of nitrogen content, pig manure showed a slightly higher nitrogen concentration. With regards to sulphur content, pig manure had the highest percentage while others were observed to have less than 1% concentration. With regard to the trace/other elements concentration, wheat showed the lowest concentration however for SBV mix, BSG and pig manure showed a high percentage of trace/other elements in the composition of the feedstock.

The elemental composition observed in this study is comparable to other studies found in the literature. Chandra *et al.* (2012) observed that agricultural crop residues are mostly ligno-cellulosic in nature. They constitute about 40-45% oxygen, and 30-35% carbon on a dry weight, around 5% hydrogen, 0.5% of nitrogen and very small amounts of sulphur (0.1%) of which 30-40% forms cellulose, 25-30% hemicellulose and 15-20% constitutes lignin (Chandra *et al.*, 2012). Brewery spent grain has their major components of cellulose, non- cellulosic polysaccharides, and lignin and it may also contain some protein and lipid. The chemical composition of BSG varies with barley variety, harvest time, malting and mashing conditions, and the quality and type of additives added in the brewing process. In general, BSG is considered as a lignocellulosic material rich in protein and fibre which account for 20 and 70% of its composition respectively. Minerals, vitamins, and amino acids are also found in BSG. They include calcium, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and sulphur all in concentrations lower than 0.5% (Mussatto *et al.*, 2006). Pig manure slurry on the other hand is shown to be a feedstock with lower carbon content however with high nitrogen content (Steffen *et al.*, 1998). Dewatering the slurry and pelletising the manure changes the elemental composition of the pig manure to be a low moisture and high carbon content feedstock (Campos *et al.*, 2008).

The feedstock composition in terms of solids, ash and moisture are critical for anaerobic digestion. Feedstock with high solids and ash content can lead to process failure in AD as a result of solids sedimentation, clogging and inhibition due to the toxic materials present in the feedstock. Feedstock with high moisture content can also result in higher requirement of the feedstock for higher organic loading rates and therefore potentially causing unfavourable process economics. The distribution of organic molecules such as carbohydrates, cellulose, proteins, fats and lipids formed from the various combination of elements in the feedstock are also of great importance as their availability to the microorganisms during the AD process will lead to the formation of either volatile fatty acids or ammonia. Feedstock with high carbon content upon degradation will lead to the formation of volatile fatty acids while feedstock with high nitrogen or protein content may lead to the formation of ammonia upon degradation (Steffen *et al.*, 1998). Higher volatile fatty acids are reported to cause accumulation of solids in the digester while ammonia concentration is shown to cause foaming in the digesters. Therefore, it is important to have an optimum carbon to nitrogen ratio for balanced AD process in the digester.

7.3.1.3 Carbon to nitrogen ratio

Carbon to nitrogen ratio in the selected feedstock in this study was found to be high in the agricultural crop- based residues and lowest in pig manure. Grass exhibited the highest C/N ratio of 42 followed by the SBV mix, BSG, maize, and wheat (35, 35, 22, 20, 10 respectively). As shown in the table above, the values found in this study is similar to those reported in the literature for grass, sugar beet, pig manure, and BSG however higher values are reported for wheat and maize (Chandra *et al.*, 2012, Amon *et al.*, 2007, Suhartini *et al.*, 2014, Steffen *et al.*, 1998, Thomas and Rahman, 2006, Mussatto *et al.*, 2006). As reviewed in literature, nutrient ratio in the feedstock is important for optimal degradation process with the optimum range of C/N ratio for effective AD shown to be between 20 – 30. From the values observed in this study for the selected feedstock high methane production was expected from the agricultural crop waste residues, sugar beet vegetable mix and BSG however lower methane production from pig manure.

7.3.2 Biochemical methane production of organic feedstock

A. Specific methane yield

In this study, of the feedstock tested, BSG exhibited the highest overall SMP at $0.456\ lCH_4/kg\ VSadded$. Pig Manure had the lowest SMP of $0.130\ lCH_4\ /kg\ VSadded$. The specific methane yield of agricultural crop waste residues (wheat, maize, grass) were observed to be similar. The yields obtained for BSG is higher than those reported by Thomas and Rahman (2006), while the yields for pig manure is lower than the reported values in the literature (Steffen *et al.*, 1998). The difference in the yields obtained for BSG in this study could be attributed because of the variation in the brewing process adopted in the brewery from where it was sourced. In addition, depending on the beer produced, BSG may also contain residues from malted barley, or non-malt sources of fermentable sugars such as wheat, rice or maize added during mashing hence increasing the methane yield from easily digestible fraction of sugars present in the feedstock (Mussatto *et al.*, 2006). In the case of pig manure, the decrease in the yield could be attributed to the use of pig manure pellets instead of pig slurry with higher solids content resulting in poorer digestion. Higher solids content in pig manure is previously reported in the literature to be difficult to be digested during methane potential tests (Campos *et al.*, 2008). For SBV mix, the yield is lower than reported by Suhartini *et al.* (2014) and (Lehtomäki *et al.*, 2008) while for wheat, grass, and maize, SMP yields are similar to those reported in the literature (Chandra *et al.*, 2012, Lehtomäki *et al.*, 2008, Cirne *et al.*, 2007).

7.3.2.1 Rate of methane production

For all of the feedstock except for pig manure, exponential methane production was observed during the biochemical methane potential test. However, rate of methane production for each feedstock was observed to be different. All feedstock reached their maximum methane production within the first 3 – 5 days of the test. The rate of methane production was faster for SBV mix and BSG where maximum methane production occurred within the first day of the test. Wheat and maize reached their maximum production at day 2 – 3 while pig manure was shown to have a small lag phase and lowest methane production among all of the feedstock tested.

This can be attributed to the improved degradation by the microorganisms in the digester. As discussed previously, the ease of degradation in the digester is, to some extent, dependent on the biochemical composition of the feedstock. The presence of higher amounts of fermentable sugars and moisture in BSG makes it more readily accessible by the microorganisms in the digester hence a better feedstock for anaerobic digestion with higher SMP yields and faster rate of methane production (Xiros and Christakopoulos, 2012). Sugar beet – vegetable mix is also very similar with sugars, starch and carbohydrates increasing the rate of methane production while for agricultural crop waste residues, even though they have shown faster rates of methane production in this study their ligno-cellulosic nature often pose a problem in AD processes utilising such biomass feedstock (Scarlat *et al.*, 2010). For pig manure, as it has shown higher nitrogen and sulphur content in this study, inhibition of methane formation could be due to ammonia formation or from the toxicity of other solids in the feedstock because of ammonia formation or from the toxicity of other solids in the feedstock (Cuetos *et al.*, 2008).

It is clear from the discussion that crop waste residues have a high potential for methane production in mono digestion. However, because of their ligno-cellulosic nature, they often require pre-treatment to optimise performances which can add to overall cost of processing be an expensive route (Steffen *et al.*, 1998). These materials also have a high solids content of 10-50% and in order to optimise their digestion, the material needs to be homogenised with other materials with higher water content (Lehtomäki *et al.*, 2008). This could be an important benefit for use of *S. Latissima* which has a relatively high moisture content could enhance AD performance under co-digestion conditions. In the case of pig manure, it is recommended that it should be co-digested with waste that has a higher carbon content improve the C:N ratio (Cuetos *et al.*, 2008). The relatively high nitrogen and fibre content of the BSG can also be optimised using co-digestion resulting in higher methane production rates and yields than observed for digestion of the individual feedstock alone.

From the results and discussion presented here, it is clear that the organic feedstock requires further optimisation for methane production through either pre-treatment or co-digestion routes. Reviewing the co-digestion practices in the last 5 years, Mata Alvarez *et al.* (2014) notes that the topic of co-digestion has been dominating the literature since 2010 with the majority of publications focusing not only traditional feedstock such as agricultural wastes, sewage sludge etc. but also newer feedstock such as fats and oils, greases, complex mixtures and micro and macro algae biomass. However, there are limited studies reported for brown algae biomass species *S. Latissima*. In addition, from a practical perspective, if biomass is to contribute to the EU target of two thirds of the renewable energy share by 2020 then it should consider the use of all available resources in a sustainable way without causing negative impacts (Scarlat *et al.*, 2010).

7.3.3 Biochemical methane production on co-digestion with *S. Latissima*

As discussed in literature, macroalgae biomass have high percentages of carbohydrates, storage sugars and special compounds like phenolic compounds and cellulose based compounds called alginates for structural support. The carbohydrate content in the macroalgae biomass is easily degradable and the percentage of lignin is lower compared to land-based biomass (Xia *et al.*, 2016). This unique characteristic of higher C% and its accessible form in the biomass make it increasingly favourable for co-digestion practices. In this study, *S. Latissima* is observed to have a methane yield of $0.391\ L\ CH_4\ /kg\ VS\ added$. In agreement with literature, it was observed that harvest time, localised environmental conditions and growth type has an impact on the characteristics of the macroalgae biomass which in turn determines the variation in the biomethane potential of the feedstock. It was also observed that volatile solids and ash has an inverse relationship with the methane production potential of macroalgae biomass. Therefore, summer harvest from Ventry Harbour location, with lowest volatile solids and ash content was chosen for co-digestion. For the co-digestion experiments, the organic feedstock and macroalgae was mixed in a 70:30 ratio where 70% was the organic feedstock and 30% was made up of *S. Latissima*.

The following sections will discuss the rate of methane production and specific methane production yields obtained during co-digestion of feedstock with *S. Latissima*.

7.3.3.1 Rate of methane production

The rate of methane production for organic feedstock co-digested with *S. Latissima* was observed to be similar to rates of methane production of feedstock in isolation. However, when comparing the volume of methane produced during the first five days of digestion it could be observed that all feedstock produced more methane when co-digested than in isolation. All of the feedstock reached maximum methane production during the first five days of digestion. The rate of methane production for *S. Latissima* with sugar beet-vegetable mix is faster and diminishes more quickly to plateau around day 3 while the rate of methane production of *S. Latissima* with wheat takes longer to plateau around day 4. *S. Latissima* with pig manure consistently has a slower rate of methane production in comparison to other organic feedstock even while in co-digestion. This shows that co-digestion has been mostly favourable for the tested organic feedstock and the methane production pattern is dominated by the major component in the mix. This is in agreement with literature where degradation rates are suggested to be specific to each substrate and dependent on the inherent properties and composition of the co-substrates (Kouas *et al.*, 2017, Xie *et al.*, 2017). Apart from the easily accessible sugars and carbohydrates already present in the main organic feedstock, the composition of macroalgae biomass would have contributed to the increased methane production observed for co-digestion. As already suggested in the characteristics results, *S. Latissima* is also formed of easily degradable carbohydrates and as the co-digestion utilised summer harvest, there is also reduced inhibition from inorganic components within the biomass, however the micronutrients in the biomass can potentially balance the digester stability. This is also in agreement with other co-digestion studies utilising macroalgae biomass which suggest that, as macroalgae contain relatively high fractions of sugars and hemicellulose, this also favours the enzymes activity to the feedstock resulting in improved hydrolysis yield in co-digestion (Jard *et al.*, 2012, Costa *et al.*, 2012). The rapid degradation of feedstock demonstrated in the faster rates of methane production is a very useful indicator to as it can also provide a rough estimate of retention times required for complete digestion of a given feedstock (Costa *et al.*, 2012a). In this study all of the methane production of all of the feedstock in co-digestion reached their complete digestion by around day 20 where methane volumes were less than 10 ml/day. The increase or decrease in methane production to ascertain synergistic or antagonistic effects of co-digestion was evaluated using the specific methane yields.

7.3.3.2 Specific methane yield

Percentage increase or decrease in specific methane yields for the organic feedstock in isolation were compared to those in co-digestion. A percentage increase was observed for wheat, maize, SBV mix and pig manure, however there was no difference observed for grass and a decrease in yield was observed for BSG. The highest percentage increase was observed for co-digestion with pig manure. Even though the percentage difference is calculated for specific methane yields, the factor of mixing ratio in co-digestion is not fully considered to understand the effect of addition of *S. Latissima* to an organic feedstock. Therefore, expected specific methane yields from co-digestion is calculated by incorporating the mixing ratios to the specific methane yields of feedstock in isolation. From those estimated values, net percentage increase or decrease for co-digestion was measured. This was found to be in agreement with the calculation suggested in the study by Labatut *et al.*, 2011. Interestingly the highest net percentage increase was observed for wheat followed by SBV mix. The decreasing order of net percentage increase in co-digestion can be given as Wheat > SBV mix > Maize > Grass > BSG > Pig manure. Pig manure was now observed to show a decrease of 17% in the methane production while in co-digestion.

Co-digestion is reported to enhance the degradation of individual substrates in the mix, i.e. as a result of synergistic effects, or decrease the degradation of the substrates resulting from antagonistic effects (Mata-Alvarez *et al.*, 2011). Synergism could be observed as an additional methane yield for co-digestion samples over the weighted average of the specific methane yield of the individual substrates and antagonism will be translated in to lower methane yields in co-digestion compared to the weighted averages (Nielfa *et al.*, 2015). For co-digestion of wheat with *S. Latissima* the increase of methane yield has been synergistic as the percentage of increase (21%) is greater than standard deviation of 0.033 (7%) and the weighted averages of the individual yields of the substrates. The specific methane yield obtained for wheat in this study of $0.472\ lCH_4\ /kgVSadded$ is higher than reported for *S. Latissima* co-digested with wheat straw in the literature where a value of $0.275\ lCH_4\ kg/\ VSadded$ is obtained for a blend of 75:25 (Vivekanand *et al.*, 2012). Synergism is also observed for sugar beet – vegetable mix where percentage of increase observed was 15% higher than the SMP observed for monodigestion of the feedstock. As for grass and maize, the calculated values were within the standard deviation limits; hence no conclusions could be made for the co-digestion combination. Co-digestion of pig manure with *S. Latissima* had been clearly antagonistic in nature with a decrease of 17%. Brewery spent grain also showed a decrease of 1% from the mono digestion specific methane production values. As there are no reported studies on co-digestion of *S. Latissima* with these feedstock these values could be very informative to future co-digestion research involving such feedstock.

Literature suggests that synergistic effects can be influenced by the different components in the co-digested mixture as they are biodegraded in the digesters. In a mixture of co-digestion, the degradation of the feedstock can occur in three routes: i) the feedstock influence each other for enhanced methane production or biodegradation, or ii) the individual feedstock degrade independently in the mixture with no significant increase in methane production or biodegradability or finally where the feedstock has a competitive effect in the degradation during co-digestion and results in either no increase or even decrease in methane production and degradability (Nielfa *et al.*, 2015). In the case of macroalgae, literature has suggested that co-digestion with a complementary feedstock can induce synergetic effect on the biodegradability of both the feedstock with an increase in methane yield and production rate owing to their enhanced carbon and nitrogen concentrations as a result of mixing (Oliveira *et al.*, 2014).

7.3.4 Impact of carbon to nitrogen on the methane production on co-digestion

As described in the literature, one of the important parameters for characterising the suitability of a feedstock for AD is carbon to ratio. Wastes with higher C/N ratios are reported to produce methane more rapidly (Shanmugam and Horan, 2009). In the case of macroalgae, the literature generally recommends co-digestion with a feedstock with higher carbon concentration and lower nitrogen concentrations (Oliveira *et al.*, 2014). An optimum C/N ratio is still debated but a range of 20-30 is found to be accepted and a ratio close to the optimum range is potentially considered highly suitable for AD (Nielsen *et al.*, 2012).

As a mono-digestion feedstock *S. Latissima* has a C/N ratio of 17. Wheat and maize had a C/N ratio closer to 20 while BSG and SBV mix closer to 35. Grass is an outlier with the highest C/N ratio of 42 (higher than from the values reported in the literature) while the lowest noted for pig manure with a value of 10. In co-digestion with *S. Latissima* the C/N ratios for wheat and maize almost remain the same however for SBV mix, grass and BSG the values become closer to the optimum range. The value for pig manure still remained low at 10. This could suggest that the higher methane production observed for SBV mix could also have been due to the optimised C/N ratios.

To explore this further a selection of feedstock for AD were chosen to evaluate their C/N ratio as a single feedstock and then theoretically estimated for their C/N ratios to assess suitability for co-digestion with *S. Latissima* Figure 52.

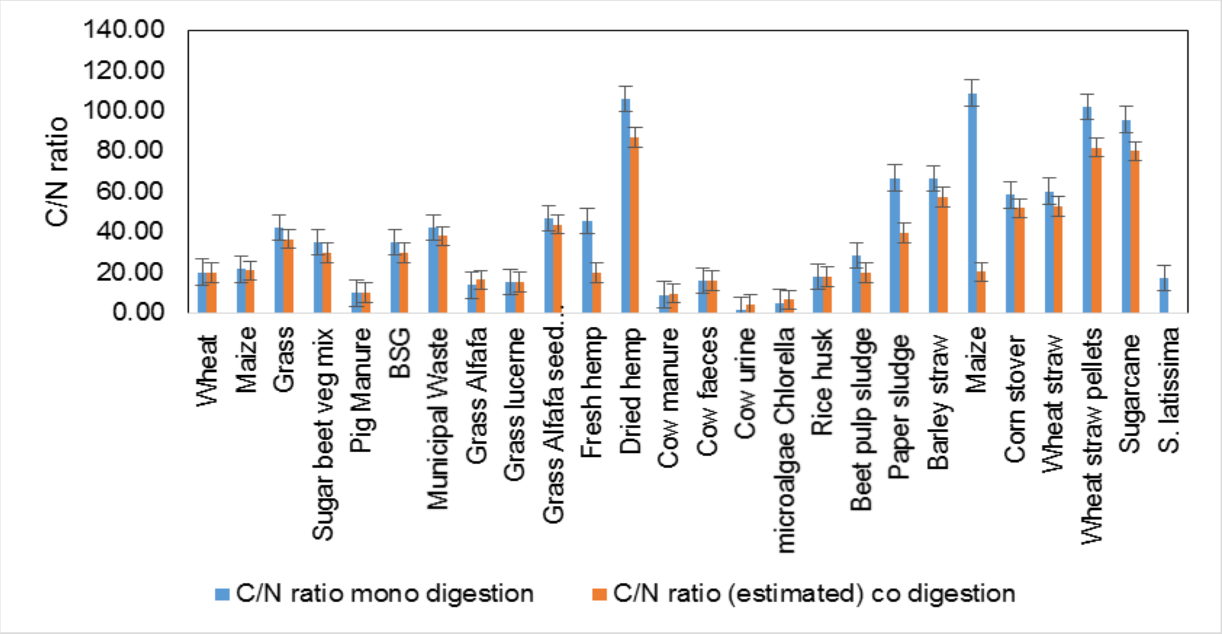

Figure 52 Estimation of C/N ratio for various feedstock with *S. Latissima* from literature

It can be seen that addition of *S. Latissima* reduces the combined C/N ratio for a number of feedstock. For feedstock such as cow urine, it seems to increase the ratio while for grass Lucerne and pig manure the values are identical. The majority of the agricultural feedstock exhibits a theoretical C/N ratio between 20 – 30 upon co-digestion with *S. Latissima* such as wheat, maize, grass, rice, and beet pulp sludge. This co-digestion of *S. Latissima* with agricultural wastes could therefore be beneficial in terms of enhanced methane production. These are estimated or theoretical values, and only the experimental values can show the true impact of co-digesting the feedstock with macroalgae biomass such as *S. Latissima*. Even though the biomass has a C/N ratio nearer to the optimum range, summer biomass of *S. Latissima* also has lower protein content which makes it feasible to use in combination with other feedstock. However, macroalgae is in its early developmental stage during winter with higher concentration of proteins for its cellular growth which can result in higher N% during those seasons. The seasonal supply of macroalgae could be an issue for continuous digestion operations not only because of the reduced-availability but also on the basis of their optimal biochemical composition. In addition, the presence of high concentrations of inorganic elements (e.g. sodium, potassium, calcium ions and chloride and sulphate as counter ions) in winter months, could also be detrimental to anaerobic digestion (Schiener *et al.*, 2015, Alaswad *et al.*, 2015).

7.3.5 Seasonal supply of *S. Latissima* for co-digestion practice

Co-digestion is favoured for technical reasons as it can overcome inherent problems related to feedstock such as lack of micronutrients, imbalanced C/N ratio and unfavourable (high/low) organic loading rates. In addition, it can also aid in utilising the spare capacity of AD infrastructure at wastewater treatment plants etc. to treat a variety of feedstock through co-digestion and generate supplementary revenue via gate fees or service charges, whilst producing electricity and heat. Equally, co-digestion can help lower capital investment for any additional waste management facilities (Xie *et al.*, 2017).

This research investigated the anaerobic co-digestion potential of macroalgae biomass for the species of *S. Latissima* harvested in summer. Synergetic effects observed for the enhanced methane production and rates could be attributed to optimisation of C/N ratios and the inherent composition of the mixed feedstock. The BMP tests demonstrated *S. Latissima* enhanced methane production while co-digested with SBV mix and wheat. Feedstock such as wheat, maize, grass, sugar beet etc. are already identified as potential biomass feedstock for increasing the energy yield from agricultural residues in the EU for meeting the renewable energy targets for 2020 (Scarlat *et al.*, 2010). In this study, localised environmental conditions are found significant in defining the macroalgae characteristics and thereby its methane potential. Therefore, specific wastes available in a particular location can be targeted for co digestion with macroalgae biomass and it being a seasonal feedstock, with its high availability during summer, co-digestion can be a favoured practice in summer but it can be less effective in winter months where biomass will be less available due to its growth cycle.

This is very promising as recent research on the environmental life cycle assessment of macroalgae AD (which also considered co-digestion as an approach) recommended that energy production from macroalgae via AD is sustainable if it is regionally accessible and it can also be used as substitutes for energy crops in conventional AD plant (Ertem *et al.*, 2017).

The main limitation of this study was that the results were based on the batch BMP studies. BMP tests are a useful tool for determining the best feedstock configuration for co-digestion. However, over estimation of the methane yields are reported due to methodical errors such as instrumental or human errors (Kreuger *et al.*, 2011), or due to lack of capability to include biodegradability or kinetics factors for predicting the methane productions from the configured co-digestion mixtures. BMP assays are good indicators of methane potential of a feedstock, however results are not easily transferable to full scale processes where operation is generally continuous and dynamics of loading, gas production and the biochemical environment are completely variable (Nielfa *et al.*, 2015). In addition, the substrate to inoculum ratio are also to be chosen carefully to utilise these assays to replicate information for adaptation into large scale AD applications (Holliger *et al.*, 2016). In this study, a substrate to inoculum ratio of 1/4 was used as per recommendations found in the literature by Angelidaki *et al.*, 2009 for batch BMP tests. To evaluate the dynamics of the process and provide greater understanding of the performance of individual feedstock under co-digestion conditions, continuous studies are required. For the purposes of this study organic feedstock were collected over a fixed season. Therefore, to assess the percentage of variability within the tested organic feedstock, co-digestion needs to be conducted with samples collected over time or season to show realistic variability of performance. In short, further studies are required to understand the extent of variability within co-digestion of macroalgae biomass.

Anaerobic digestion has been reported to be a low cost renewable energy technology. Wider utilisation of new feedstock like macroalgae biomass at full, however, is highly dependent on the overall economic viability of the process. This study also evaluated the techno-economic factors to be considered when utilising *S. Latissima* as a feedstock for anaerobic digestion. The economic model developed by EnAlgae was used as a template for the techno-economic analysis in this study. The results of the techno-economic analyses carried out as a part of this study will be discussed in the next chapter.

8 Results – Techno-economic feasibility of *S. Latissima*

There are few techno-economic assessments performed on macroalgae biomass to date in the literature. Production of biofuels and chemicals from macroalgae biomass is definitely shown to be one of the promising systems for future. However, without an integrated bio-refinery approach the process is not shown to be feasible as costs and investments are higher for a macroalgae based system (Konda *et al.*, 2015). The technical feasibility of macroalgae biomass for biogas production is recognised in the literature, however fewer studies are available on the economic feasibility of the AD technology for biomass utilisation (Montingelli *et al.*, 2016). Therefore, in this study, techno-economic analysis of AD of *S. Latissima* was performed to identify the effect of AD technology on the overall economics of the process – i.e. the benefits and challenges on the economics of the process utilising *S. Latissima*.

The different steps performed for the techno-economic analysis in this study are shown below (and described in Methodology Section 3.6): -

- Stakeholder identification
- System boundary and process flow
- Results for macroalgae biomass as a mono-digestion feedstock for AD
- Results for macroalgae biomass as a co-digestion feedstock for AD

8.1 Stakeholder identification

Stakeholder identification is a key step in a techno-economic assessment or LCA study. New perceptions and views on the sustainability of macroalgae biomass can be aided through discussions with stakeholders with varying interests. The EnAlgae project (2015) carried out a workshop with existing and potential stakeholders to gain a better understanding of experiences, attitudes, and to gain insight into the sustainability of macroalgae cultivation and uses of the biomass.

The stakeholder assessment carried out by EnAlgae is given in Figure 53.

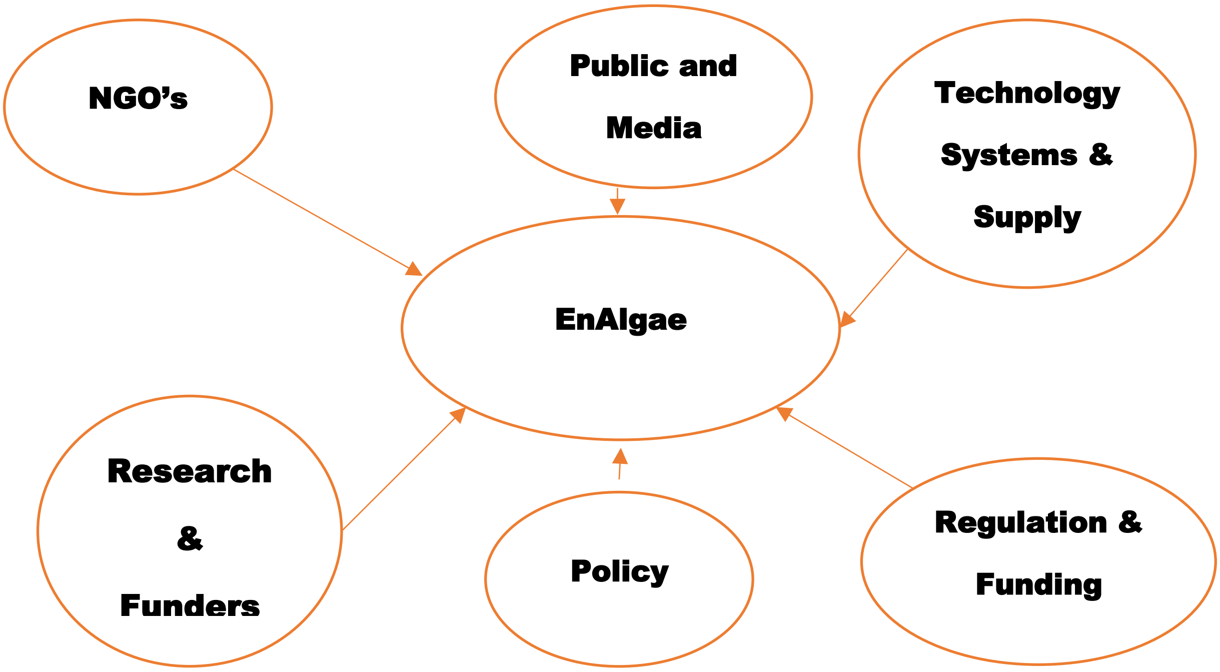

Figure 53 Stakeholder assessment by EnAlgae

The main stakeholders identified in the project were the research and funders, NGO's, public and media, regulators and planners, and policy makers. A dynamic discussion was based on 5 main questions: -

- Could ecosystem services provided by the cultivation of macroalgae biomass compensate the unfavourable energy balance?
- Sustainability around biomass - Wild harvest or cultivated?
- Is macroalgae biomass cultivation in competition to other users of the marine ecosystem?
- Would macroalgae biomass cultivation bring jobs only to the rural areas - low income jobs?
- Perceptions around non-native species of macroalgae biomass (Sprujit, 2015)

These questions were identified as the main discussion questions because the critical parameters to consider for the techno-economic feasibility of the processes involving macroalgae biomass were the selected species; native or non-native, methods of cultivation implemented for bioenergy production, and harvesting techniques as harvesting of wild stocks is still the main source of biomass in Europe. Developing sustainable harvesting of wild resources would also prevent any negative impact on the other living organisms in the ecosystem.

The questions were also important to understand the scope of employability that macroalgae biomass cultivation and related processes for bioenergy production would help develop in the region (Parker *et al.*, 2015).

The return on investment (ROI) from macroalgae biomass is shown not to be favourable and hence it is challenging to improve the small-scale production of biomass in near future. However, a two-step harvesting process was suggested by the EnAlgae project to compensate the energy balance, where first harvest will be for the high value compound extraction, while second harvest will be purely for bioenergy production. As the biomass are in the long lines, the growing seedlings will be of the same growth stage and hence the same age when harvested. Still negative economic impacts due to reduced amount of harvested biomass in the first harvest in April as the biomass is still in its growth phase is regarded as a main obstacle to implement the two step harvest process. There were concerns raised over the competition for space by other users of the marine ecosystem if used for extensive macroalgae biomass cultivation. With regards to harvest, less than 1% is produced through cultivation systems and in North West Europe, the majority of macroalgae biomass is produced from wild harvest. At present, macroalgae biomass cultivation is seasonal, and has similarities with mussel cultivation. Therefore, it is also assumed that there is not a requirement for highly skilled or qualified personnel for macroalgae biomass harvesting thus the development of the industry involving biomass would be creating jobs. The EnAlgae study also highlighted that there was uncertainty whether wages for this type of specific employment would be equal to those gained in the fishery business. However, the project proposed that if large scale cultivation farms are deployed this can lead to higher paid employment e.g. via biomass processing specialists, jobs in logistics aiding the transport of biomass, and also jobs in industries utilising the biomass for the production of high value products. Lastly in the findings of the report, the use of non-native species was not at all encouraged amongst stakeholder's discussions as there was consensus about non-native species weakening the genetic integrity of local species and this affecting the wider ecosystem biodiversity (Sprujit, 2015).

Based on the findings from the EnAlgae project, the stakeholder analysis for this study was carried out. For this research, a stakeholder template developed by Prof. Mark Reed was utilised (Reed, 2016). Discussion with the research organisers, AD operators, and the macroalgae biomass farmers was facilitated while on site visits, through formal and informal emails and telephone conversations. The general interests, expectations and concerns regarding the utilisation of macroalgae production for energy were ascertained. The key findings from the research discussions are shown in the following sections. The stakeholders and participants of this research study are shown in Figure 54 and the details are discussed in the sections below.

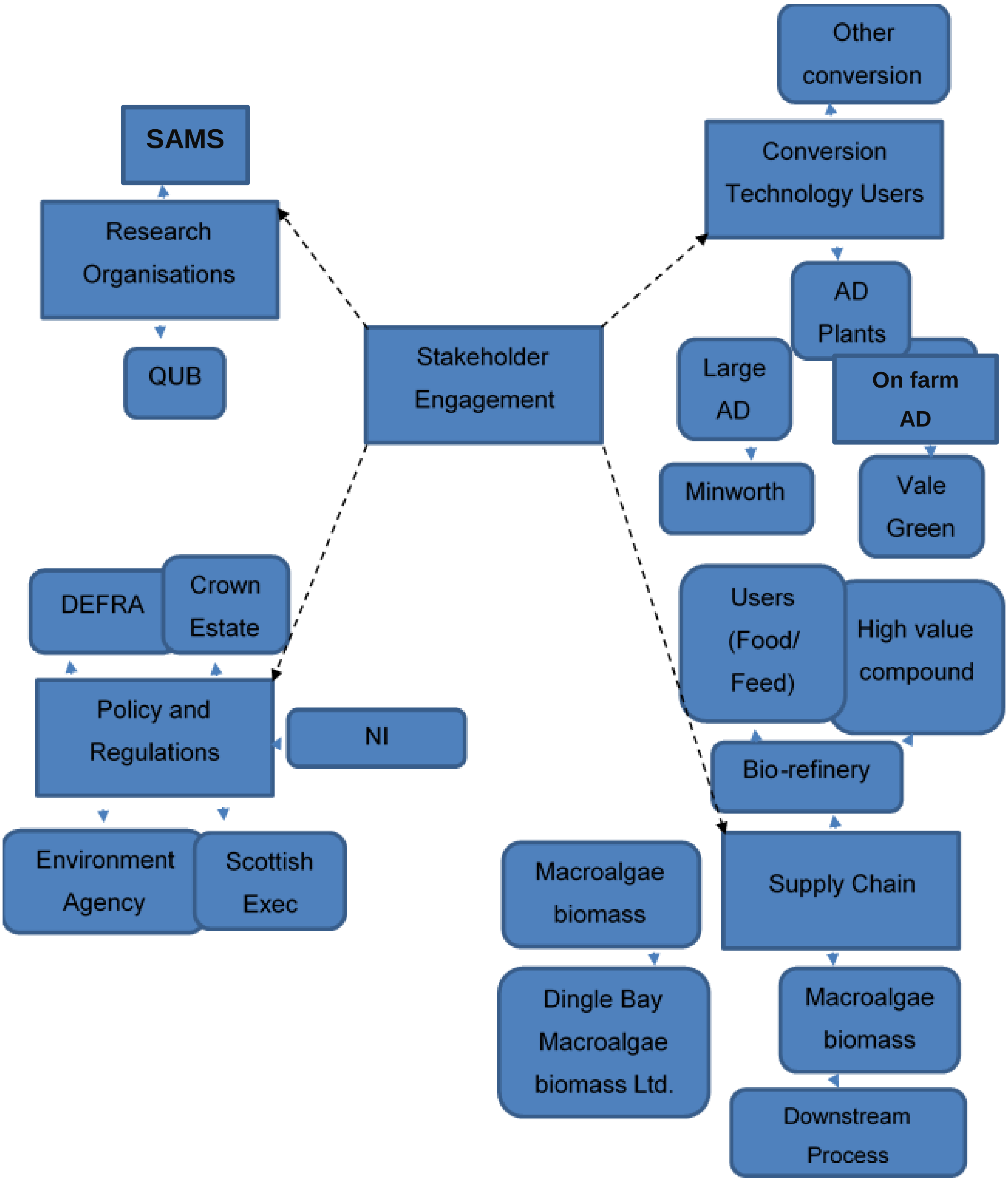

Figure 54 Stakeholder assessment in this study

As shown in the figure above, the participants of this study were mainly research organisations (QUB, SAMS), macroalgae biomass farmers (Dingle bay Macroalgae biomass ltd.) and on farm (Vale Green Energy) and large scale AD (Minworth) operators. Therefore, the techno-economic discussions were mainly around the sustainability of macroalgae biomass in terms of cost of the biomass, utilisation of the biomass for bioenergy production (mainly AD), and generation of energy through co-digestion of wastes.

The main questions for the techno-economic discussion of this study were:

- How does the cost of macroalgae biomass affect the biomass utilisation for AD?
- Will co-digestion make the bioenergy production from macroalgae biomass sustainable?
- Will the digestate sale as fertiliser be beneficial for the overall economics of the system?

Sensitivity analyses were performed for determining the variation in cost of the macroalgae biomass, with the market price for the macroalgae biomass and other feedstock, and scenarios with no digestate for comparing results. The system boundary and assumptions for this study are discussed in the sections below.

8.2 System boundary and process flow

The process flow sheet for the techno economic assessment carried out in this study is given in the figure below. Most of the reported T-E studies on macroalgae have focused on the cultivation processes of the biomass (Czyrnek-Deletre, 2017, Murray *et al.*, 2013) . Therefore, our study has only focused on the utilisation of the biomass for bioenergy production using AD processes.

Our study has utilised the Techno economic model (WP2A7.07 model methane V2.3) developed by Chris de visier *et al.* as a part of the EnAlgae Project. The basis of the new model including parameters, constants and the investments for the development of the model were derived from the work by Sinnott *et al.*, 2005. The model was initially developed as an economic model developed for analysing the co-digestion potential of algae with other 4 co-substrates to either produce electricity with a CHP (Combined Heat and Power) or green gas. The model assumed a 50-50% ratio of the co-substrates with the main feedstock.

The model was modified for our study to incorporate macroalgae biomass and 6 other co-substrates including maize, grass, wheat, sugar beet-vegetable mix and pig manure and brewery spent grain. The ratio assumed was 30% (macroalgae biomass) and 70% (co-substrate). There was no pre-treatment considered and the generated biomethane was utilised for CHP purposes. The capacity of biogas was assumed at 1000,000 (m^3 per year) with a CHP production of 2400 MWh/year. The life span of the digester was assumed at 25 years with a life span of CHP engine at 10 years. The investments of the model include the cost of a co-digester, a CHP unit, and a biogas process unit.

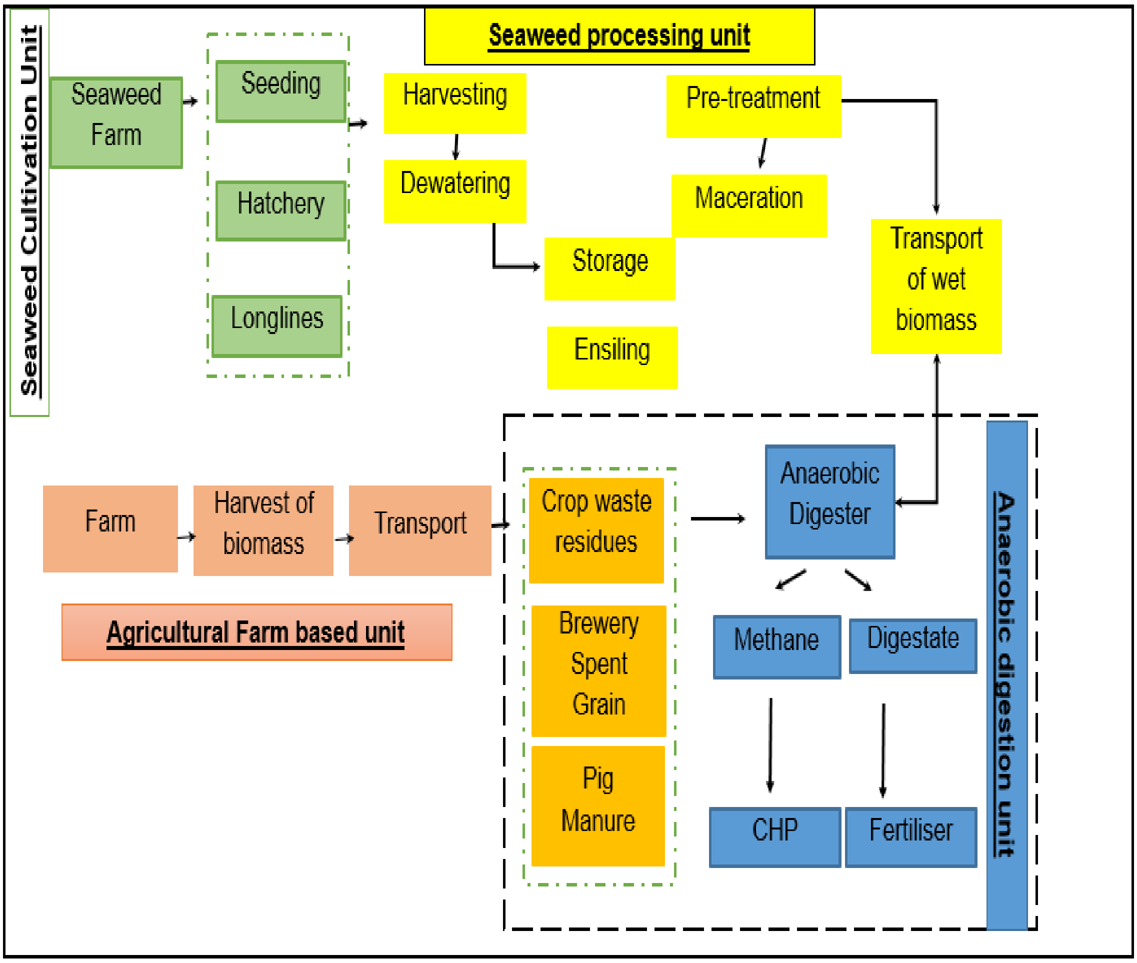

Figure 55 System boundary and process flow for this study

The boundary for the system Figure 55 (shown by dotted lines) is assumed to start at the AD Plant (anaerobic digestion unit) where the wet biomass would be transported to the sites from the seaweed cultivation and processing units for digestion. The agricultural farm based unit will be producing the other organic waste feedstock such as the crop waste residues, brewery spent grain and pig manure which also will be transported to the AD unit for co-digestion. The digesting time for the feedstock is assumed to be 30 days.

The two main products of an AD process; methane is utilised for CHP purposes and digestate is utilised as a fertiliser for CHP respectively. The different scenarios assumed for the techno-economic analysis are broadly divided into two.

- Macroalgae biomass as a mono-digestion feedstock
- Macroalgae biomass as a co-digestion feedstock

This techno-economic assessment is specifically on species *S. Latissima*. The digester characteristics for the macroalgae biomass and the other organic feedstock were taken from the experimental data (BMP experiments chapter 7 section 7.2.4) In northwest Europe the species is cultivated in long lines with a yield of 10 kg/m long line with a growing period of 20 weeks at sea. The price of electricity is assumed at 0.11 €/kWh which is the lowest of the electricity prices in a European member state in 2017 (Eurostat, 2017). The efficiency of the assessment is mainly reported in terms of the return on investment (ROI, %) and payback time (year).

Return on investment was cacluated using the below formula

Return on investment =

$$\frac{(Total\ returns - Total\ costs) - (Depreciation\ of\ asset - Rent\ on\ debt\ capital)}{Total\ investment}$$

Equation 13 Calculation for return on investment

Payback time = $\frac{Total\ investment}{(Total\ returns - Total\ costs) + (Rent\ on\ debt\ capital + Depreciation)}$

Equation 14 Calculation for payback time

The cost considerations included raw materials (organic feedstock costs), investment, depreciation, insurance, rent on capital, land costs, maintenance costs, land cost and labour cost. The total returns mainly involved the selling of the green gas into the grid unless the sale of digestate was considered as a route of income.

The digestate from the co-digestion is assumed to be sold at 5 Euros per tonne (WRAP, 2015). All the co-substrates are assumed to be organic wastes that can be either utilised in an on farm AD plant or be treated as organic waste with an applied gate fees of 29 euros per tonne. All quantities are reported in tonnes and prices in euros.

The results for the analysis is discussed in the following sections.

8.3 Results - Techno-economic assessment

The techno-economic assessment is performed on the macroalgae biomass with the highest methane potential from the BMP tests and the substrate on which semi continuous digestion trials were performed for this study. This was *S. Latissima* obtained from Ventry Harbour. The results for macroalgae biomass, as a mono-digestion and co-digestion feedstock, will be presented separately. The sensitivity analyses results are also presented in the following sections.

The digester details are given in Table 44.

Table 44 Digester details

Digester			
inflation	2.00%	per year	Sinott *et al.*, 2005
life span digester	25	years	
life span CHP	10	years	
rent on debt capital	5.50%		
Capacity biogas	1,000,000	m^3/year	
CHP electricity production	2520	MWh/year	
Digesting time	30	days	
Green Gas price	0.22	Euros/Nm^3	

Green gas price is taken from the techno economic model from EnAlgae. The price of green gas was validated by comparing the price of the natural gas and the quality of green gas. The price of green gas fluctuates every year and the price used for calculation takes into account the gas quality grid in comparison to natural gas (Lems, 2010, Eurostat, 2018). Digesting time was informed by the methane potential tests carried out as a part of this research.

The assumptions for the assessment are given in Table 45.

Table 45 Assumptions and parameters for the techno economic analysis

Parameter			
Methane	0.668	kg/m^3	Sinott *et al.*, 2005
CO_2	1.842	kg/m^3	
Biogas	1.138	kg/m^3	
electric efficiency CHP	36.00%		
heat efficiency CHP	60.00%		
CH_4 content	60%		
CO_2 content	40%		
electric efficiency CHP	36.00%		
number of operational hours per year	8400	per year	
heating value methane	36.5	MJ/m^3	

Based on the digester capacity of 1000,000 m^3/year used in this study, for the selected feedstock of maize, grass, wheat, sugar beet-vegetable mix, BSG and pig manure, the quantity of feedstock, the macroalgae biomass required for each co-digestion, and the amount of digestate generated for each co-digestion scenario is shown in Figure 56.

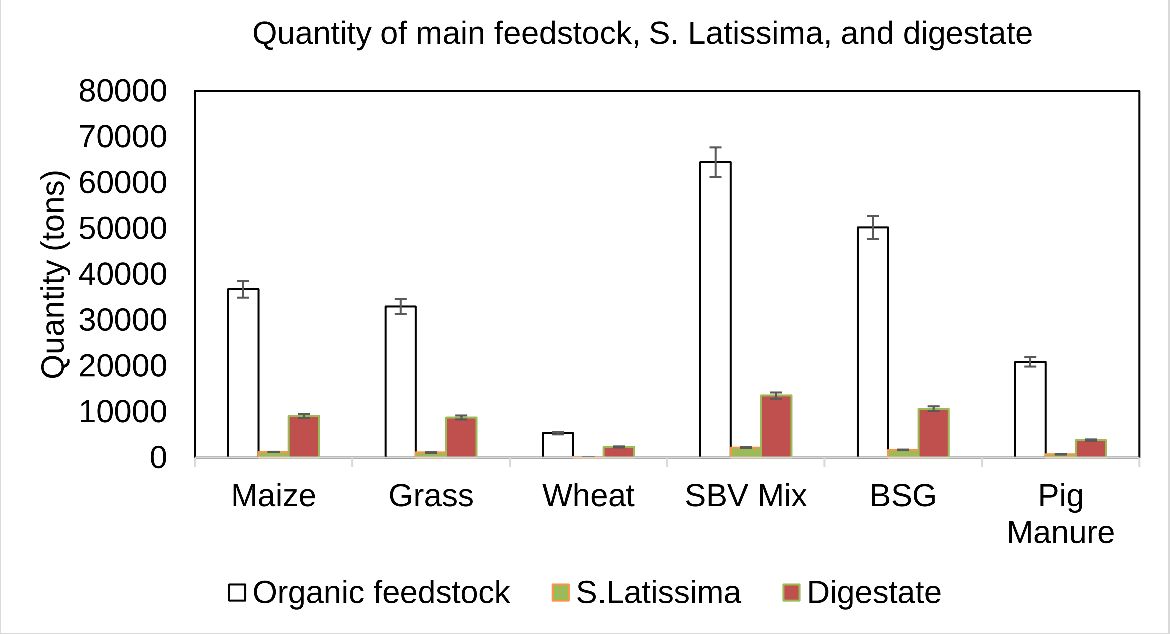

Figure 56 Quantity of feedstock and digestate for each codigestion scenario

The biomethane production potential for the feedstock is calculated on the basis of volatile solids. As shown in the figure above, the highest amount of bulk feedstock required to maintain the digester output of 1million m^3 biogas per year is observed for the sugar beet – vegetable mix (64461 tons) and the lowest for wheat residues (5330 tons). This can be related to their VS concentrations (as observed in co-digestion results chapter) where the VS concentration of SBV mix is observed to be 22.5%WW and for wheat it is observed to be 84.6%WW. In addition, the highest methane production per tonne feedstock is observed for wheat residues with macroalgae biomass.

In terms of the digestate production from the feedstock used for digestion, it is also possible to estimate the digestate residual from the model. The model estimates the amount of digestate produced as a result of co-digestion from the amount of feedstock utilised in the input (as dry matter (dm %)) left after the quantity of feedstock converted into biogas. Clearly the volume of digestate produced is correlated to the overall quantity of feedstock used. Therefore, it can be observed from the figure that sugar beet vegetable mix evolved the greatest quantity of digestate (13527 tons) and the lowest for wheat residues (3775 tons).

8.4 *S. Latissima* as a mono digestion feedstock

For *S. Latissima* as a mono digestion feedstock, the economics were calculated with varying macroalgae biomass prices. The prices assumed were 0, 50, 250 and 1000 Euros per tonne respectively. The prices represented the scenarios where macroalgae biomass was free (0 euros), 50 Euros (Cost of brown algae biomass dry basis per tonne in the literature (Dave *et al.*, 2013), 250 Euros, (Price suggested by Crown Report if intended for bioenergy production, Kelly *et al.*, 2008) and 1000 Euros (current price of biomass for high value products extraction, Vandendaele, 2013).

Results in this study showed that the only positive return on investment (2%) for macroalgae biomass as a mono digestion feedstock was achieved when the feedstock was free (0 Euros per tonne). The values for ROI became increasingly negative as the prices were increased from 50 to 1000 Euros per tonne. The graph for macroalgae biomass as a mono-digestion feedstock is shown in Figure 57.

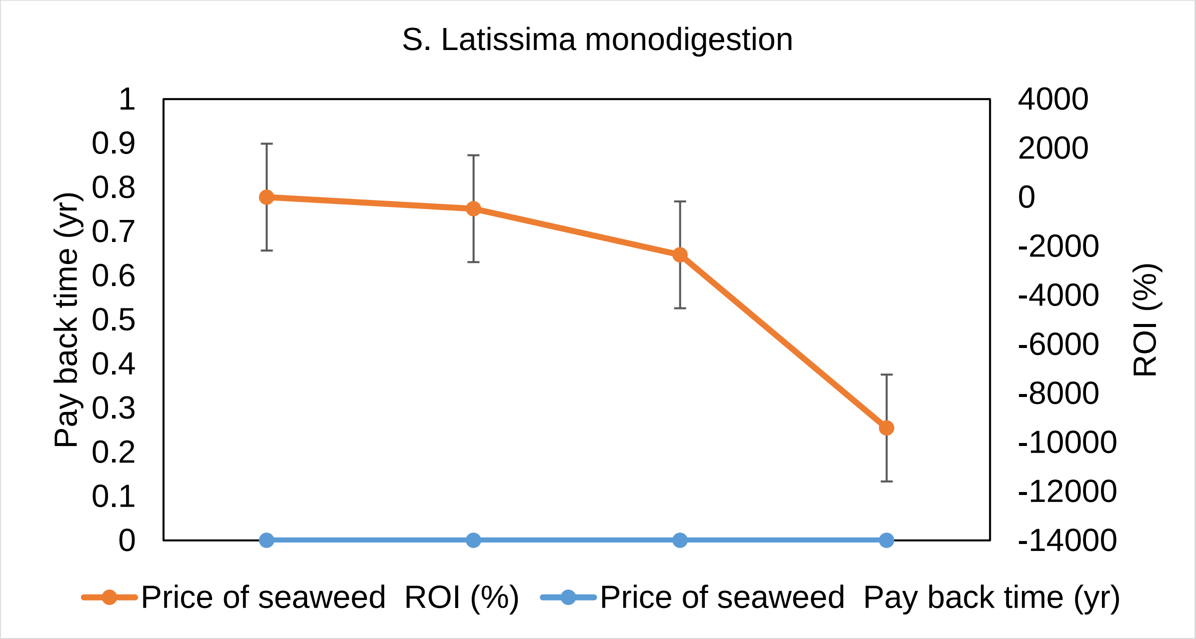

Figure 57 Assessment of *S. Latissima* as a monodigestion feedstock

The economic benefits as discussed in (Section 1.15) are analysed using internal rate of return (IRR), return on investment (ROI) and payback period which vary with different AD plants. From the literature it is seen that choice of feedstock is critical for the positive returns on any AD plant. In this study, we have analysed ROI and payback period for *S. Latissima* as a mono digestion and co-digestion feedstock. These parameters were chosen as this study focuses only on the selectivity of the best feedstock combination from the range of feedstock tested to produce the highest ROI in the shortest payback period. For a typical AD plant, an IRR higher than of 8% was suggested to provide profits with a payback period of 17 years using the North Atlantic species of *Laminaria digitata* (Dave *et al.*, 2013). The price of seaweed has a limited effect on the payback time in consideration with the other cost factors and hence payback time is very small as shown in Figure 57.

In this study, the graph shows negative ROI, which can mean lower profits or negative returns during the initial years of the investment which is common at the start of any project. This also shows that for macroalgae biomass based mono-digestion, the process is not economically viable and a positive rate of return on investment of 2% with payback time of less than a year is only possible when the macroalgae biomass is free of any cost. However, this can only be made possible if macroalgae is available in bulk and is cheaper to harvest, which at present is not practical.

8.5 Base case scenario for co-digestion

The base case scenario for co-digestion was considered in order to better understand the impact of macroalgae biomass co-digestion on the overall economics of the AD process. The assumptions made were as follows: -

- Macroalgae biomass price is 50 Euros per tonne
- The other feedstock digested with macroalgae biomass are cost free (0 Euros per tonne)
- No gate fee for the main feedstock
- Digestate priced at 5 Euros per tonne

The results obtained for the base case scenario are given in Figure 58 .

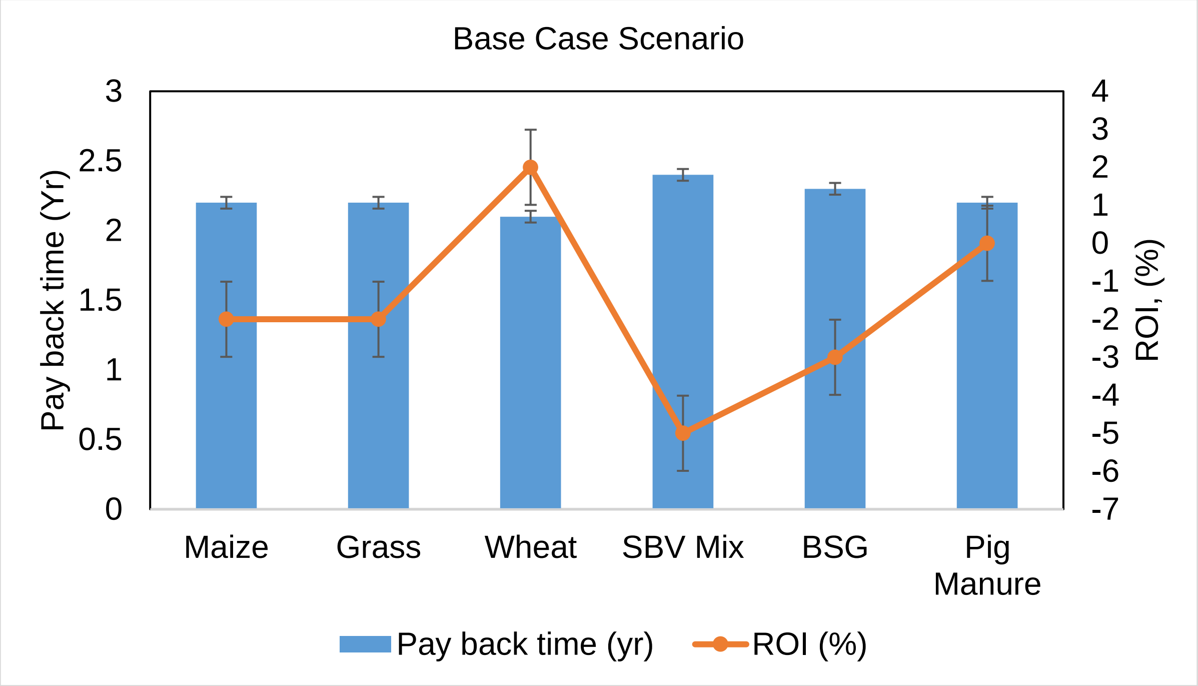

Figure 58 Base case scenario

It can be observed from the results above that such a scenario was not economically favourable even when macroalgae biomass is co-digested with other organic feedstock. The only feasible co-digestion scenario was observed for wheat residues with a ROI of 2% and a payback time of 2 years. The highest quantity of digestate was observed for sugar beet mix of 13527 tonnes. Assuming a 5 Euros per tonne digestate value this would result in a contribution of 11k Euros to the process economics (which was 17% of the total returns for sugar beet mix).

8.6 Co-digestion with gate fees for the organic wastes

As the base case scenario for co-digestion was considered to be not economically viable, modifications were made to the original assumptions. The main feedstock (70%) in co-digestion including the crop waste residues, brewery spent grain and pig manure are all organic wastes in nature. Typically, in an AD plant, where these wastes are admitted for digestion they are to be paid a price of (29 Euros per tonne, WRAP 2015) as gate fees unless they are being used in an on farm AD where the feedstock is in surplus. This was included in the model to analyse any improvements to the economics of the whole system. The macroalgae biomass was priced at 50 Euros per tonne and the digestate was priced at 5 Euros per tonne. The results are shown in Figure 59.

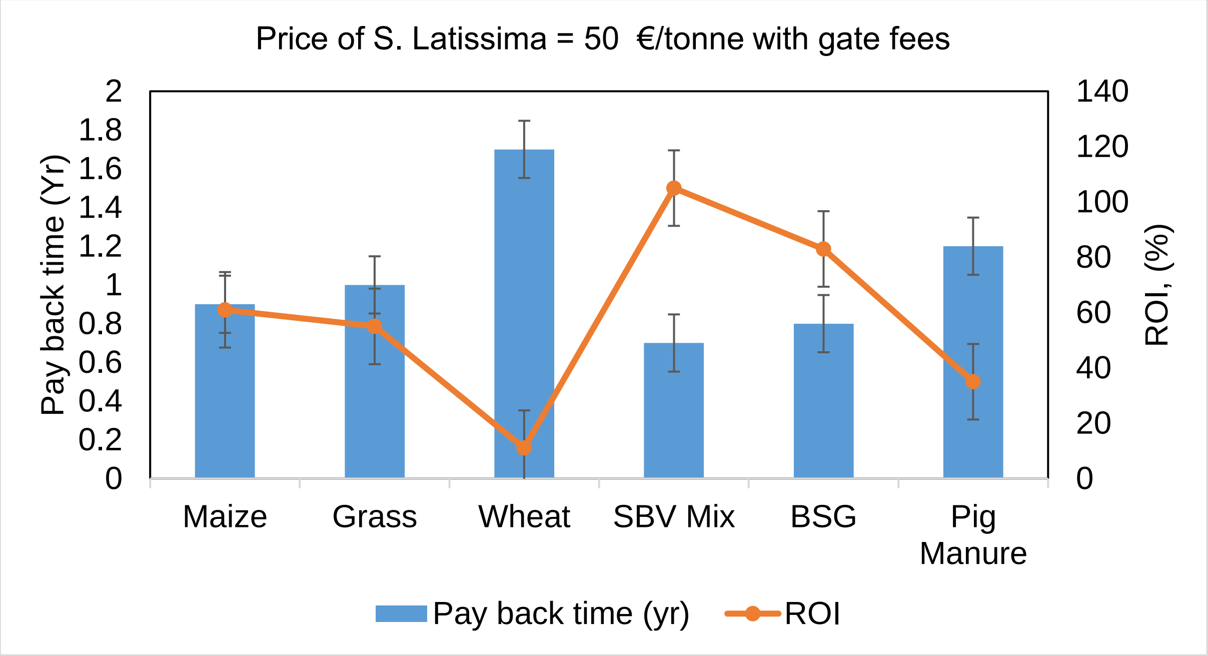

Figure 59 Scenario for macroalgae biomass priced at 50 Euros per tonne

It can be seen that the gate fees for the organic wastes provided a positive variation in the ROI, generating the highest returns for the sugar beet-vegetable mix (105%), and the lowest for the pig manure (35%). The payback time was lowest for the sugar beet-vegetable mix (0.7 years) and the highest for wheat (1.7 years). In this scenario, digestate sale at 5 Euros per tonne did not have any impact on the process economics.

8.7 Sensitivity analysis – Price of the macroalgae biomass

The sensitivity analyses were done in two parts.

- Difference in price of macroalgae biomass without gate fees for the main feedstock
- Difference in price of macroalgae biomass with gate fees for the main feedstock

The first analysis involved changing the price of the macroalgae biomass from 0, 50, 250 and 1000 Euros per tonne with no gate fees for the organic waste main feedstock. The values obtained for ROI (%) with varying macroalgae prices are given in Table 46.

Table 46 Sensitivity analysis of varying macroalgae prices with no gate fees

Return on investment, ROI (%) Gate fees 0€/tonne						
Price of macroalgae biomass	Maize	Grass	Wheat	SBV mix	BSG	Pig Manure
0 €/tonne	2	2	2	1	2	2
50 €/tonne	-2	-2	2	-5	-3	0
250 €/tonne	-16	-14	0	-30	-22	-8
1000 €/tonne	-70	-63	-8	-125	-96	-39

When the values were plotted in a graph the following figure is obtained (Figure 60).

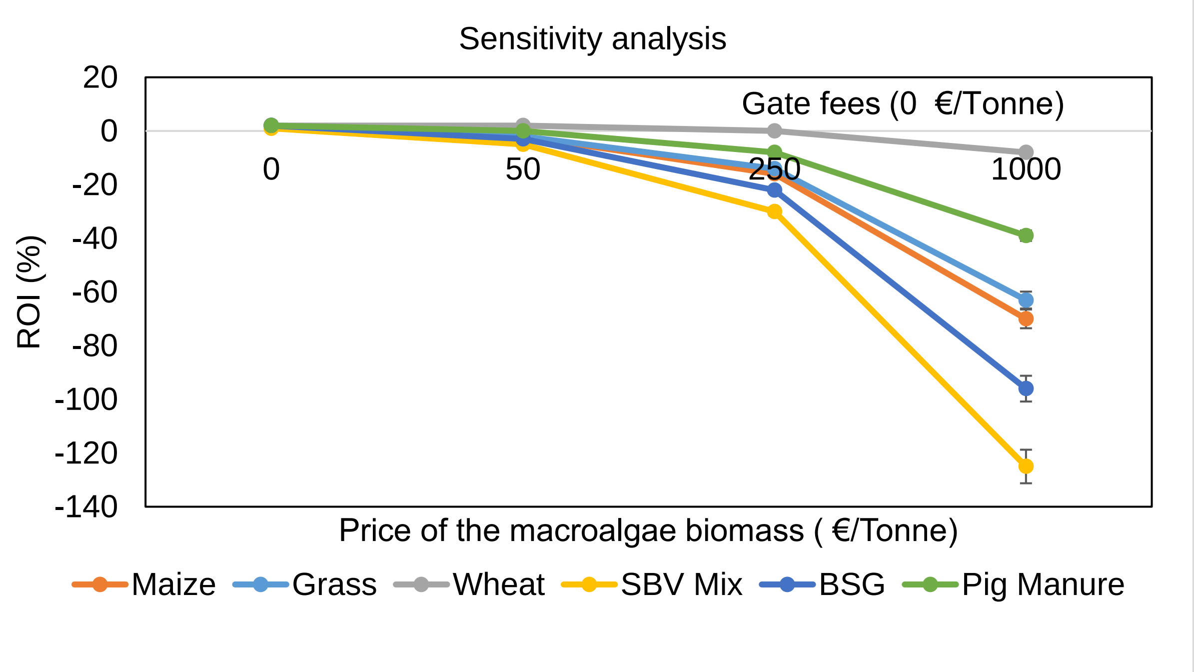

Figure 60 ROI for varying macroalgae prices with no gate fees

As observed in the figure, except for co-digestion with wheat, no other feedstock had a positive ROI for the increased macroalgae biomass prices. As the prices for the macroalgae biomass increases, the returns are observed to be negative as expected. Wheat residues has the lowest sensitivity (2% to -8%) observed as the price of the macroalgae biomass increased however for sugar beet-vegetable mix the sensitivity is higher as the ROI is observed to decrease from 1% to -135%.

8.7.1 Sensitivity analyses – Return on investment with gate fees for the organic feedstock

This sensitivity analysis involved varying the price of the macroalgae biomass with gate fees attached for the main feedstock and observing the return on investment for each co-digestion scenario (Table 47). The gate fees were fixed at 29 Euros per tonne for all of the feedstock and the price of the macroalgae biomass was varied from 0, 50, 250 and 1000 euros per tonne.

Table 47 ROI for varying macroalgae prices with gate fees at 29 Euros per tonne

Return on investment, ROI (%) Gate fees 29€/tonne						
Price of macroalgae biomass	Maize	Grass	Wheat	SBV mix	BSG	Pig Manure
0 €/tonne	64	58	11	111	88	38
50 €/tonne	61	55	11	105	83	35
250 €/tonne	46	42	9	80	63	27
1000 €/tonne	-8	-7	1	-15	-10	-3

The obtained results are shown in the figure below (Figure 61).

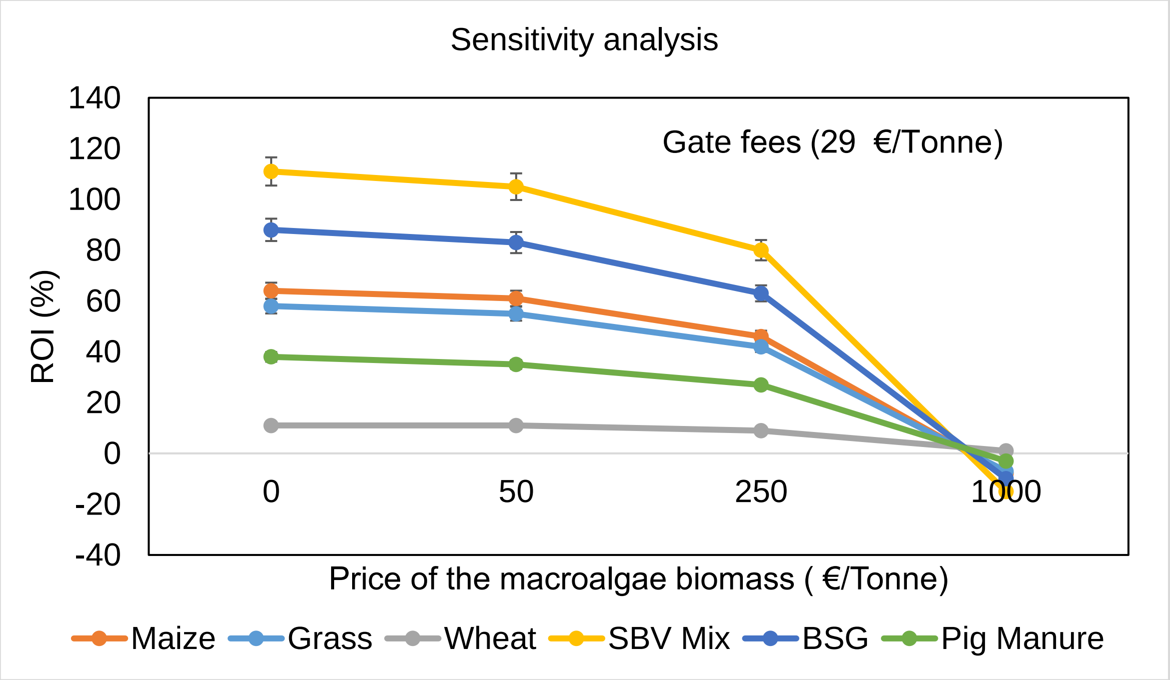

Figure 61 ROI for varying macroalgae prices with gate fees at 29 Euros per tonne

As observed in the graph above, even with the gate fees for the organic feedstock, high feedstock costs associated with the price of macroalgae biomass is prohibitive for the overall economic feasibility of the AD plant AD with co-digestion practices. Ideally, it could be recommended that the price of the macroalgae biomass should be between 50 and 250 Euros for a positive ROI if intended to perform co-digestion with macroalgae biomass for these main feedstock.

Sugar beet – vegetable mix (105%) demonstrated the highest methane production yield while wheat residues demonstrated the lowest with the ROI percentages (11%). All of the feedstock had positive ROI when cost of the macroalgae biomass is free (as expected) however only wheat had a positive ROI for the highest macroalgae biomass price (1000 Euros/Tonne).

8.7.2 Sensitivity analyses – Payback time with gate fees for the organic feedstock

This sensitivity analyses produced the pattern of payback time (years) for each co-digestion with gate fees fixed for the main organic feedstock at 29Euros/tonne (Table 48).

Table 48 Pay back time for varying macroalgae prices with gate fees at 29 Euros per tonne

Pay Back time (years) With gate fees at 29€/tonne						
Price of macroalgae biomass	Maize	Grass	Wheat	SBV mix	BSG	Pig Manure
0 €/tonne	0.9	1	1.7	0.6	0.7	1.2
50 €/tonne	0.9	1	1.7	0.7	0.8	1.2
250 €/tonne	1.1	1.1	1.8	0.8	0.9	1.4
1000 €/tonne	2.6	2.5	2.1	3.2	2.8	2.3

The result is shown in the graph below (Figure 62).

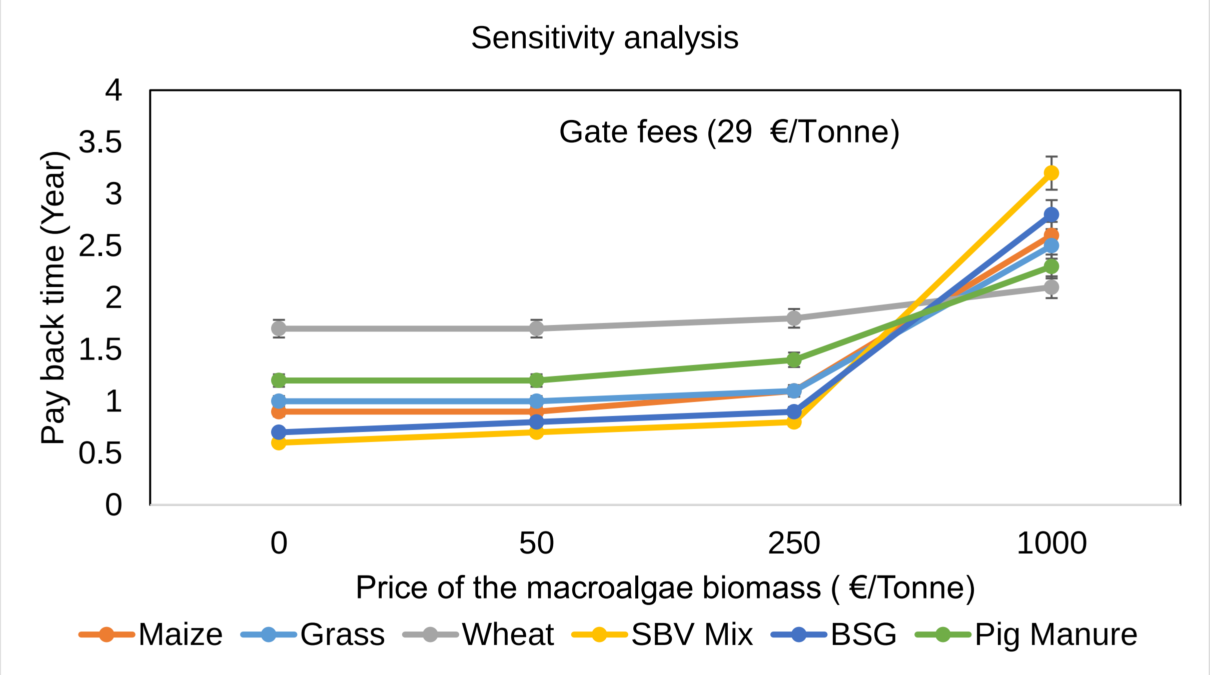

Figure 62 Pay back time for varying macroalgae prices with gate fees at 29 Euros per tonne

As expected, as the macroalgae biomass price increased, the payback time also increased for each substrate in co-digestion. Among the substrates, wheat residue exhibited the least variability in payback time 1.7 and 2.1 years across the various macroalgae biomass prices. Sugar beet-vegetable mixture was found to have the lowest payback time for the price of 0, 50 and 250 Euros (macroalgae biomass) however the highest payback time of 3.2 years if the macroalgae biomass is priced at 1000 Euros per tonne.

8.8 Discussion

Anaerobic digestion has been used in the UK for sewage sludge management in the UK since the mid twentieth century however in the recent decades it has been more prevalent in treating all kinds of wastes including food waste, farm waste, green garden waste and other organic wastes such as industrial processing wastes and municipal solid wastes. Regulations such as Landfill directive (1999), Waste Directive (European Commission, 2008), Renewable energy Directive (EU Commission, 2009) and Waste framework directive (European Commission, 2009) has attributed to the progress in waste treatment sector including wide scale adoption of AD in to small and medium scale in the UK. In the International Energy Agency report (IEA Task 37) UK was identified with around 214 AD plants with 40% treating organic wastes. The UK Department of Environment, Food and Rural Affairs (DEFRA) included increased energy generation from waste through AD in the UK government's structural reform plan in 2010 (Anaerobic digestion strategy and action plan, DEFRA 2011) and it was estimated that the potential for AD in the heat and electricity in the UK is between 3 and 5 TWh by 2020. Moreover to further increase the development of AD in the UK, the Government's waste and resources action program (WRAP) also set up a loan of £10 m over 4 years to help stimulate the investment in AD infrastructure (Evangelisti *et al.*, 2014). Waste management is the main benefit and motivation of AD, and electricity and heat generation, digestate sale are considered as extra options for income generation through AD. For larger deployment of AD, on farm AD and centralised AD farms have been more frequently proposed in the recent years in Europe and in the UK (Cave, 2013). Currently UK AD industry has sufficient capacity to power 1.2 million homes, and reducing emissions by 1% per year, however it has the potential to reduce emissions by 5% if it reaches its full potential whilst improving energy and food security and improving air quality by converting organic wastes into renewable heat, and power, cleaner transport fuel and a nutrient rich biofertiliser (ADBA, 2018b). According to ADBA (2018) AD map, there are 357 agricultural, 45 industrial, 109 municipal/ commercial, and 162 sewage sludge AD plants in the UK out of which 563 focus on renewable electricity production, 97 on biomethane production and only 38 on renewable heat production. These are figures based on operational plants and do not include the plants given licenses or under construction (ADBA, 2018a).

Agricultural AD has been adopted widely in the UK treating a variety of feedstock including energy crops and crop waste residues in on farm AD plants. An example is Vale Green Energy, with 2 AD plants at Spring Hill and Rotherdale. The feedstock used for biogas production is from energy crops including grass, maize, sugar beet and wheat. The input to the digesters is around 140 tonnes per day, generating biomethane of 7800 m^3/day at Springhill and up to 22000 m^3/day at Rotherdale digester plant into the national gas grid (Vale green Energy website, 2018).

Utilisation of distillery waste and brewery waste for AD has also been exhibited at small and medium scale for biomethane and electricity generation. For example, Adnams Brewery (96m^3 biomethane/hour), Dailuaine Distillery (0.5 MWe), Girvan distillery (5.5 MWe, 2500 m^3 biomethane/hour), Glenfiddich distillery (3.5 MWe, 1500m^3 biomethane/hour), Heineken Royal Brewery (0.4 MWe), Lancaster Brewery (0.1 MWe), Sharps Brewery (0.2 MWe) etc.

JJ Power Ltd based in Gloucestershire in the UK utilises dilute slurry from 400 sow pig unit in addition to 100 beef cows and chicken litter for electricity generation of 350kW on a daily basis. The additional feedstock used include forage maize, and hybrid rye as well as a small amount of pasteurised food waste from neighbouring areas. The farm has been successful in utilising dilute liquid slurry and even though energy generation is not comparatively higher but is a typical example of small scale on farm AD digester utilising pig slurry. The digestate generated is used as an effective fertiliser. Another case study is of Geotech AD plant at Harper Adams Energy in Edgmont, Newport, Shropshire where renewable electricity is produced from a mix of feedstock including 8500 tonnes of municipal food waste from households, restaurants, and 17000 tonnes of cow and pig slurry from campus on farm's 400 cow dairy unit and 2500 pigs (ADBA, 2017).

Defra's 'Anaerobic Digestion Shared Goals' aspires a target of 1000 farm based AD plants in the UK by 2020. Even though the numbers have increased in the last few years, achieving the target still seems unachievable unless it is adopted at small scale i.e. farm based AD plant. European AD knowledge especially the Danish and German case studies will be invaluable which has had solid governmental support for decades. However, the case is different in the UK owing to the difference in average type and size of farms, farming practices and environmental incentives. Therefore to achieve the targets and for sustainable energy generation through AD it is essential to highlight the benefits of AD to the farming community, where AD will have the greatest effect, reducing the environmental impact of farm pollution and also in a cost effective way for the farmer (Bywater, 2011). In this context, it is self-explanatory that there is a requirement for more analysis on the efficiency of the technical aspect of AD process hand in hand with the economic feasibility of the technology in a UK environment especially for a newer feedstock such as macroalgae biomass.

Therefore, in this study, stakeholder analyses focused on three main questions:

- How does the cost of macroalgae biomass affect the biomass utilisation for AD?
- Will co-digestion make the bioenergy production from macroalgae biomass sustainable?
- Will the digestate sale as fertiliser be beneficial for the overall economics of the system?

These questions were viewed from different perspectives with the stakeholders involved in this study. The stakeholders who participated in this study were Queen's University Belfast (Research Organisation - macroalgae biomass cultivation team), SAMS (Laboratory and analyses organisation - macroalgae biomass cultivation farmers and analysts team), Dingle Bay macroalgae biomass ltd (macroalgae biomass farmer in Ireland), Severn Trent WTTP – Minworth (wastewater treatment utility and operators of large scale AD plant) and Vale Green Energy (On farm AD operators).

Scottish association of marine sciences (SAMS), based in Oban, Scotland, is an independent non-profit Scottish charity organisation undertaking research into all aspects of marine systems, with specialist interest in ocean processes, climate change, marine conservation, blue economy of aquaculture, marine biotechnology, ocean energy and fisheries (SAMS website, 2018). Queen's University Belfast, based in Belfast, Northern Ireland, has had a long history of macroalgae research based at Queen's Marine Laboratory with expertise in local species, hatchery cultivation and extending long line cultivation of macroalgae biomass in Strangford Lough. They have been involved with interesting projects such as EnAlgae (Interreg IVB funded project) and currently running Sea gas project (BBSRC/EPSRC funded) of growing of Kelp species for AD and for a wide range of human nutritive and pharmaceutical products (Queen's University website, 2018).

Vale Green Energy as discussed above is an energy plant based in Worcestershire, UK, utilising AD and solar power for sustainable energy production (Vale Green Energy website, 2018). Severn Trent Wastewater treatment plant based in Minworth, West Midlands UK, is the second largest sewage treatment works in the country. The plant has sludge digestion, combined heat and power generation to produce a stabilised sludge product and biogas. The digestate or the sludge product is used for spreading in agriculture and biogas is exported to the gas grid via a gas to grid plant and combusted to generate heat for installation purposes or power in the wider use in the wastewater treatment works (Water, 2017).

Stakeholder analysis in this study did not involve any formal interview rather a much more informal approach was used to understand their knowledge and understanding of AD, its implementation, benefits and challenges. A lot of information was collected during the site visits for sample collection, and through email conversations. The responses obtained from the stakeholders are described according to their area of expertise and valued as practitioner's perspectives on AD and its adoption in the UK.

Cost was a predominant factor for macroalgae biomass cultivators (QUB, SAMS, and Dingle Bay farm) as it determined the route to market and overall economics generated from the biomass. According to the macroalgae biomass farmers, the biomass that is currently intended for human consumption or nutrition products provides a higher economic yield. However, if the biomass is intended for bioenergy generation, the stakeholders held the opinion that the market price would need to be lowered (to approximately less than 50 Euros per tonne) from currently around 100 euros per kilo. At both QUB and SAMS, macroalgae biomass is grown only for research purposes, therefore commercial level information on the economics could not be retrieved for the purpose of this study.

Vale green Energy supported the concept of co-digestion of macroalgae biomass with agricultural crop wastes as they currently practice co-digestion of various different crops and wastes generated from the farm. The energy generated on site is currently utilised for various purposes and the site operators were keen to test the probability of using a seasonal feedstock such as macroalgae biomass. However, large scale AD users such as Minworth were more cautious of the proposed approach to co-digestion predominantly because of the scale of its application. They recommended pilot scale adoptions of the technology prior to large scale AD.

With regards to the digestate, vale green energy was supportive as they currently used AD digestate as fertiliser on their site. However, currently the price of digestate is considered low in the market therefore the stakeholders agreed that specific high value N, P, K (Nitrogen, Phosphorus and Potassium respectively) should be extracted from the digestate first to enhance the overall process economics. Macroalgae biomass cultivators also held the opinion that if macroalgae biomass waste was utilised as a fertiliser in IMTA (integrated multi trophic aquaculture) systems, it could generate more income for the macroalgae biomass cultivators and fish and oyster farmers equally. SAMS have previously published a number of studies for the ideal IMTA system with combined macroalgae biomass cultivation.

The following sections will discuss the variability found among the feedstock in terms of the economics and the sensitivity analyses performed on the price of macroalgae biomass and its impact on the digester economics.

8.9 Impact of feedstock variability on process economics

The process economics of macroalgae biomass utilisation (as the single feedstock for AD) was found to be economically unviable. Even when macroalgae biomass was priced at 0 Euros per tonne, an ROI of only 2% could be generated. As the price of macroalgae biomass increased, the ROI demonstrated increasingly negative values (up to -9000%). This is in agreement with the literature where macroalgae with lesser solids content is shown to require more quantities in wet weight to feed the digester which will be reflected in higher feedstock costs resulting in negative rates on investment if used for biogas production. In addition, there is little established data on long term anaerobic digestion of macroalgae biomass and little agreement on the yields of macroalgae biomass per hectare per annum which differs by geographical location, and related environmental conditions, nutrient levels, methods of cultivation and harvest type (Murphy *et al.*, 2015).

To improve the digester economics, co-digestion of different feedstock was considered in this study. The agricultural crop waste residues analysed included wheat, maize, sugar beet – vegetable mix, and grass. Pig manure pellets and brewery spent grain was also analysed in this techno-economic study. A base case scenario where macroalgae biomass was priced at 50 Euros per tonne (Dave *et al.*, 2013) was chosen for all the scenarios considered and compared in this study.

8.9.1 Agricultural crop waste feedstock and Macroalgae biomass

When the feedstock was compared assuming macroalgae biomass price is 0 Euros per tonne, wheat residues, sugar beet mix, maize and grass demonstrated a2% on ROI with a payback time of 2 years. In the base case scenario where macroalgae biomass is priced at 50 Euros per tonne, among the agricultural crop wastes, only wheat was observed to have a positive ROI (2%) with a payback time of 2 years. This can be linked to the characteristics of wheat biomass with its higher volatile solids content (84%WW) and lower ash content of 1.4%WW. All the other feedstock had negative ROI values with a payback time between 2-3 years. In addition, as macroalgae biomass price increased from 50 to 250 and 1000 Euros, the scenarios became less economically viable with negative returns and longer payback times. Agricultural crop wastes include the non-food based portion including the leaves, stalks, cobs trimmings, husk and straw, grasses and animal waste. A large quantity of crop waste is produced annually around the world and is disposed of in the open environment. The disposal of the crop waste residues is not considered a sustainable practice and direct combustion of biomass results in emission of 1599kg CO_2 per tonne of dry biomass (Chandra *et al.*, 2012). Therefore, with the aid of co-digestion practices these wastes can be utilised to extract their energy potential with a potential of application in on farm AD facilities.

8.9.2 Pig Manure and Macroalgae biomass

For pig manure, when the organic feedstock is free, a ROI of 2% with a payback time of 2.1 years was observed When the base case scenario is considered, pig manure has a 0% ROI with a payback time of 2.2 years, and as price of macroalgae biomass increased, (for 250 euros - -8%, 2.2 years; for 1000 euros - -39% and 13.4 years) negative returns are observed with longer payback times. In our study, the co-digestion of *S. Latissima* and pig manure is shown to have a lower C/N ratio of 10. Co-digestion is a complex process and literature suggest that the performance efficiency of co-digestion is dependent on a variety of factors including the physical and chemical characteristics of the wastes chosen, design and configuration of the AD process, inoculum quality, C/N ratio, organic loading rates, volatile solids and volatile fatty acids content, total ammonia content, pH, temperature of the reactors and mixing (Nalo *et al.*, 2014). Pig slurry pellets used in this study are very different to the traditional animal slurry used for digestion. The total solids content in a slurry is 0.9%WW where as in pellets used in this study is observed to be 90%WW. Even though separation of solid and liquid fraction in a pig slurry is recommended for slurry management, anaerobic digestion of solid fraction is found to be inhibited due to its high solids content (Campos *et al.*, 2008). Therefore, it could be suggested that pig manure pellets are not an appropriate substrate to be mixed with macroalgae biomass and if intended for co-digestion, C/N ratio should be balanced for the optimum process efficiency. This will reflect on the overall methane production from the mix and thereby making the process economically viable.

8.9.3 Brewery spent grain and Macroalgae biomass

Brewery spent grain is an emerging potential feedstock for AD, and therefore considered for co-digestion with macroalgae biomass. For Brewery Spent Grain, 2% ROI with 2 years' payback time is observed when the macroalgae biomass is free. For the base case scenario, -3% ROI with a payback time of 2.2 years is observed. The pattern observed for other feedstock is repeated for BSG as -22% ROI and 4 years' payback time is observed when macroalgae biomass is 250 Euros per tonne and -96%

ROI when macroalgae biomass is 1000 euros per tonne. This could suggest that, for effective co-digestion of feedstock such as BSG, more research is required to optimise effective digestion of the biomass. BSG is currently perceived as a waste that brewers want to dispose due to their environmental challenges of odours with disintegration and increase in rodents in their premises however biovalerisation of BSG is found to hold a strategic economic position in the EU due to its availability and various potential uses in food industry (Vitanza *et al.*, 2016). Currently it is used as an animal feed which most of the breweries sell to the farmers with no cost. However the farmers will be responsible for transporting the wet BSG to the farms which upon analysis can cost up to £38/ton in the UK market (Ben-Hamed *et al.*, 2011). So utilising for AD can be a better option for economic as well as environmental benefits however BSG is predominantly a ligno-cellulosic biomass which could potentially be a limiting factor for full utilisation of the biomass for AD and pre-treatment may be required to enhance the digestibility of the biomass. However, from an economical point of view, addition of a pre-treatment technology can only lead to an increase in the investments which can challenge the economic viability of the process.

The key observations at this stage are as follows: -

- Wheat was the only organic feedstock found to be economically viable for co-digestion with macroalgae biomass at a 70 (organic feedstock):30 (*S. Latissima*) ratio used in this study with higher percentages of macroalgae biomass further reducing the economic viability of co-digestion
- The current market price of macroalgae biomass is the predominant factor determining the economic viability of the whole process.
- As the price of macroalgae biomass increased, the process becomes less economically viable for co-digestion with increasingly negative returns and longer payback times.

8.10 Sensitivity analysis

Even though a variety of techno-economic studies have focused on the co-digestion of traditional feedstock, fewer studies have focused on the techno-economics of co-digestion with newer feedstock such as macroalgae biomass. There are, therefore, limited studies to compare against the results obtained in this study. Sensitivity analyses were performed on the variability of the market price for macroalgae biomass, income from sale of digestate (as a fertiliser) and also variability in gate fees for the organic feedstock chosen for this study.

8.10.1 Impact of market price on the economics of macroalgae biomass co-digestion.

The prices adopted in this study were 0, 50, 250, and 1000 Euros per tonne as described previously in Section 8.4. For mono-digestion of macroalgae, the process was not found economically viable if the biomass itself is costed into the model due to negative return percentages and longer payback times. A positive return was observed when macroalgae biomass is free, however this is not currently achievable where macroalgae biomass is specifically cultivated for energy due to the high capital and operational cost of the cultivation/harvest techniques. Therefore, in our study the most economically viable and realistic price range for the macroalgae biomass was found to be between 50 and 250 Euros for positive returns. Macroalgae biomass for high value products can achieve a market price of approximately 1000 Euros per tonne. This is the case for biomass which is utilised for human consumption or pharma/nutraceutical grade products. However, literature (The Crown Estate Report) suggests that if macroalgae biomass is to be used for bioenergy generation the price of the biomass should be less than 250 Euros per tonne (Kelly and Dworjanyn, 2008). This is also in agreement with other studies conducted on brown algae biomass for species *Laminaria digitata* by Dave *et al.* (2013). Since *S. Latissima* is also a brown algae biomass, economic feasibility with positive return on investment using AD can be possible only if the price is below 250 Euros. Other techno economic feasibility studies on the biofuel production from macroalgae biomass has reported that adopting a biorefinery approach with understanding in the growth factors of the biomass and better management of aquaculture systems are essential for efficient biofuel production utilising the biomass (Roesijadi *et al.*, 2008). Therefore by lowering production costs and increasing area under cultivation biofuel production from macroalgae biomass can be made economically feasible (Soleymani and Rosentrater, 2017). The price of macroalgae biomass for bioenergy purposes can be reduced by having two harvests one in early spring for the biomass where high quality biomass can be sold for high value products manufacture and use the later summer harvests with epiphytic growth for bioenergy production (Sprujit, 2015). Utilising summer harvests can be very advantageous for AD due to the higher percentage of storage carbohydrates in the biomass which would result in higher methane production in AD (Manns *et al.*, 2017). In addition, studies on *S. Latissima* have also shown that biomass which are fouled with epiphytes can still be used for methane production, therefore there may be an opportunity to capture this poorer quality/ wasted biomass for AD. Even though properties of macroalgae biomass residues after transesterification were found to be a suitable material as fuel pellets, cost effective utilisation of these residues using AD are still needed and yet to be explored after other biofuel processes such as fermentation and extraction of chemicals from the biomass (Maceiras *et al.*, 2011, Wei *et al.*, 2013).

8.10.2 Cost of digestate variation and the impact on the process economics

Digestate is mostly perceived as a burden to be disposed in an AD operation plant although often AD plant operators need to have clear pathways for reutilisation of digestate. However, the market for digestate is not optimised for untreated digestate leading to upgrading and nutrient recovery practices. Primarily because of its low economic value and high volumes, digestate is suggested to play an important role in the process economics. There have been studies in the literature to show that digestate of agricultural origin can be used for growing microalgae biomass with good levels of bioremediation levels thus providing a better way of digestate treatment and avoiding problems by direct land applications (Gerardo *et al.*, 2015). Recycling nutrients from digestate with algal technology is still at its early stage however development of this new technology can support continual growth in AD deployment, new markets and novel uses for digestate without causing a negative environmental impact (Stiles *et al.*, 2018).

Research studies into utilising the digestate from macroalgae AD has often recommended its usage as a soil conditioner or fertiliser (Vanegas and Bartlett, 2015). The study by Ramirez (2015) investigated the effect of the digestate generated from the AD of brown algae *Laminaria digitata* on seed germination and plant growth of sunflower and found that higher concentrations of (20%, 50% and 100%) digestate improved the growth rate of plants. This was attributed to the effect of micro and macro nutrients present in the digestate that stimulated plant development. However, in the latter stage higher dosage of digestate reflected a reduction of growth in plants where it was attributed to either the accumulation of inhibitory compounds from the digestate or nutrient overloading. Overall the use of macroalgae digestate use as a fertiliser was found feasible offering potential for an additional income of revenue owing to the nutrients embedded in the digestate (Ramírez, 2015).

This study explored the impact of market price variability for digestate on the overall economics of co-digestion. It was found that for biomass priced at 50 Euros and digestate sale at 5 Euros per tonne produced little impact on the return on investments. Clearly any increased income from the sale of the digestate will positively impact on the overall profit however the market price for this product is still too low. The other benefit the AD plant can have is possibly use the digestate as a biofertiliser and have reduction in costs related to buying synthetic fertiliser for agricultural purposes.

Scenarios which included the processing of digestate for nutrient recovery were outside of the scope of this study. However, it can be postulated that if products such as nitrates, phosphates and potassium were efficiently and economically recovered from the digestate, then higher profits could potentially be achieved. Food based digestate can have high nutrient content such as readily available N (202kg/ha), Phosphate (16.3kg/ha) and potash (61.5 kg/ha). Nitrates and phosphates are valued higher from the digestate at around 0.95£/kg nutrient for ammonium nitrate (35%N), 0.89 £/kg nutrient for phosphate (46% P2O5) and 0.55£/kg nutrient for potash (60% K2O). However contributions from other inorganic nutrients such as sulphur, magnesium etc. are reported harder to value (Wallace *et al.*, 2011). Even though nitrates are valued the most and are present in the readily available form for plants in digestate under the European Commission nitrates directive, in a nitrate vulnerable zone the amount of N that can be returned to land is restricted limiting application of nitrogen rich digestate in such risk prone regions. In addition, digestate may also contain potentially toxic elements or compounds such as lead, zinc and copper which will vary depending on the feedstock used and process stabilisation nutrients used in AD plants (Stiles *et al.*, 2018).

8.10.3 Gate fees for the organic waste feedstock and the impact on the process economics

A gate fees of 29 Euros was used for studying the effect of gate fees on the process economics. According to the literature, gate fees have decreased in the last few years from 40 Euros per tonne to 29 Euros per tonne (Dick *et al.*, 2016). This techno-economic study only considered a fixed rate for gate fees of 29 Euros and the variation of gate fees was not studied. This was found to be the best feasible scenario for co-digestion with *S. Latissima* in our study. The sensitivity analyses were showed that co-digestion was highly favourable with high rates of returns when the price of macroalgae biomass was 50 Euros per tonne. The most favourable combination for of co-digestion identified in this study was *S. Latissima* plus sugar beet-vegetable mix with a ROI of 105% and a payback time of 0.7 years. The second most favourable combination was BSG with 83% ROI and 0.8 years of payback time. The lowest ROI (11%) and highest payback time (1.7 years) was observed for wheat residues. The results are promising illustrating that given optimised gate fees (50E/ton) a profitable scenario can be achieved for co-digestion of *S. Latissima* with sugar beet-vegetable mix (a traditional AD feedstock) as well as newly emerging biomass sources such as Brewery spent grain. In the UK, BSG has shown an availability of 250 million tonnes per year (Thomas & Rahman, 2006, Kerby and Vriesekoop, 2017) and already Sugar beet is used for AD as a whole crop or from residues after sugar processing. A total volume of 500,000 tonnes are currently produced in the UK alone from the sugar processing waste (Suhartini, 2014). Sugar beet is a root crop very similar to parsnip and is grown widely in the temperate climates of Europe and North America (Sugar). This also suggests that seasonal feedstock such as macroalgae biomass if intended for bioenergy production can produce high rates of methane using the co-digestion mechanisms.

Currently majority of the macroalgae biomass is used for human consumption or high value products extraction. For full utilisation of the biomass for bioenergy production has challenges to overcome before becoming a fully functional reality. For commercial realisation of macroalgae industry in the UK, there are technical challenges as well as legislative challenges to overcome. The magnitude of the environmental impacts associated with macroalgae cultivation or harvest, the transport of the biomass, upstream and downstream processing for bio-products and bioenergy production also need to be considered (Roberts and Upham, 2012). Macroalgae is shown in the literature to have high potential as a feedstock for biorefinery to produce bio materials and bioenergy. In the era of biorefinery, flexibility of process design is also important as the desired product can change depending upon location and market. The main bottle neck is the processing economics and with the current low oil prices it is even more difficult to make algal fuel cost competitive to fossil fuels. Gradually the expenditure has to be lowered with increased outcomes and thereby narrowing the economic gap (Jiang *et al.*, 2016).

AD of *S. Latissima* is shown to have comparable methane potentials to energy crops in this study and is found to be feasible as a co-digestion feedstock (Bauer *et al.*, 2010). For the techno-economic analysis, this study only considered the economic feasibility of an AD process utilising *S. Latissima* as a mono-digestion feedstock and as co-digestion feedstock. The results were represented as a percentage of return and payback time. The study boundary did not consider transportation or cultivation costs into the system boundary. Transportation is a key factor for the development of a macroalgae supply chain as transportation between cultivation sites, processing sites and post processing sites can have potential environmental and economic impact on the utilisation of biomass. Therefore for future environmental and economic assessment of bioenergy production from macroalgae biomass, it is important to consider the transportation to further develop the macroalgae industry in a sustainable fashion (Gegg and Wells, 2017).

9 Conclusions

Reflecting on the experience of doing PhD, I have come to the realisation that I have had a thorough learning process for the researcher in me, whether it had been with the procurement of the samples, planning and performing the experiments, data analysis and finally writing the thesis. Though the learning process has been challenging at times, I had to motivate myself more to get the work completed and now I can truly say that on the whole I have enjoyed the journey. The skills that I have attained through this PhD such as presentation and communication skills, research skills, scientific lab skills are invaluable to both the student and professional in me. In addition, working under my supervisory team for the last few years has given me the opportunity to develop as an independent researcher. Therefore, in conclusion, I would like to say that for me, doing a PhD has been a gratifying experience.

To summarise, this research investigated the potential of third generation biomass feedstock, Saccharina latissima for methane production by anaerobic digestion technology. The literature review identified gaps where environmental conditions of the macroalgae biomass cultivation systems were not researched previously in relation to the methane production potential of the biomass. In addition, no previous work had compared wild and cultivated biomass of the macroalgae species for either their characteristics and methane production. In continuous digestion, the parameters controlling the digestion of the *S. Latissima* was also understood to a limited extend. The co-digestion potential of *S. Latissima* also is an under researched area with no techno-economic feasibility advising the practical application of AD in the UK. Therefore, the key findings of this research are given below.

- *S. Latissima* can be considered as a biomass feedstock for AD. The choice of cultivation site is perhaps the most important consideration as environmental conditions are shown to be influential on biomass characteristics. The environmental conditions in a specific location are complex and inter-related and have a combined effect on biomass growth and subsequent biochemical composition. *S. Latissima,* harvested at different points of the year, exhibit significant differences in their characteristics. From an AD perspective, it is better to target the biomass with highest carbon percentages. Wild macroalgae biomass was found variable in their biomass characteristics owing to their age and maturity in comparison to cultivated biomass [Results – Chapter 4].

- Biochemical methane potential of *S. Latissima* has been found to vary with localised environmental conditions, harvest times and growth type. Methane yields were found to have an inverse relationship with the volatile solids and ash percentages of the biomass. Results showed that environmental conditions favouring production of higher volatile solids, and inorganic content in the biomass may not necessarily produce biomass with higher methane potentials. Methane production of *S. Latissima* harvested from different times were also found significantly different from each other. When sourcing biomass for anaerobic digestion, the biomethane yields from wild and cultivated samples are found not significantly different to each other. However, due to their variable characteristics and negative impact on the ecological balance of marine ecosystem, wild biomass is not advised for anaerobic digestion [Results – Chapter 5].
- Semi continuous digestion of *S. Latissima* was performed for three hydraulic retention times for a total of 105 days. Higher organic loading rate is feasible in the first HRT however leads to accumulation and lowered methane production towards the third HRT. Alkalinity values were found to be lower than desirable which suggests buffer addition ($NaOH$,KOH) might be necessary for full scale applications. Trace elements addition was found to enhance digestion reflected as relatively higher methane yields. Increasing the temperature of digestion to thermophilic conditions was not found to be favourable in this study. Both methane production (as biogas volume) and methane concentrations were significantly lower than mesophilic digesters. This could be also because the bacteria could acclimatise to mesophilic temperatures easily than thermophilic temperatures. However thermophilic digesters exhibited higher volatile solids reduction and dewaterability rates than mesophilic digesters [Results – Chapter 6].
- Co-digestion with *S. Latissima* at a ratio of 70:30 (macroalgae: feedstock) ratios was shown to increase the rate of methane production and methane yields for all feedstock except pig manure and brewery spent grain. The highest net percentage increase in methane yield was obtained for the co-digestion of macroalgae biomass with wheat and the lowest net percentage reduction for pig manure. This was attributed to the biochemical composition of the feedstock, where easily accessible carbohydrates in macroalgae and wheat resulted in higher methane yields. The carbon to nitrogen ratio of the combined feedstock

is found to be important for effective co-digestion with *S. Latissima* and the optimal range is found to be between 20 and 30. A synergistic effect was observed when co-digesting *S. Latissima* with wheat and sugar beet – vegetable mix, however antagonistic effects were observed during co-digestion with pig manure. Even though co-digestion is technically favourable, the seasonal supply of macroalgae biomass might affect wider applications especially in winter where the biomass will be scarce [Results – Chapter 7].

- According to the techno-economic assessment, mono digestion of *S. Latissima* was not found to be economically viable. This was primarily due to the high costs associated with the procurement of macroalgae biomass. However, co-digestion with sugar beet – vegetable mix was found to be the most economically viable scenario when the gate fees of the organic feedstock were set at 29 Euros per tonne and macroalgae biomass priced at 50 Euros per tonne. Sale of unprocessed digestate (which currently has a low market value of 5 Euros per tonne) was also not found to improve the process economics in any of the scenarios [Results – Chapter 8].

To conclude, this study showed that the species *S. Latissima* is feasible for anaerobic digestion in North West Europe region however there needs to be significant research efforts in exploring other suitable species for AD to enhance macroalgae biomass utilisation in full scale AD operations. Further advancements in biorefinery approach will also increase the overall economic viability of AD processes using macroalgae biomass.

10 Recommendations

Macroalgae biomass species, Saccharina latissima is shown to be feasible for methane production via anaerobic digestion however the adoption of technology is still at its nascent stages here in the UK and north western Europe due to lack of commercial level cultivation farms for the biomass which limits the amount of biomass that can be harvested year around for renewable energy production. So ideally, such a technology will work efficiently in places around the world such as South East Asia., where there is already a large amount of macroalgae biomass available through competent cultivation farms for different macroalgae species. Even then the technology needs to be tailored to suit their needs for human consumption and energy production simultaneously. The easiest way to encourage new feedstock utilisation such as macroalgae biomass into the existing AD plants could be through co-digestion mechanisms. A continuous and supportive policy framework is also essential for such technologies to be able to contribute to the national and international energy targets.

Therefore, recommendations from this research study are as follows: -

- Technical recommendations

1. Further research is required to fully understand the impact of inter related environmental conditions on macroalgae biomass characteristics which are important when identifying the optimal cultivation site for bioenergy production.
2. Significant research is needed to identify the impact of *S. Latissima* growth cycle on biochemical composition of *S. Latissima* especially on the carbohydrates, lipids and fats profiles to be evaluated over longer periods of time to ascertain long term variability in the biomass and therefore optimise harvesting practices.
3. For continuous applications involving *S. Latissima* monitoring of volatile fatty acids profile would be recommended to identify optimal organic loading rates and corresponding retention times. Costs permitting, trace elements customised according to macroalgal biochemical composition should be added to avoid combined inhibitive effects from the different elements in the biomass and the prepared solution.

- Implementation recommendations

1. To achieve favourable process economics for anaerobic digestion of *S. Latissima* it is recommended that further research efforts should identify ways of lowering the costs of cultivation and thereby reducing the cost of macroalgae biomass.

2. In order to optimise co-digestion practices using macroalgae biomass, further studies should determine suitable feedstock other than those used in this study to be digested with macroalgae biomass and/or utilising nutrient rich macroalgae biomass as a supplement addition for balancing C/N ratios for digestion involving multiple feedstock.

- Policy recommendations

1. Finally, if intended for bioenergy production in future, a recommendation would be to develop policies to incentivise cultivation of macroalgae biomass and thereby making biomass an attractive feedstock for wider applications.

11 Publications

- R. Paul, S. Suhartini, L. Melville, and M. Sulu. “Semi continuous digestion of Saccharina latissima: A comparison of mesophilic and thermophilic digestion.” (2018) – ADNET Conference, University of York, UK
- R. Paul, S. Suhartini, L. Melville, and M. Sulu. “Anaerobic digestion of Saccharina latissima – Challenges and prospects of utilising seaweed as a novel feedstock for co-digestion” (2017), 15th IWA World conference on Anaerobic Digestion, Beijing, China.
- R. Paul, S. Suhartini, L. Melville, and M. Sulu. “Feasibility of cultivated Saccharina latissimi as a novel co-digestion feedstock” (2017) –ADNET Conference, University of Birmingham, UK
- R. Paul, S. Suhartini, L. Melville, and M. Sulu. “Anaerobic co-digestion of Saccharina latissima and agricultural crop waste residues for increased methane production” (2016) –ADNET Conference, University of Birmingham, UK
- R. Paul, L. Melville, and M. Sulu, "Anaerobic Digestion of Micro and Macro Algae, Pre-treatment and Co-Digestion-Biomass — A Review for a Better Practice," International Journal of Environmental Science and Development vol. 7, no. 9, pp. 646-650, 2016.
- R. Paul, L. Melville, and M. Sulu. Anaerobic Digestion of Micro and Macro Algae, Pre-treatment and Co-Digestion-Biomass – A review for Better practice, ICBEE 2014: September, Paris, France.
- R. Paul, L. Melville, (2012) Energy from Algae - A Biorefinery Concept. 2nd International Conference on Energy, Environment and Sustainable Development 2012 (EESD2012), Feb 27-29, 2012 Mehran University of Engineering and Technology, Jamshoro, Pakistan.
- R. Paul, L. Melville, (2011), ‘Bioenergy from Microalgae – A bio-refinery approach’, Kerala Environment Congress, 2011, India.

12 References

ABBASI, T., TAUSEEF, S. M. & ABBASI, S. A. 2012. Anaerobic digestion for global warming control and energy generation-An overview. *RENEWABLE & SUSTAINABLE ENERGY REVIEWS,* 16**,** 3228-3242.

ABOUELENIEN, F., NAMBA, Y., KOSSEVA, M. R., NISHIO, N. & NAKASHIMADA, Y. 2014. Enhancement of methane production from co-digestion of chicken manure with agricultural wastes. *Bioresource technology,* 159**,** 80-87.

ADAMS, J., TOOP, T., DONNISON, I. S. & GALLAGHER, J. A. 2011. Seasonal variation in Laminaria digitata and its impact on biochemical conversion routes to biofuels. *Bioresource Technology,* 102**,** 9976-9984.

ADBA. 2017. *Case study: JJ Power Ltd* [Online]. Available: http://adbioresources.org/library/case-studies/case-study-james-hart/ [Accessed July, 2017].

ADBA. 2018a. *AD Map* [Online]. UK: ADBA. Available: http://adbioresources.org/map [Accessed January, 2018].

ADBA 2018b. ADBA Market Report UK: Anaerobic Digestion and Bioresources Association.

AE LEE, J. & BRINKHUIS, B. H. 1988. SEASONAL LIGHT AND TEMPERATURE INTERACTION EFFECTS ON DEVELOPMENT OF LAMINARIA SACCHARINA (PHAEOPHYTA) GAMETOPHYTES AND JUVENILE SPOROPHYTES 1. *Journal of Phycology,* 24**,** 181-191.

ALASWAD, A., DASSISTI, M., PRESCOTT, T. & OLABI, A. G. 2015. Technologies and developments of third generation biofuel production. *Renewable and Sustainable Energy Reviews,* 51**,** 1446-1460.

ALLEN, E., WALL, D. M., HERRMANN, C. & MURPHY, J. D. 2016. A detailed assessment of resource of biomethane from first, second and third generation substrates. *Renewable Energy,* 87**,** 656-665.

ALLEN, E., WALL, D. M., HERRMANN, C., XIA, A. & MURPHY, J. D. 2015. What is the gross energy yield of third generation gaseous biofuel sourced from seaweed? *Energy,* 81**,** 352-360.

AMON, T., AMON, B., KRYVORUCHKO, V., ZOLLITSCH, W., MAYER, K. & GRUBER, L. 2007. Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. *Agriculture, Ecosystems and Environment,* 118**,** 173-182.

ANASTASAKIS, K. 2011. *The potential of the production of fuels and chemicals from marine biomass.* University of Leeds.

ANASTASAKIS, K. & ROSS, A. 2011. Hydrothermal liquefaction of the brown macro-alga Laminaria saccharina: effect of reaction conditions on product distribution and composition. *Bioresource technology,* 102**,** 4876-4883.

ANDERSON, M. J., CONNELL, S. D., GILLANDERS, B. M., DIEBEL, C. E., BLOM, W. M., SAUNDERS, J. E. & LANDERS, T. J. 2005. Relationships between taxonomic resolution and spatial scales of multivariate variation. *Journal of Animal Ecology,* 74**,** 636-646.

ANGELIDAKI, I., ALVES, M., BOLZONELLA, D., BORZACCONI, L., CAMPOS, J., GUWY, A., KALYUZHNYI, S., JENICEK, P. & VAN LIER, J. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays.

ANGELIDAKI, I. & SANDERS, W. T. M. 2004. Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science & Bio-technology,* 3**,** 117-129.

APHA, A. 1992. WPCF, 1992. *Standard methods for the examination of water and wastewater,* 18**,** 518-523.

APPELS, L., LAUWERS, J., DEGRÃ¨VE, J., HELSEN, L., LIEVENS, B., WILLEMS, K., VAN IMPE, J. & DEWIL, R. 2011. Anaerobic digestion in global bio-energy production: potential and research challenges. *Renewable and Sustainable Energy Reviews,* 15**,** 4295-4301.

APUL, O. G., DOGAN, I. & SANIN, F. D. 2009. Can Capillary Suction Time be an Indicator for Sludge Disintegration? *Journal of Residuals Science & Technology,* 6.

ARAUJO, R., ASSIS, J., AGUILLAR, R., AIROLDI, L., BARBARA, I., BARTSCH, I., BEKKBY, T., CHRISTIE, H., DAVOULT, D. & DERRIEN-COURTEL, S. 2016. Status, trends and drivers of kelp forests in Europe: an expert assessment. *Biodiversity and Conservation,* 25**,** 1319-1348.

ARIS, C. 2014. Wind Power Reassessed: A review of the UK wind resource for electricity generation. *Scientific Alliance Report for the Adam Smith Institute, London: www. adamsmith. org/wp-content/uploads/2014/10/Assessment7. pdf Google Scholar*.

ARO, E.-M. 2016. From first generation biofuels to advanced solar biofuels. *Ambio,* 45**,** 24-31.

ARVANITIS, K. 2016. MACROALGAE IN THE BIOREFINERY: A SUBSTANCE FLOW ANALYSIS AND ENVIRONMENTAL ASSESSMENT OF AN EXTRACTION PROCESS OF THE MAJOR COMPONENTS IN SACCHARINA LATISSIMA.

AZEVEDO, I. C., MARINHO, G. S., SILVA, D. M. & SOUSA-PINTO, I. 2016. Pilot scale land-based cultivation of Saccharina latissima Linnaeus at southern European climate conditions: Growth and nutrient uptake at high temperatures. *Aquaculture,* 459**,** 166-172.

BANKS, C. J., ZHANG, Y., JIANG, Y. & HEAVEN, S. 2012. Trace element requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresource technology,* 104**,** 127-135.

BATSTONE, D. J., KELLER, J., ANGELIDAKI, I., KALYUZHNYI, S. V., PAVLOSTATHIS, S. G., ROZZI, A., SANDERS, W. T. M., SIEGRIST, H. & VAVILIN, V. A. 2002. The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science and Technology,* 45**,** 65-73.

BAUER, A., LEONHARTSBERGER, C., BÖSCH, P., AMON, B., FRIEDL, A. & AMON, T. 2010. Analysis of methane yields from energy crops and agricultural by-products and estimation of energy potential from sustainable crop rotation systems in EU-27. *Clean Technologies and Environmental Policy,* 12**,** 153-161.

BEAVIS, A. & CHARLIER, R. H. 1987. AN ECONOMIC APPRAISAL FOR THE ONSHORE CULTIVATION OF LAMINARIA SPP. *Hydrobiologia,* 151**,** 387-398.

BELL, G. & REDPATH MUSEUM, M. U., 859 SHERBROOKE STREET WEST, MONTREAL, QUEBEC, CANADA H31 1B1 1997. The evolution of the life cycle of brown seaweeds. *Biological Journal of the Linnean Society,* 60**,** 21-38.

BEN-HAMED, U., SEDDIGHI, H. & THOMAS, K. 2011. Economic returns of using brewery's spent grain in animal feed. *World Academy of Science, Engineering and Technology,* 50**,** 695-698.

BENABDALLAH EL HADJ, T., ASTALS, S., GALI, A., MACE, S. & MATA-ALVAREZ, J. 2009. Ammonia influence in anaerobic digestion of OFMSW. *Water science and technology,* 59**,** 1153-1158.

BIKKER, P., VAN KRIMPEN, M. M., VAN WIKSELAAR, P., HOUWELING-TAN, B., SCACCIA, N., VAN HAL, J. W., HUIJGEN, W. J., CONE, J. W. & LÓPEZ-CONTRERAS, A. M. 2016. Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels. *Journal of applied phycology,* 28**,** 3511-3525.

BIOPROCESS CONTROL. 2017. Available: http://www.bioprocesscontrol.com/ [Accessed June, 2017].

BIXLER, H. J. & PORSE, H. 2011. A decade of change in the seaweed hydrocolloids industry. *Journal of applied Phycology,* 23**,** 321-335.

BOLD, H. C. & WYNNE, M. 1978. Introduction to algae: structure and reproduction. Upper Sadle River. NJ: Prentice-Hall Inc.

BLACK, W. 1948. The seasonal variation in chemical constitution of some of the sub-littoral seaweeds common to scotland. Part I. laminaria cloustoni. *Journal of Chemical Technology and Biotechnology,* 67**,** 165-168.

BLACK, W. Á. 1950. The seasonal variation in weight and chemical composition of the common British Laminariaceae. *Journal of the Marine Biological Association of the United Kingdom,* 29**,** 45-72.

BOCSI, R., HANAK, L., HORVATH, G. & HODAI, Z. Processing residuals from microalga technology. 2013. IEEE, 1-5.

BOLZONELLA, D. 2011. Monitoring parameters in anaerobic digestion processes. *Jyvaskyla Summer School*.

BOLZONELLA, D., INNOCENTI, L., PAVAN, P., TRAVERSO, P. & CECCHI, F. 2003. Semi-dry thermophilic anaerobic digestion of the organic fraction of municipal solid waste: focusing on the start-up phase. *Bioresource Technology,* 86**,** 123-129.

BORINES, M. G., DE LEON, R. L. & CUELLO, J. L. 2013. Bioethanol production from the macroalgae Sargassum spp. *Bioresource technology,* 138**,** 22-29.

BOUALLAGUI, H., TOUHAMI, Y., CHEIKH, R. B. & HAMDI, M. 2005. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. *Process Biochemistry,* 40**,** 989-995.

BOUALLAGUI, H., LAHDHEB, H., BEN ROMDAN, E., RACHDI, B. & HAMDI, M. 2009. Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. Journal of Environmental Management, 90, 1844-1849.

BRABER, K. 1995. Anaerobic digestion of municipal solid waste: a modern waste disposal option on the verge of breakthrough. *Biomass and bioenergy,* 9**,** 365-376.

BRACMORT, K. 2015. The Renewable Fuel Standard (RFS): Cellulosic Biofuels. Congressional Research Service, Washington, DC, accessed Apr, 3, 2018.

BRENNAN, L. & OWENDE, P. 2010. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and sustainable energy reviews,* 14**,** 557-577.

BRIAND, X. & MORAND, P. 1997. Anaerobic digestion of Ulva sp. 1. Relationship between Ulva composition and methanisation. *Journal of Applied Phycology,* 9**,** 511.

BUNDSCHUH, J., YUSAF, T., MAITY, J. P., NELSON, E., MAMAT, R. & MAHLIA, T. I. 2014. Special Section: Algae-biomass for fuel, electricity and agriculture. *Energy (Oxford),* 78**,** 1-113.

BYWATER, A. 2011. A Review of Anaerobic Digestion Plants on UK Farms-Barriers, Benefits and Case Studies A report prepared with financial support from the Frank Parkinson Agricultural trust. *Warwickshire: RASE*.

CALLAGHAN, F., WASE, D., THAYANITHY, K. & FORSTER, C. 2002. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and bioenergy,* 22**,** 71-77.

CAMPOS, E., ALMIRALL, M., MTNEZ-ALMELA, J., PALATSI, J. & FLOTATS, X. 2008. Feasibility study of the anaerobic digestion of dewatered pig slurry by means of polyacrylamide. *Bioresource Technology,* 99**,** 387-395.

Capuzzo, E. and McKie, T., 2016. Seaweed in the UK and abroad–status, products, limitations, gaps and Cefas role. Cefas Contract Report FC002I.

CARPENTIER, B., FESTINO, C. & AUBART, C. 1988. Anaerobic digestion of flotation sludges from the alginic acid extraction process. Biological wastes, 23, 269-278.

CAVE, S. 2013. Anaerobic Digestion across the UK and Europe. *Nothern Ireland Assembly*.

CECCHI, F., PAVAN, P. & MATA-ALVAREZ, J. 1996. Anaerobic co-digestion of sewage sludge: application to the macroalgae from the Venice lagoon. *Resources, Conservation and recycling,* 17**,** 57-66.

CHANDRA, R., TAKEUCHI, H. & HASEGAWA, T. 2012. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews,* 16**,** 1462-1476.

CHANG, H. N., KIM, N.-J., KANG, J. & JEONG, C. M. 2010. Biomass-derived volatile fatty acid platform for fuels and chemicals. *Biotechnology and Bioprocess engineering,* 15**,** 1-10.

CHEN, Y., CHENG, J. J. & CREAMER, K. S. 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology,* 99**,** 4044-4064.

CHRISTIE, H., FREDRIKSEN, S. & RINDE, E. 1998. Regrowth of kelp and colonization of epiphyte and fauna community after kelp trawling at the coast of Norway. *Recruitment, Colonization and Physical-Chemical Forcing in Marine Biological Systems.* Springer.

CHYNOWETH, D. P., OWENS, J. M. & LEGRAND, R. 2001. Renewable methane from anaerobic digestion of biomass. *Renewable energy,* 22**,** 1-8.

CIRNE, D., PALOUMET, X., BJÖRNSSON, L., ALVES, M. & MATTIASSON, B. 2007. Anaerobic digestion of lipid-rich waste—effects of lipid concentration. *Renewable energy,* 32**,** 965-975.

CLERICI, A. & ALIMONTI, G. World energy resources. 2015 Les Ulis. EDP Sciences, 1001.

COCHRANE, K., DE YOUNG, C., SOTO, D. & BAHRI, T. 2009. Climate change implications for fisheries and aquaculture. *FAO Fisheries and aquaculture technical paper,* 530**,** 212.

COLLET, P., HÉLIAS, A., LARDON, L., RAS, M., GOY, R.-A. & STEYER, J.-P. 2011. Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresource technology,* 102**,** 207-214.

COMMISSION, E. 2018. Renewable energy directive - Energy - European Commission.

COSTA, J., GONÇALVES, P., NOBRE, A. & ALVES, M. 2012. Biomethanation potential of macroalgae Ulva spp. and Gracilaria spp. and in co-digestion with waste activated sludge. *Bioresource technology,* 114**,** 320-326.

CUETOS, M. J., GÓMEZ, X., OTERO, M. & MORÁN, A. 2008. Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). *Biochemical Engineering Journal,* 40**,** 99-106.

CZYRNEK-DELETRE, M. 2017. Holistic sustainability assessment of biomethane systems.

D'ALESSANDRO, E. B. & ANTONIOSI FILHO, N. R. 2016. Concepts and studies on lipid and pigments of microalgae: A review. *Renewable and Sustainable Energy Reviews,* 58**,** 832-841.

DA SILVA MARINHO, G. M. 2016. Bioremediation capacity, nutritional value and biorefining of macroalga Saccharina latissima.

DAVE, A., HUANG, Y., REZVANI, S., MCILVEEN-WRIGHT, D., NOVAES, M. & HEWITT, N. 2013. Techno-economic assessment of biofuel development by anaerobic digestion of European marine cold-water seaweeds. *Bioresource Technology,* 135**,** 120-127.

DAVIS, R., ADEN, A. & PIENKOS, P. T. 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy,* 88**,** 3524-3531.

DAVIS, T. A., VOLESKY, B. & MUCCI, A. 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water research, 37, 4311-4330.

DE LA ROCHA, S. R. D., SANCHEZ-MUNIZ, F., GÃ³MEZ-JUARISTI, M. N. & MARÃ-N, M. L. 2009. Trace elements determination in edible seaweeds by an optimized and validated ICP-MS method. *Journal of Food Composition and Analysis,* 22**,** 330-336.

DECC, A. 2012. UK Bioenergy Strategy. *UK Department of Energy and Climate Change*.

DEFRA, 2011. Department for Environment, Food and Rural Affairs. *Guidance on Reporting Greenhouse Gas Removals and Emissions from Domestic Woodland Creation*.

DEFRA, 2011. Anaerobic Digestion Strategy and Action Plan: A Commitment to Increasing Energy from Waste through Anaerobic Digestion. Available http://www.defra.gov.uk/publications/files/anaerobic-digestion-strat-action-plan.pdf [Accessed January 2017).

DEMIRBAS, A. 2008. *Biodiesel: A Realistic Fuel Alterntaive for Diesel Engines,* London, Springer.

DEMIRBAS, A. 2009. *Biofuels: Securing the Planet's Future Energy Needs,* Newyork,London, Springer.

DEMIRBAS, I. & DEMIRBAS, A. H. 2007. Importance of rural bioenergy for developing countries. *Energy Conversion and Management,* 48**,** 2386-2398.

DEMIREL, B. & SCHERER, P. 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. *Reviews in Environmental Science and Bio/Technology,* 7**,** 173-190.

DEMIREL, B. & SCHERER, P. 2011. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass and Bioenergy,* 35**,** 992-998.

DEUBLEIN, D. & STEINHAUSER, A. 2011. *Biogas from waste and renewable resources: an introduction,* Weinheim, Wiley-VCH.

DHAR, B. R., NAKHLA, G. & RAY, M. B. 2012. Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. *Waste Management,* 32**,** 542-549.

DICK, H., HENNIG, A. & SCHOLES, P. 2016. Comparing the cost of alternative waste treatment options-Gate Fees report 2016. *Banbury: WRAP*.

DING, L., MA, Y., HUANG, B. & CHEN, S. 2013. Effects of seawater salinity and temperature on growth and pigment contents in Hypnea cervicornis J. Agardh (Gigartinales, Rhodophyta). *BioMed research international,* 2013.

E-OCEANIC SITE, 2016, Available: https://eoceanic.com/sailing/harbours/99/ventry_harbour [Accessed June, 2017].

EASTMAN, J. A. & FERGUSON, J. F. 1981. SOLUBILIZATION OF PARTICULATE ORGANIC-CARBON DURING THE ACID PHASE OF ANAEROBIC-DIGESTION. *Journal Water Pollution Control Federation,* 53**,** 352-366.

EDWARDS, M. & WATSON, L. 2011. Aquaculture explained cultivating Laminaria digitata. *Irish Sea Fisheries Board*.

ELBESHBISHY, E., NAKHLA, G. & HAFEZ, H. 2012. Biochemical methane potential (BMP) of food waste and primary sludge: influence of inoculum pre-incubation and inoculum source. *Bioresource technology,* 110**,** 18-25.

ELLABBAN, O., ABU-RUB, H. & BLAABJERG, F. 2014. Renewable energy resources: Current status, future prospects and their enabling technology. *Renewable and Sustainable Energy Reviews,* 39**,** 748-764.

ENERGY TECHNOLOGIES INSTITUTE, E. 2016. Bioenergy

Enabling UK biomass. UK.

ERTEM, F. C., NEUBAUER, P. & JUNNE, S. 2017. Environmental life cycle assessment of biogas production from marine macroalgal feedstock for the substitution of energy crops. *Journal of cleaner production,* 140**,** 977-985.

ESPOSITO, G., FRUNZO, L., GIORDANO, A., LIOTTA, F., PANICO, A. & PIROZZI, F. 2012a. Anaerobic co-digestion of organic wastes. *REVIEWS IN ENVIRONMENTAL SCIENCE AND BIO-TECHNOLOGY,* 11**,** 325-341.

ESPOSITO, G., FRUNZO, L., LIOTTA, F., PANICO, A. & PIROZZI, F. 2012b. Bio-methane potential tests to measure the biogas production from the digestion and co-digestion of complex organic substrates. *The Open Environmental Engineering Journal,* 5.

EU COMMISSION 1999. The European Landfill Directive 99/31/EC *In:* COMMISSION, E. (ed.). EU [Online].[Accessed June 2018]

EU COMMISSION. 2009. *Renewable energy directive - Energy - European Commission* [Online]. Available: https://ec.europa.eu/energy/en/topics/renewable-energy/renewable-energy-directive [Accessed June 2018].

EU COMMISSION 2018. Renewable energy directive - Energy - European Commission. [Online] [Accessed June 2018].

EUROSTAT, 2018, Gas proces for non-household consumers - bi annual data (from 2007 onwards) http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=nrg_pc_203&lang=en [Accessed on February 2019]

EVANGELISTI, S., LETTIERI, P., BORELLO, D. & CLIFT, R. 2014. Life cycle assessment of energy from waste via anaerobic digestion: a UK case study. *Waste management,* 34**,** 226-237.

EVANS, T. J., EVANS, A. & STREZOV, V. 2009. Assessment of sustainability indicators for renewable energy technologies. *Renewable and Sustainable Energy Reviews,* 13**,** 1082-1088.

FANTINI, M. 2017. Biomass Availability, Potential and Characteristics. *Biorefineries.* Springer.

FAO 2016. *State of World Fisheries and Aquaculture 2016*, Food & Agriculture Org. [Online].

FENG, X. M., KARLSSON, A., SVENSSON, B. H. & BERTILSSON, S. 2010. Impact of trace element addition on biogas production from food industrial waste–linking process to microbial communities. *FEMS microbiology ecology,* 74**,** 226-240.

FERNAND, F., ISRAEL, A., SKJERMO, J., WICHARD, T., TIMMERMANS, K. R. & GOLBERG, A. 2017. Offshore macroalgae biomass for bioenergy production: Environmental aspects, technological achievements and challenges. *Renewable and Sustainable Energy Reviews,* 75**,** 35-45.

FERNANDEZ, B., PORRIER, P. & CHAMY, R. 2001. Effect of inoculum-substrate ratio on the start-up of solid waste anaerobic digesters. *WATER SCIENCE AND TECHNOLOGY,* 44**,** 103-108.

FOGED, H., FLOTATS RIPOLL, X., BONMATÍ BLASI, A., PALATSI CIVIT, J., MAGRÍ ALOY, A. & SCHELDE, K. M. 2012. Inventory of manure processing activities in Europe.

FORBORD, S., SKJERMO, J., ARFF, J., HANDÅ, A., REITAN, K. I., BJERREGAARD, R. & LÜNING, K. 2012. Development of Saccharina latissima (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. *Journal of Applied Phycology,* 24**,** 393-399.

FORTES, M. & LÜNING, K. 1980. Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgoländer Meeresuntersuchungen,* 34**,** 15.

FOXON, T. J., GROSS, R., CHASE, A., HOWES, J., ARNALL, A. & ANDERSON, D. 2005. UK innovation systems for new and renewable energy technologies: drivers, barriers and systems failures. *Energy Policy,* 33**,** 2123-2137.

FRID, C., GARWOOD, P. & ROBINSON, L. 2009. Observing change in a North Sea benthic system: a 33 year time series. *Journal of Marine Systems,* 77**,** 227-236.

FRID, C. L. 2011. Temporal variability in the benthos: Does the sea floor function differently over time? *Journal of Experimental Marine Biology and Ecology,* 400**,** 99-107.

GAO, X., ENDO, H., TANIGUCHI, K. & AGATSUMA, Y. 2013. Combined effects of seawater temperature and nutrient condition on growth and survival of juvenile sporophytes of the kelp Undaria pinnatifida (Laminariales; Phaeophyta) cultivated in northern Honshu, Japan. *Journal of applied phycology,* 25**,** 269-275.

GARCIA, R., PIZARRO, C., LAVIN, A. G. & BUENO, J. L. 2012. Characterization of Spanish biomass wastes for energy use. *BIORESOURCE TECHNOLOGY,* 103**,** 249-258.

GASPARATOS, A., DOLL, C. N. H., ESTEBAN, M., AHMED, A. & OLANG, T. A. 2017. Renewable energy and biodiversity: Implications for transitioning to a Green Economy. *Renewable and Sustainable Energy Reviews,* 70**,** 161-184.

GE, H., JENSEN, P. D. & BATSTONE, D. J. 2011. Relative kinetics of anaerobic digestion under thermophilic and mesophilic conditions. *Water Science and Technology,* 64**,** 848-853.

GEGG, P. & WELLS, V. 2017. UK macro-algae biofuels: a strategic management review and future research agenda. *Journal of Marine Science and Engineering,* 5**,** 32.

GERARD, V. 1986. Photosynthetic characteristics of giant kelp (Macrocystis pyrifera) determined in situ. Marine Biology, 90, 473-482.

GERARDI, M. H. 2003. *The Microbiology of Anaerobic Digesters,* New Jersey, John Wiley & Sons.

GERARDO, M. L., ALJOHANI, N. H., OATLEY-RADCLIFFE, D. L. & LOVITT, R. W. 2015. Moving towards sustainable resources: Recovery and fractionation of nutrients from dairy manure digestate using membranes. *Water research,* 80**,** 80-89.

GHADIRYANFAR, M., ROSENTRATER, K. A., KEYHANI, A. & OMID, M. 2016. A review of macroalgae production, with potential applications in biofuels and bioenergy. *Renewable and Sustainable Energy Reviews,* 54**,** 473-481.

GHOSH, A., KHANRA, S., MONDAL, M., HALDER, G., TIWARI, O. N., SAINI, S., BHOWMICK, T. K. & GAYEN, K. 2016. Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: A review. *Energy Conversion and Management,* 113**,** 104-118.

GINLEY, D. S. & KAHEN, D. I. 2012. *Fundamentals of materials for energy and environmental sustainability,* Cambridge, Cambridge University Press.

GONZALEZ-FERNANDEZ, C., SIALVE, B., BERNET, N. & STEYER, J. P. 2012. Impact of microalgae characteristics on their conversion to biofuel. Part II: Focus on biomethane production. *BIOFUELS BIOPRODUCTS & BIOREFINING-BIOFPR,* 6**,** 205-218.

GRIFFIN, L. P. Anaerobic digestion of organic wastes: the impact of operating conditions on hydrolysis efficiency and microbial community composition. Masters Abstracts International, 2012.

GUIRY, M. D. & MORRISON, L. 2013. The sustainable harvesting of Ascophyllum nodosum (Fucaceae, Phaeophyceae) in Ireland, with notes on the collection and use of some other brown algae. *Journal of applied phycology,* 25**,** 1823-1830.

GUO, Z., LIU, Y., GUO, H., YAN, S. & MU, J. 2013. Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production. Journal of Environmental Sciences (China), 25 Suppl 1, S85.

GUNASEELAN, V. N. 1997. Anaerobic digestion of biomass for methane production: a review. *Biomass and bioenergy,* 13**,** 83-114.

GURUNG, A., VAN GINKEL, S. W., KANG, W.-C., QAMBRANI, N. A. & OH, S.-E. 2012. Evaluation of marine biomass as a source of methane in batch tests: A lab-scale study. *Energy,* 43**,** 396-401.

HANNA INSTRUMENTS, Available: https://www.hannainstruments.co.uk/25-vial-thermo-reactor.html/ [Accessed June, 2017].

HAN, W., FANG, J., LIU, Z. & TANG, J. 2016. Techno-economic evaluation of a combined bioprocess for fermentative hydrogen production from food waste. *Bioresource Technology,* 202**,** 107-112.

HANDÅ, A., FORBORD, S., WANG, X., BROCH, O. J., DAHLE, S. W., STØRSETH, T. R., REITAN, K. I., OLSEN, Y. & SKJERMO, J. 2013. Seasonal-and depth-dependent growth of cultivated kelp (Saccharina latissima) in close proximity to salmon (Salmo salar) aquaculture in Norway. *Aquaculture,* 414**,** 191-201.

HANSEN, T. L., SCHMIDT, J. E., ANGELIDAKI, I., MARCA, E., JANSEN, J. L. C., MOSBÃ¦K, H., CHRISTENSEN, T. H., INSTITUTIONEN FÃ¶R BYGG- OCH, M. T., DEPARTMENT OF CHEMICAL, E., FACULTY OF ENGINEERING, L. T. H., DEPARTMENT OF, B., ENVIRONMENTAL, T., VATTENFÃ¶RSÃ¶RJNINGS-OCH, A., LUNDS, U., INSTITUTIONER VID, L. T. H., LUNDS TEKNISKA, H. G., LUND, U., DEPARTMENTS AT, L. T. H., WATER, ENVIRONMENTAL, E. & INSTITUTIONEN FÃ¶R, K. 2004. Method for determination of methane potentials of solid organic waste. *Waste Management,* 24**,** 393-400.

HANSSEN, J. F., INDERGAARD, M., Ã˜STGAARD, K., BÃ¦VRE, O. A., PEDERSEN, T. A. & JENSEN, A. 1987. Anaerobic digestion of Laminaria spp. and Ascophyllum nodosum and application of end products. Biomass, 14, 1-13.

HANSSON, G. 1983. Methane production from marine, green macro-algae. Resources and conservation, 8, 185-194.

HARTMANN, H. & AHRING, B. K. 2006. Strategies for the anaerobic digestion of the organic fraction of municipal solid waste: an overview. *Water science and technology,* 53**,** 7-22.

HARUN, R., DAVIDSON, M., DOYLE, M., GOPIRAJ, R., DANQUAH, M. & FORDE, G. 2011. Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. *Biomass and Bioenergy,* 35**,** 741-747.

HASANUZZAMAN, M., ZUBIR, U. S., ILHAM, N. I. & CHE, H. S. 2017a. Global electricity demand, generation, grid system, and renewable energy polices: a review. WILEY INTERDISCIPLINARY REVIEWS-ENERGY AND ENVIRONMENT, 6.

HIERHOLTZER, A. 2013. *Investigating factors affecting the anaerobic digestion of seaweed: modelling and experimental approaches.* University of Abertay Dundee.

HINKEN, L., URBAN, I., HAUN, E., WEICHGREBE, D. & ROSENWINKEL, K.-H. 2008. The valuation of malnutrition in the mono-digestion of maize silage by anaerobic batch tests. *Water science and Technology,* 58**,** 1453-1459.

HINKS, J., EDWARDS, S., SALLIS, P. J. & CALDWELL, G. S. 2013. The steady state anaerobic digestion of Laminaria hyperborea–Effect of hydraulic residence on biogas production and bacterial community composition. *Bioresource technology,* 143**,** 221-230.

HO, D. P., NGO, H. H. & GUO, W. 2014. A mini review on renewable sources for biofuel. *BIORESOURCE TECHNOLOGY,* 169**,** 742-749.

HOLLIGER, C., ALVES, M., ANDRADE, D., ANGELIDAKI, I., ASTALS, S., BAIER, U., BOUGRIER, C., BUFFIÈRE, P., CARBALLA, M. & DE WILDE, V. 2016. Towards a standardization of biomethane potential tests. *Water Science and Technology,* 74**,** 2515-2522.

HOLM-NIELSEN, J. B., AL SEADI, T. & OLESKOWICZ-POPIEL, P. 2009. The future of anaerobic digestion and biogas utilization. *Bioresource technology,* 100**,** 5478-5484.

HORAN, N., SMYTH, M. & MAY, A. Optimisation of digester performance and gas yield through analysis of VFA speciation. 16th European Bio-solids and Organic Resources Conference, 2011.

HORNUNG, A. 2014. *Transformation of biomass: theory to practice*, John Wiley & Sons.

HUANG, L., HWANG, A. & PHILLIPS, J. 2011. Effect of temperature on microbial growth rate–mathematical analysis: the Arrhenius and Eyring–Polanyi connections. *Journal of food science,* 76.

HUBLIN, A., SCHNEIDER, D. R. & DŽODAN, J. 2014. Utilization of biogas produced by anaerobic digestion of agro-industrial waste: Energy, economic and environmental effects:. *http://dx.doi.org/10.1177/0734242X14539789*.

HUGHES, A. D., KELLY, M. S., BLACK, K. D. & STANLEY, M. S. 2012. Biogas from Macroalgae: is it time to revisit the idea? *BIOTECHNOLOGY FOR BIOFUELS,* 5**,** 86-86.

IEA 2006. Energy Technology Perspectives. Scenarios & Strategies to 2050. IEA/OECD, Paris.

IEE TASK 5.1, R. 2012. European Case Studies of Anaerobic Digestion Plants Showcasing their Monitoring practices. *Severn Wye Report.*

IGONI, A. H., AYOTAMUNO, M., EZE, C., OGAJI, S. & PROBERT, S. 2008. Designs of anaerobic digesters for producing biogas from municipal solid-waste. *Applied energy,* 85**,** 430-438.

INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE. WORKING GROUP, I. 2007. Climate change 2007: mitigation of climate change : working group III contribution to the Fourth Assessment Report of the IPCC, Cambridge, Cambridge University Press.

JANG, H. M., PARK, S. K., HA, J. H. & PARK, J. M. 2013. Microbial community structure in a thermophilic aerobic digester used as a sludge pretreatment process for the mesophilic anaerobic digestion and the enhancement of methane production. *Bioresource technology,* 145**,** 80-89.

JARD, G., JACKOWIAK, D., CARRÃ¨RE, H., DELGENES, J., TORRIJOS, M., STEYER, J. & DUMAS, C. 2012. Batch and semi-continuous anaerobic digestion of Palmaria palmata: comparison with Saccharina latissima and inhibition studies. *Chemical engineering journal,* 209**,** 513-519.

JARD, G., MARFAING, H., CARRÈRE, H., DELGENES, J., STEYER, J. & DUMAS, C. 2013. French Brittany macroalgae screening: composition and methane potential for potential alternative sources of energy and products. *Bioresource technology,* 144**,** 492-498.

JIANG, R., INGLE, K. N. & GOLBERG, A. 2016. Macroalgae (seaweed) for liquid transportation biofuel production: what is next? *Algal Research,* 14**,** 48-57.

JIANG, Y., HEAVEN, S. & BANKS, C. J. 2012. Strategies for stable anaerobic digestion of vegetable waste. *Renewable Energy,* 44**,** 206-214.

JIMENEZ, J., LATRILLE, E., HARMAND, J., ROBLES, A., FERRER, J., GAIDA, D., WOLF, C., MAIRET, F., BERNARD, O. & ALCARAZ-GONZALEZ, V. 2015. Instrumentation and control of anaerobic digestion processes: a review and some research challenges. *Reviews in Environmental Science and Bio/Technology,* 14**,** 615-648.

JOHANSSON, T. B., REDDY, A. K., KELLY, H., WILLIAMS, R. H. & BURNHAM, L. 1993. *Renewable energy: sources for fuels and electricity*, Island press.

JOHN, R. P., ANISHA, G., NAMPOOTHIRI, K. M. & PANDEY, A. 2011. Micro and macroalgal biomass: a renewable source for bioethanol. *Bioresource technology,* 102**,** 186-193.

JOHNSTONE, N., HAÅ¡Ä• IÄ• , I. & POPP, D. 2010. Renewable Energy Policies and Technological Innovation: Evidence Based on Patent Counts. *Environmental and Resource Economics,* 45**,** 133-155.

JUNG, K. A., LIM, S.-R., KIM, Y. & PARK, J. M. 2013. Potentials of macroalgae as feedstocks for biorefinery. *Bioresource technology,* 135**,** 182-190.

KADAM, R. & PANWAR, N. 2017. Recent advancement in biogas enrichment and its applications. *Renewable and Sustainable Energy Reviews,* 73**,** 892-903.

KAYHANIAN, M. & RICH, D. 1995. Pilot-scale high solids thermophilic anaerobic digestion of municipal solid waste with an emphasis on nutrient requirements. *Biomass and bioenergy,* 8**,** 433-444.

KELLY, E. 2005. The role of kelp in the marine environment. Galway: Department of Environment,

Heritage and Local Government, Dublin, Ireland.

KELLY, M. S. & DWORJANYN, S. 2008. The potential of marine biomass for anaerobic biogas production. Scotland: The Crown Estate.

KERBY, C. & VRIESEKOOP, F. 2017. An overview of the utilisation of brewery by-products as generated by british craft breweries. *Beverages,* 3**,** 24.

KERRISON, P. D., STANLEY, M. S., EDWARDS, M. D., BLACK, K. D. & HUGHES, A. D. 2015. The cultivation of European kelp for bioenergy: Site and species selection. *Biomass and Bioenergy,* 80**,** 229-242.

KHALID, A., MUHAMMAD, A., MUZAMMIL, A., MAHMOOD, T., DAWSON, L. 2011. The anaerobic digestion of solid organic waste. 31. Available: https://www.sciencedirect.com/science/article/pii/S0956053X11001668?via%3Dihub.

KHANAL, S. K. 2011. *Anaerobic biotechnology for bioenergy production: principles and applications*, John Wiley & Sons.

KIM, D. H. & H. HYUN, S. 2004. *Kinetics of Thermophilic Anaerobic Digestion and Effects of Propionate on Thermophilic Anaerobic Digestion*.

KIM, S. & DALE, B. E. 2004. Global potential bioethanol production from wasted crops and crop residues. *Biomass and bioenergy,* 26**,** 361-375.

KLESSMANN, C., HELD, A., RATHMANN, M. & RAGWITZ, M. 2011. Status and perspectives of renewable energy policy and deployment in the European Union-What is needed to reach the 2020 targets? *ENERGY POLICY,* 39**,** 7637-7657.

KONDA, N. M., SINGH, S., SIMMONS, B. A. & KLEIN-MARCUSCHAMER, D. 2015. An investigation on the economic feasibility of macroalgae as a potential feedstock for biorefineries. *BioEnergy Research,* 8**,** 1046-1056.

KORPINEN, S., HONKANEN, T., VESAKOSKI, O., HEMMI, A., KOIVIKKO, R., LOPONEN, J. & JORMALAINEN, V. 2007. Macroalgal communities face the challenge of changing biotic interactions: review with focus on the Baltic Sea. *AMBIO: A Journal of the Human Environment,* 36**,** 203-211.

KOUAS, M., TORRIJOS, M., SOUSBIE, P., STEYER, J.-P., SAYADI, S. & HARMAND, J. 2017. Robust assessment of both biochemical methane potential and degradation kinetics of solid residues in successive batches. Waste Management, 70, 59-70.

KOUTINAS, A. A., YEPEZ, B., KOPSAHELIS, N., FREIRE, D. M. G., DE CASTRO, A. M., PAPANIKOLAOU, S. & KOOKOS, I. K. 2016. Techno-economic evaluation of a complete bioprocess for 2,3-butanediol production from renewable resources. *Bioresource Technology,* 204**,** 55-64.

KRAAN, S. 2013. Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production. Mitigation and Adaptation Strategies for Global Change, 18, 27-46.

KREUGER, E., NGES, I. A. & BJÖRNSSON, L. 2011. Ensiling of crops for biogas production: effects on methane yield and total solids determination. *Biotechnology for biofuels,* 4**,** 44.

LABATUT, R. A., ANGENENT, L. T. & SCOTT, N. R. 2011. Biochemical methane potential and biodegradability of complex organic substrates. *Bioresource technology,* 102**,** 2255-2264.

LAURENS, L., M.L. 2017. State of Technology Review – Algae Bioenergy

An IEA Bioenergy Inter-Task Strategic Project. USA: IEA.

LCK 014, HACH LANGE, Available: https://uk.hach.com/ [Accessed June, 2017].

LEE, R. A. & LAVOIE, J.-M. 2013. From first-to third-generation biofuels: Challenges of producing a commodity from a biomass of increasing complexity. *Animal Frontiers,* 3**,** 6-11.

LEE, J.-Y., YOO, C., JUN, S.-Y., AHN, C.-Y. & OH, H.-M. 2010. Comparison of several methods for effective lipid extraction from microalgae. Bioresource technology, 101, S75-S77.

LEHTOMÄKI, A., HUTTUNEN, S., LEHTINEN, T. M. & RINTALA, J. A. 2008. Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresource Technology,* 99**,** 3267-3278.

LEHTOMÄKI, A. & BJÖRNSSON, L. 2006. Two-stage anaerobic digestion of energy crops: methane production, nitrogen mineralisation and heavy metal mobilisation. Environmental technology, 27, 209-218.

LEMS, R., & DIRKSE, E.H.M. 2010. Small Scale biogas upgrading: Green gas with the DMT Carborex-MS system. In 15th European Biosolids and Organic Resources Conference, organised by Aqua Enviro Technology Transfer. Available http://www. dirksemilieutechniek. com/dmt/do/download/_/true/210371/DMT_Carborex_MS_Small_sc ale_biogas_upgrading_Green_gas_1-11-2010. pdf. Accessed (Volume. 15, p.2012)

LETTINGA, G., REBAC, S. & ZEEMAN, G. 2001. Challenge of psychrophilic anaerobic wastewater treatment. *TRENDS in Biotechnology,* 19**,** 363-370.

LEVIDOW, L. & PAPAIOANNOU, T. 2013. State imaginaries of the public good: shaping UK innovation priorities for bioenergy. *Environmental Science & Policy,* 30**,** 36-49.

LEVIN, L. A. & SMITH, C. R. 1984. Response of background fauna to disturbance and enrichment in the deep sea: a sediment tray experiment. Deep Sea Research Part A. Oceanographic Research Papers, 31, 1277-1285.

LEVLIN, E. Conductivity measurements for controlling municipal waste-water treatment. Proceedings of a Polish-Swedish-Ukrainian Seminar, Utron, 2010.

LEWIS, D. & SMITH, D. 1967. Sugar alcohols (polyols) in fungi and green plants: I. Distribution, physiology and metabolism. New Phytologist, 66, 143-184.

LI, Q., DU, W. & LIU, D. 2008. Perspectives of microbial oils for biodiesel production. *Applied microbiology and biotechnology,* 80**,** 749-756.

LINKE, B. 2006. Kinetic study of thermophilic anaerobic digestion of solid wastes from potato processing. *Biomass and Bioenergy,* 30**,** 892-896.

LTINING, K. 1988. Photoperiodic control of sorus formation in the brown alga Laminaria saccharina. *Mar. Ecol. Prog. Ser,* 45**,** 137-144.

LUND, H. 2007. Renewable energy strategies for sustainable development. *Energy,* 32**,** 912-919.

LÜNING, K. 1980. CRITICAL LEVELS OF LIGHT AND TEMPERATURE REGULATING THE GAMETOGENESIS OF THREE LAMINARIA SPECIES (PHAEOPHYCEAE) 1. *Journal of Phycology,* 16**,** 1-15.

LÜDTKE, M., BARESEL, C., NORDBERG, Å., WITKIEWICZ, A. & THUNBERG, A. 2017a. Impact of Temperature-Dependent Reaction Rates on Methane Yields in Intermittently Fed Mesophilic Sludge Digestion. Journal of Environmental Engineering, 144, 04017085.

LÜDTKE, M., NORDBERG, Å. & BARESEL, C. 2017b. Experimental power of laboratory-scale results and transferability to full-scale anaerobic digestion. Water Science and Technology, 76, 983-991.

MACEIRAS, R., RODRI, M., CANCELA, A., URRÉJOLA, S. & SÁNCHEZ, A. 2011. Macroalgae: raw material for biodiesel production. Applied Energy, 88, 3318-3323.

MADSEN, M., HOLM-NIELSEN, J. B. & ESBENSEN, K. H. 2011. Monitoring of anaerobic digestion processes: A review perspective. *Renewable and Sustainable Energy Reviews,* 15**,** 3141-3155.

MAKARA, A. & KOWALSKI, Z. 2015. Pig manure treatment and purification by filtration. *Journal of environmental management,* 161**,** 317-324.

MANNS, D., NIELSEN, M. M., BRUHN, A., SAAKE, B. & MEYER, A. S. 2017. Compositional variations of brown seaweeds Laminaria digitata and Saccharina latissima in Danish waters. *Journal of Applied Phycology,* 29**,** 1493-1506.

MANZANO-AGUGLIARO, F., ALCAYDE, A., MONTOYA, F. G., ZAPATA-SIERRA, A. & GIL, C. 2013. Scientific production of renewable energies worldwide: An overview. *RENEWABLE & SUSTAINABLE ENERGY REVIEWS,* 18**,** 134-143.

MARINHO, G. A. S., ALVARADO-MORALES, M. & ANGELIDAKI, I. 2016. Valorization of macroalga Saccharina latissima as novel feedstock for fermentation-based succinic acid production in a biorefinery approach and economic aspects. *Algal Research,* 16**,** 102-109.

MARTINEZ-VAL, J. M. 2013. Energy for Sustainable Development: A systematic approach for a badly defined challenge. *ENERGY CONVERSION AND MANAGEMENT,* 72**,** 3-11.

MATA-ALVAREZ, J., DOSTA, J., MACÉ, S. & ASTALS, S. 2011. Codigestion of solid wastes: a review of its uses and perspectives including modeling. *Critical reviews in biotechnology,* 31**,** 99-111.

MATA-ALVAREZ, J., MACE, S. & LLABRES, P. 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology,* 74**,** 3-16.

MATSUI, T. & KOIKE, Y. 2010. Methane fermentation of a mixture of seaweed and milk at a pilot-scale plant. *Journal of bioscience and bioengineering,* 110**,** 558-563.

MCHUGH, D. J. 2003. *A guide to the seaweed industry*, Food and Agriculture Organization of the United Nations Rome.

MCKENDRY, P. 2002a. Energy production from biomass (part 1): overview of biomass. OXFORD: Elsevier Ltd.

MCKENDRY, P. 2002b. Energy production from biomass (part 2): conversion technologies. OXFORD: Elsevier Ltd.

MCKENDRY, P. 2002c. Energy production from biomass (part 3): gasification technologies. OXFORD: Elsevier Ltd.

MCKENNEDY, J. & SHERLOCK, O. 2015. Anaerobic digestion of marine macroalgae: A review. *Renewable and Sustainable Energy Reviews,* 52**,** 1781-1790.

MERCURI, E. G. F., KUMATA, A. Y. J., AMARAL, E. B. & VITULE, J. R. S. 2016. Energy by Microbial Fuel Cells: Scientometric global synthesis and challenges. *RENEWABLE & SUSTAINABLE ENERGY REVIEWS,* 65**,** 832-840.

MERZOUK, A. & JOHNSON, L. E. 2011. Kelp distribution in the northwest Atlantic Ocean under a changing climate. *Journal of Experimental Marine Biology and Ecology,* 400**,** 90-98.

METTLER TOLEDO, Available: https://www.mt.com/gb/en/home.html [Accessed June, 2017].

MILANO, J., ONG, H. C., MASJUKI, H., CHONG, W., LAM, M. K., LOH, P. K. & VELLAYAN, V. 2016. Microalgae biofuels as an alternative to fossil fuel for power generation. Renewable and Sustainable Energy Reviews, 58, 180-197.

MILLEDGE, J., SMITH, B., DYER, P. & HARVEY, P. 2014a. Macroalgae-Derived Biofuel: A Review of Methods of Energy Extraction from Seaweed Biomass. *Energies,* 7**,** 7194-7222.

MILLEDGE, J. J., SMITH, B., DYER, P. W. & HARVEY, P. 2014b. Macroalgae-derived biofuel: a review of methods of energy extraction from seaweed biomass. *Energies,* 7**,** 7194-7222.

MITCHELL, C. & CONNOR, P. 2004. Renewable energy policy in the UK 1990–2003. *Energy Policy,* 32**,** 1935-1947.

MOEN, E., HORN, S. & Ã˜STGAARD, K. 1997. Biological degradation of Ascophyllum nodosum. *Journal of Applied Phycology,* 9**,** 347-357.

MOHTASHAM, J. 2015. Renewable Energies. *Energy Procedia,* 74**,** 1289-1297.

MONTINGELLI, M., TEDESCO, S. & OLABI, A. 2015. Biogas production from algal biomass: A review. *Renewable and Sustainable Energy Reviews,* 43**,** 961-972.

MONTINGELLI, M. E., BENYOUNIS, K. Y., STOKES, J. & OLABI, A. G. 2016. Pretreatment of macroalgal biomass for biogas production. *Energy Conversion and Management,* 108**,** 202-209.

MOODY, L., BURNS, R., WU-HAAN, W. & SPAJIC, R. Use of biochemical methane potential (BMP) assays for predicting and enhancing anaerobic digester performance. Proceedings of the 44th Croatian and the 4th International Symposium on Agriculture, Opatija, Croatia, 16-20 February 2009., 2009.

MÜLLER, R., LAEPPLE, T., BARTSCH, I. & WIENCKE, C. 2009. Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Botanica Marina,* 52**,** 617-638.

MURPHY, J. D., DROSG, B., ALLEN, E., JERNEY, J., XIA, A. & HERRMANN, C. 2015. A perspective on algal biogas. *IEA Bioenergy***,** 1-38.

MURRAY, S., GROOM, R. E., HANNA, J.-A. & WATSON, C. 2013. BioMara Project Processing Technology Review. *Queen's University Belfast.*

MUSSATTO, S. I., DRAGONE, G. & ROBERTO, I. C. 2006. Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science,* 43**,** 1-14.

NAIK, S., GOUD, V. V., ROUT, P. K., JACOBSON, K. & DALAI, A. K. 2010. Characterization of Canadian biomass for alternative renewable biofuel. *Renewable Energy,* 35**,** 1624-1631.

NALO, T., TASING, K., KUMAR, S. & BHARTI, A. 2014. Anaerobic digestion of municipal solid waste: a critical analysis. *Int J Innov Res Sci Eng Technol,* 3**,** 2347-6710.

NETO, A. M. P., SOTANA DE SOUZA, R. A., LEON-NINO, A. D., DA COSTA, J. D. A. A., TIBURCIO, R. S., NUNES, T. A., SELLARE DE MELLO, T. C., KANEMOTO, F. T., SALDANHA-CORRÊA, F. M. P. & GIANESELLA, S. M. F. 2013. Improvement in microalgae lipid extraction using a sonication-assisted method. *Renewable Energy,* 55**,** 525-531.

NIELFA, A., CANO, R. & FDZ-POLANCO, M. 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports,* 5**,** 14-21.

NIELSEN, M., BRUHN, A., RASMUSSEN, M., OLESEN, B., LARSEN, M. & B. MÃ¸LLER, H. 2011. *Cultivation of Ulva lactuca with manure for simultaneous bioremediation and biomass production*.

NIELSEN, M. M., BRUHN, A., RASMUSSEN, M. B., OLESEN, B., LARSEN, M. M. & MØLLER, H. B. 2012. Cultivation of Ulva lactuca with manure for simultaneous bioremediation and biomass production. *Journal of applied phycology,* 24**,** 449-458.

NIELSEN, M. M., KUMAR, J. P., SOLER-VILA, A., JOHNSON, M. P. & BRUHN, A. 2016. Early stage growth responses of Saccharina latissima spores and gametophytes. Part 1: inclusion of different phosphorus regimes. *Journal of applied phycology,* 28**,** 387-393.

NZIHOU, A. 2010. Toward the Valorization of Waste and Biomass. *Waste and Biomass Valorization,* 1**,** 3-7.

OKONKWO, U. C., ONOKPITE, E. & ONOKWAI, A. O. 2016. Comparative study of the optimal ratio of biogas production from various organic wastes and weeds for digester/restarted digester. *Journal of King Saud University-Engineering Sciences*.

OLENIN, S., ALEMANY, F., CARDOSO, A., GOLLASCH, S., GOULLETQUER, P., LEHTINIEMI, M., MCCOLLIN, T., MINCHIN, D., MIOSSEC, L. & AMBROGI, A. O. 2010. Marine strategy framework directive. *Task Group,* 2.

OLISCHLÄGER, M., IÑIGUEZ, C., GORDILLO, F. J. L. & WIENCKE, C. 2014. Biochemical composition of temperate and Arctic populations of Saccharina latissima after exposure to increased pCO2 and temperature reveals ecotypic variation. *Planta,* 240**,** 1213-1224.

OLIVEIRA, J., ALVES, M. & COSTA, J. 2014. Design of experiments to assess pre-treatment and co-digestion strategies that optimize biogas production from macroalgae Gracilaria vermiculophylla. *Bioresource technology,* 162**,** 323-330.

OMETTO, F., BERG, A., BJÃ¶RN, A., SAFARIC, L., SVENSSON, B., KARLSSON, A. & EJLERTSSON, J. 2017. Inclusion of Saccharina latissima in conventional anaerobic digestion systems. *Environmental Technology*, 1-42.

OSPAR 2017. Condition of Benthic Habitat Communities: the Common Conceptual Approach. North Atlantic, EU.

ØSTERGAARD, P. A. 2009. Reviewing optimisation criteria for energy systems analyses of renewable energy integration. *Energy,* 34**,** 1236-1245.

ØSTGAARD, K., INDERGAARD, M., MARKUSSEN, S., KNUTSEN, S. H. & JENSEN, A. 1993. Carbohydrate degradation and methane production during fermentation ofLaminaria saccharina (Laminariales, Phaeophyceae). *Journal of applied phycology,* 5**,** 333-342.

OTSUKA, K. & YOSHINO, A. A fundamental study on anaerobic digestion of sea lettuce. 2004. IEEE, 1770-1773 Vol.3.

PAHL, G. 2005. *Biodiesel: Growing a New Energy Economy,* United States, Chelsea Green Publishing.

PAINULY, J. P. 2001. Barriers to renewable energy penetration; a framework for analysis. *Renewable Energy,* 24**,** 73-89.

PARKER, B., SPRUIJT, J., SCHIPPERUS, R., KOOTSTRA, M. & DE VISSER, C. 2015. AlgaEconomics: bio-economic production models of micro-algae and downstream processing to produce bio energy carriers.

PARAWIRA, W., READ, J. S., MATTIASSON, B. & BJÖRNSSON, L. 2008. Energy production from agricultural residues: high methane yields in pilot-scale two-stage anaerobic digestion. Biomass and Bioenergy, 32, 44-50.

PARR INSTRUMENT COMPANY, UK, Available: https://www.parrinst.com/products/oxygen-bomb-calorimeters/6100-compensated-jacket-calorimeter/ [Accessed June, 2017].

PETEIRO, C. & FREIRE, Ó. 2013. Biomass yield and morphological features of the seaweed Saccharina latissima cultivated at two different sites in a coastal bay in the Atlantic coast of Spain. *Journal of applied phycology,* 25**,** 205-213.

PHILIPPART, C. J., ANADÓN, R., DANOVARO, R., DIPPNER, J. W., DRINKWATER, K. F., HAWKINS, S. J., OGUZ, T., O'SULLIVAN, G. & REID, P. C. 2011. Impacts of climate change on European marine ecosystems: observations, expectations and indicators. *Journal of experimental marine biology and ecology,* 400**,** 52-69.

POBEHEIM, H., MUNK, B., JOHANSSON, J. & GUEBITZ, G. M. 2010. Influence of trace elements on methane formation from a synthetic model substrate for maize silage. *Bioresource technology,* 101**,** 836-839.

POPP, D., HASCIC, I. & MEDHI, N. 2011. Technology and the diffusion of renewable energy. *Energy Economics,* 33**,** 648-662.

PRAJAPATI, S. K., MALIK, A., VIJAY, V. K. & SREEKRISHNAN, T. R. 2015. Enhanced methane production from algal biomass through short duration enzymatic pretreatment and codigestion with carbon rich waste. *RSC ADVANCES,* 5**,** 67175-67183.

QUEENS UNIVERSITY WEBSITE, Available: http://qub.ac.uk/research-centres/QueensUniversityMarineLaboratory/ [Accessed June, 2017].

RAJVANSHI, S. & SHARMA, M. P. 2012. Micro algae: a potential source of biodiesel. *Journal of Sustainable Bioenergy Systems,* 2**,** 49.

RAMÍREZ, C. H. V. 2015. Biogas Production from Seaweed Biomass: a Biorefinery Approach.

RAPOSO, F., DE LA RUBIA, M., FERNÁNDEZ-CEGRÍ, V. & BORJA, R. 2012. Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renewable and Sustainable Energy Reviews, 16, 861-877.

RAO, M. S. & SINGH, S. P. 2004. Bioenergy conversion studies of organic fraction of MSW: kinetic studies and gas yieldâ□“organic loading relationships for process optimisation. Bioresource Technology, 95, 173-185.

RATCLIFF, J., WAN, A., EDWARDS, M., SOLER-VILA, A., JOHNSON, M., ABREU, M. H. & MORRISON, L. 2016. Metal content of kelp (Laminaria digitata) co-cultivated with Atlantic salmon in an Integrated Multi-Trophic Aquaculture system. *Aquaculture,* 450, 234-243.

REED, M. S. 2016. *The research impact handbook*, Fast Track Impact.

ROBERTS, D., DAVIES, C., MITCHELL, A., MOORE, H., PICTON, B., PORTIG, A., PRESTON, J., SMYTH, D., STRONG, D. & VIZE, S. 2004. Strangford Lough Ecological Change Investigation (SLECI). *Belfast: Oueens University*.

ROBERTS, T. & UPHAM, P. 2012. Prospects for the use of macro-algae for fuel in Ireland and the UK: An overview of marine management issues. *Marine Policy,* 36, 1047-1053.

ROESIJADI, G., COPPING, A., HUESEMANN, M., FORSTER, J. & BENEMANN, J. 2008. Techno-economic feasibility analysis of offshore seaweed farming for bioenergy and biobased products. *Independent research and development report IR# PNWD-3931, Battelle Pacific Northwest Division,* 115.

ROESIJADI, G., JONES, S. B., SNOWDEN-SWAN, L. J. & ZHU, Y. 2010. Macroalgae as a biomass feedstock: a preliminary analysis. Pacific Northwest National Laboratory (PNNL), Richland, WA (US).

ROŠ, M. & ZUPANČIČ, G. D. 2003. Thermophilic anaerobic digestion of waste activated sludge. *Acta chimica slovenica,* 50, 343358.

ROSILLO-CALLE, F. & WOODS, J. 2012. *Biomass Assessment Handbook. [Online]*, Routledge.

ROSS, A. B., JONES, J. M., KUBACKI, M. L. & BRIDGEMAN, T. 2008. Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresource Technology,* 99, 6494-6504.

RUPÉREZ, P., AHRAZEM, O. & LEAL, J. A. 2002. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed Fucus vesiculosus. Journal of agricultural and food chemistry, 50, 840-845.

RZAMA, A., BENHARREF, A., ARREGUY, B. & DUFOURC, E. J. 1995. Volatile compounds of green microalgae grown on reused waste water. *Phytochemistry,* 38, 1375-1379.

SANDERSON, J., CROMEY, C., DRING, M. & KELLY, M. 2008. Distribution of nutrients for seaweed cultivation around salmon cages at farm sites in north–west Scotland. *Aquaculture,* 278, 60-68.

SANNA, A. 2014. Advanced biofuels from thermochemical processing of sustainable biomass in Europe. *BioEnergy Research,* 7, 36-47.

SARKER, S., BRUHN, A., WARD, A. J. & MÃ¸LLER, H. B. 2012. Bio-fuel from anaerobic co-digestion of the macro-algae Ulva lactuca and Laminaria digitata. *Renewable Energy and Energy Efficiency, Biogas and biofuel production technologies. Retrieved from http://www. yumpu. com/en/biofuel*.

SCARLAT, N., MARTINOV, M. & DALLEMAND, J.-F. 2010. Assessment of the availability of agricultural crop residues in the European Union: potential and limitations for bioenergy use. *Waste management,* 30, 1889-1897.

SAMS WEBSITE, 2017, SCOTTISH ASSOCIATION OF MARINE SCIENCES, [Online] Available: https://www.sams.ac.uk/ [Accessed June, 2017].

SCHIENER, P. 2014. Bioethanol production from macroalgae. University of Aberdeen.

SCHIENER, P., BLACK, K. D., STANLEY, M. S. & GREEN, D. H. 2015. The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta. *Journal of applied phycology,* 27**,** 363-373.

SCHWEIZER-RIES, P. 2008. Energy sustainable communities: Environmental psychological investigations. *Energy Policy,* 36**,** 4126-4135.

Sea temperatures, 2017, [Online] Available: https://www.seatemperature.org/europe/united-kingdom/ [Accessed June, 2017]

SEN, S., GANGULY, S., DAS, A., SEN, J. & DEY, S. 2016. Renewable energy scenario in India: Opportunities and challenges. *JOURNAL OF AFRICAN EARTH SCIENCES,* 122**,** 25-31.

SHANMUGAM, P. & HORAN, N. 2009. Simple and rapid methods to evaluate methane potential and biomass yield for a range of mixed solid wastes. *Bioresource technology,* 100**,** 471-474.

SHIN, K.-S., KIM, C.-H., LEE, S.-E. & YOON, Y.-M. 2011. *Biochemical Methane Potential of Agricultural Waste Biomass*.

SIALVE, B., BERNET, N. & BERNARD, O. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances,* 27**,** 409-416.

SIBIYA, N. & MUZENDA, E. A review of biogas production optimization from grass silage. International Conference on Chemical Engineering and Advanced Computational Technologies, Nov, 2014. 24-25.

SINOTT, RK., (2005) Chemical engineering design, 4 edn. Coulson & Richardson's chemical engineering series. Coulson & Richardson's, New York, USA, p 1055

SJØTUN, K., FREDRIKSEN, S., LEIN, T. E., RUENESS, J. & SIVERTSEN, K. Population studies of Laminaria hyperborea from its northern range of distribution in Norway. Fourteenth International Seaweed Symposium, 1993. Springer, 215-221.

SMALE, D. A., BURROWS, M. T., MOORE, P., O'CONNOR, N. & HAWKINS, S. J. 2013. Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast A tlantic perspective. *Ecology and evolution,* 3**,** 4016-4038.

SMITH, S. & NUNN, J. 1985. Mollusca found in Clachan Sound. Conchologists' Newsletter, 95, 316-319.

SOCOTEC, UK, Available: https://www.socotec.co.uk/ [Accessed June, 2017].

SODE, S., BRUHN, A., BALSBY, T. J., LARSEN, M. M., GOTFREDSEN, A. & RASMUSSEN, M. B. 2013. Bioremediation of reject water from anaerobically digested waste water sludge with macroalgae (Ulva lactuca, Chlorophyta). Bioresource technology, 146, 426-435.

SOLEYMANI, M. & ROSENTRATER, K. A. 2017. Techno-economic analysis of biofuel production from macroalgae (seaweed). *Bioengineering,* 4**,** 92.

SONG, Y.-C., KWON, S.-J. & WOO, J.-H. 2004. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic-and thermophilic digestion of sewage sludge. *Water research,* 38**,** 1653-1662.

SORENSEN, B. 2011. *Renewable Energy,* USA, Elsevier.

SØRENSEN, B. 2011. *Renewable energy: physics, engineering, environmental impacts, economics & planning,* London, Academic.

SORRELL, S. 2015. Reducing energy demand: A review of issues, challenges and approaches. *Renewable and Sustainable Energy Reviews,* 47**,** 74-82.

SOUTH, G. R. & WHITTICK, A. 2009. *An Introduction to Phycology*, Wiley.

SPRUJIT, J. 2015. Inventory of North-West European algae initiatives, Public Output report of the EnAlgae project. Swansea.

SRIRANGAN, K., AKAWI, L., MOO-YOUNG, M. & CHOU, C. P. 2012. Towards sustainable production of clean energy carriers from biomass resources. *APPLIED ENERGY,* 100**,** 172-186.

STEFFEN, R., SZOLAR, O. & BRAUN, R. 1998. Feedstocks for anaerobic digestion. *Institute of Agrobiotechnology Tulin, University of Agricultural Sciences, Vienna*.

STILES, W. A. V., STYLES, D., CHAPMAN, S. P., ESTEVES, S., BYWATER, A., MELVILLE, L., SILKINA, A., LUPATSCH, I., FUENTES GRÜNEWALD, C., LOVITT, R., CHALONER, T., BULL, A., MORRIS, C. & LLEWELLYN, C. A. 2018. Using microalgae in the circular economy to valorise anaerobic digestate: challenges and opportunities. *Bioresource Technology,* 267**,** 732-742.

STRÖMBERG, S., NISTOR, M. & LIU, J. 2014. Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests. *Waste management,* 34**,** 1939-1948.

SUÁREZ, A., NIELSEN, K., KÖHLER, S., MERENCIO, D. & REYES, I. P. 2014. Enhancement of anaerobic digestion of microcrystalline cellulose (MCC) using natural micronutrient sources. *Brazilian Journal of Chemical Engineering,* 31**,** 393-401.

SUGAR, B. 2011. UK Beet Sugar Industry: Sustainability Report 2011. British Sugar plc and NFU Sugar.

SUHARTINI, S. 2014. *The anaerobic digestion of sugar beet pulp.* University of Southampton.

SUHARTINI, S., HEAVEN, S. & BANKS, C. J. 2014. Comparison of mesophilic and thermophilic anaerobic digestion of sugar beet pulp: Performance, dewaterability and foam control. *BIORESOURCE TECHNOLOGY,* 152**,** 202-211.

TABASSUM, M. R., XIA, A. & MURPHY, J. D. 2017. Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland. *Renewable and Sustainable Energy Reviews,* 68**,** 136-146.

TAELMAN, S. E., CHAMPENOIS, J., EDWARDS, M. D., DE MEESTER, S. & DEWULF, J. 2015. Comparative environmental life cycle assessment of two seaweed cultivation systems in North West Europe with a focus on quantifying sea surface occupation. *Algal Research,* 11**,** 173-183.

TCHOBANOGLOUS, G., BURTON, F. L., STENSEL, H. D., METCALF & EDDY, I. 2003. *Wastewater engineering: treatment and reuse,* Boston;London;, McGraw-Hill.

TCP, I. R. 2017. Commercial Readiness Index Assessment – Using the method as a tool in renewable

energy policy design (RE-CRI). *IEA Renewable Energy Technology Deployment Technology Collaboration*

Programme (IEA RETD TCP). Utrecht.

TEAGLE, H., HAWKINS, S. J., MOORE, P. J. & SMALE, D. A. 2017. The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology,* 492**,** 81-98.

TEDESCO, S., BARROSO, T. M. & OLABI, A. 2014. Optimization of mechanical pre-treatment of Laminariaceae spp. biomass-derived biogas. *Renewable Energy,* 62**,** 527-534.

TEDESCO, S. & STOKES, J. 2017. Valorisation to biogas of macroalgal waste streams: a circular approach to bioproducts and bioenergy in Ireland. *Chemical Papers,* 71**,** 721-728.

TITROLINE BUFFER SOLUTIONS, Available: https://www.camlab.co.uk/titroline-easy-titrator-from-si-analytics-p13678.aspx [Accessed June, 2017].

TELMO, C. & LOUSADA, J. 2011. Heating values of wood pellets from different species. *Biomass and Bioenergy,* 35**,** 2634-2639.

THERMO SCIENTIFIC, UK [Available]: https://mafiadoc.com/thermo-scientific-heraeus-heating-and-dryingOvens_59c06f131723dd7110dd560d.html [Accessed June, 2017].

THOLLANDER, P., GLAD, W., ROHDIN, P., LINKÖPINGS, U., FILOSOFISKA, F., INSTITUTIONEN FÖR EKONOMISK OCH INDUSTRIELL, U., TEMA TEKNIK OCH SOCIAL, F., TEKNISKA, H., INSTITUTIONEN FÖR, T. & ENERGISYSTEM 2014. Exploring Transactional Analysis in Relation to Post-Graduate Supervision—A Balancing Process. *Creative Education,* 5**,** 185-196.

THOMAS, K. & RAHMAN, P. 2006. Brewery wastes. Strategies for sustainability. A review. *Aspects of Applied Biology*.

TIAN, H., DUAN, N., LIN, C., LI, X. & ZHONG, M. 2015. Anaerobic co-digestion of kitchen waste and pig manure with different mixing ratios. *Journal of bioscience and bioengineering,* 120**,** 51-57.

TRITON ELECTRONICS, UK Available: http://tritonel.com/ [Accessed June, 2017].

VALE GREEN ENERGY, [Online]. Available: http://www.valegreenenergy.co.uk/ [Accessed June, 2017].

VAN HAL, J. W., HUIJGEN, W. & LÓPEZ-CONTRERAS, A. 2014. Opportunities and challenges for seaweed in the biobased economy. *Trends in biotechnology,* 32**,** 231-233.

VANDENDAELE, P. 2013. The economics of large-scale seaweed cultivation [Online]. Belgium: At Sea Technologies. Available: http://www.idreem.eu/cms/wp-content/uploads/2015/06/Vandendaele-Patrice-ATSEA-Technologies.pdf [Accessed 2017].

VANEGAS, C. & BARTLETT, J. 2013. Green energy from marine algae: biogas production and composition from the anaerobic digestion of Irish seaweed species. *Environmental technology,* 34**,** 2277-2283.

VANEGAS, C. & BARTLETT, J. 2015. Biogas production from the anaerobic digestion of Laminaria digitata in a 10 L pilot-plant with digestate re-use as fertiliser. *International Journal of Ambient Energy,* 36**,** 183-189.

VASILAKI, G. & GARCIA, L. 2013. Biogas production from organic wastes in Sisimiut: methane potentials and co-digestion of shrimp waste, brown algae and sludge. *Artek Database: http://find. artek. byg. dtu. dk/pubs/frontpage***,** 12.

VASSILEV, S. V., BAXTER, D., ANDERSEN, L. K. & VASSILEVA, C. G. 2010. An overview of the chemical composition of biomass. *Fuel,* 89**,** 913-933.

VASSILEV, S. V. & VASSILEVA, C. G. 2016. Composition, properties and challenges of algae biomass for biofuel application: an overview. *Fuel,* 181**,** 1-33.

VAVILIN, V. A., FERNANDEZ, B., PALATSI, J. & FLOTATS, X. 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. *Waste Management,* 28**,** 939-951.

VERGARA-FERNÃ¡NDEZ, A., VARGAS, G., ALARCÃ³N, N. & VELASCO, A. 2008. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. *Biomass and Bioenergy,* 32**,** 338-344.

VERTES, A. A., 2010. *Biomass to biofuels: strategies for global industries,* Chichester, West Sussex, U.K, John Wiley & Sons.

VILG, J. V., NYLUND, G. M., WERNER, T., QVIRIST, L., MAYERS, J. J., PAVIA, H., UNDELAND, I. & ALBERS, E. 2015. Seasonal and spatial variation in biochemical composition of Saccharina latissima during a potential harvesting season for Western Sweden. Botanica marina, 58, 435-447.

VITANZA, R., CORTESI, A., GALLO, V., COLUSSI, I. & ARANA-SARABIA, D. 2016. Biovalorization of brewery waste by applying anaerobic digestion. *Chemical and biochemical engineering quarterly,* 30**,** 351-357.

VIVEKANAND, V., EIJSINK, V. G. H. & HORN, S. J. 2012. Biogas production from the brown seaweed Saccharina latissima: thermal pretreatment and codigestion with wheat straw. *Journal of Applied Phycology,* 24**,** 1295-1301.

WALKER, M., ZHANG, Y., HEAVEN, S. & BANKS, C. 2009. Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. *Bioresource Technology,* 100**,** 6339-6346.

WALKER, S., HOWELL, R., HODGSON, P. & GRIFFIN, A. 2015. Tidal energy machines: A comparative life cycle assessment study. *PROCEEDINGS OF THE INSTITUTION OF MECHANICAL ENGINEERS PART M-JOURNAL OF ENGINEERING FOR THE MARITIME ENVIRONMENT,* 229**,** 124-140.

WALLACE, P., FREDERICKSON, J., CHAMBERS, B., TAYLOR, M., LONGHURST, P., TYRRELL, S., GALE, P., GODDARD, A. & LITTERICK, A. 2011. Digestates: Realising the fertiliser benefits for crops and grassland. *Waste and Resources Action Programme, Banbury*.

WALLS, A., EDWARDS, M., FIRTH, L. & JOHNSON, M. 2018. Ecological priming of artificial aquaculture structures: kelp farms as an example. *Journal of the Marine Biological Association of the United Kingdom***,** 1-12.

WALLS, A., KENNEDY, R., EDWARDS, M. & JOHNSON, M. 2017. Impact of kelp cultivation on the Ecological Status of benthic habitats and Zostera marina seagrass biomass. *Marine pollution bulletin*.

WANG, J.-Y., ZHANG, D.-S., STABNIKOVA, O. & TAY, J.-H. 2005. Evaluation of electrokinetic removal of heavy metals from sewage sludge. Journal of Hazardous Materials, 124, 139-146.

WANG, B., NGES, I. A., NISTOR, M. & LIU, J. 2014. Determination of methane yield of cellulose using different experimental setups. *Water science and technology,* 70**,** 599.

WANG, J.-Y., ZHANG, D.-S., STABNIKOVA, O. & TAY, J.-H. 2005. Evaluation of electrokinetic removal of heavy metals from sewage sludge. *Journal of Hazardous Materials,* 124**,** 139-146.

WANG, X. J., YANG, G. H., FENG, Y. Z., REN, G. X. & HAN, X. H. 2012. Optimizing feeding composition and carbon-nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *BIORESOURCE TECHNOLOGY,* 120**,** 78-83.

WARD, A. J., HOBBS, P. J., HOLLIMAN, P. J. & JONES, D. L. 2008. Optimisation of the anaerobic digestion of agricultural resources. *Bioresource technology,* 99**,** 7928-7940.

WARD, A. J., LEWIS, D. M. & GREEN, B. 2014. Anaerobic digestion of algae biomass: A review. *ALGAL RESEARCH-BIOMASS BIOFUELS AND BIOPRODUCTS,* 5**,** 204-214.

WATER, S. T. 2017. *Minworth Thermal Hydrolysis Process Environmental Permit Variation Application, [Online] ATKINS Report, [Accessed June 2018].*

WEI, N., QUARTERMAN, J. & JIN, Y.-S. 2013. Marine macroalgae: an untapped resource for producing fuels and chemicals. *Trends in Biotechnology,* 31**,** 70-77.

WEILAND, P. 2010. Biogas production: current state and perspectives. *Applied microbiology and biotechnology,* 85**,** 849-860.

WEILAND, P., VERSTRAETE, W. & VAN HAANDEL, A. 2009. Biomass digestion to methane in agriculture: A successful pathway for the energy production and waste treatment worldwide. *Biofuels***,** 171-195.

WERNER, A. & DRING, M. 2011. Aquaculture explained no. 27: cultivating Palmaria palmata. Irish Sea Fisheries Board.

WERNER, F. J., GRAIFF, A. & MATTHIESSEN, B. 2016. Temperature effects on seaweed-sustaining top-down control vary with season. *Oecologia,* 180**,** 889-901.

WHITE N. & MARSHALL, C. E. 2007. Saccharina latissima Sugar kelp. In Tyler-Walters H. and Hiscock K. (eds) Marine Life Information Network: Biology and Sensitivity Key Information Reviews. Plymouth: Marine Biological Association of the United Kingdom.

WIDÉN, J., CARPMAN, N., CASTELLUCCI, V., LINGFORS, D., OLAUSON, J., REMOUIT, F., BERGKVIST, M., GRABBE, M., WATERS, R., UPPSALA, U., ELEKTRICITETSLÄRA, TEKNISKA, S., INSTITUTIONEN FÖR, T., FASTA TILLSTÅNDETS, F. & TEKNISK-NATURVETENSKAPLIGA, V. 2015. Variability assessment and forecasting of renewables: A review for solar, wind, wave and tidal resources. *Renewable and Sustainable Energy Reviews,* 44**,** 356-375.

WILLIAMS, C. L., WESTOVER, T. L., EMERSON, R. M., TUMULURU, J. S. & LI, C. 2016. Sources of Biomass Feedstock Variability and the Potential Impact on Biofuels Production. *BioEnergy Research,* 9**,** 1-14.

WRAP 2012. Water use in the UK food and drink industry, UK [Online] [Accessed June 2017].

WRAP 2015. Optimising the value of digestate and digestion systems. UK [Online] [Accessed June 2017].

WRAP 2017. Comparing the Costs of Alternative Waste Treatment Options. Banbury.

WORLD SEA TEMPERATURES, 2016, [Online]. Available: https://www.seatemperature.org/ [Accessed June, 2017].

XIA, A., JACOB, A., TABASSUM, M. R., HERRMANN, C. & MURPHY, J. D. 2016. Production of hydrogen, ethanol and volatile fatty acids through co-fermentation of macro-and micro-algae. *Bioresource technology,* 205**,** 118-125.

XIE, S., WICKHAM, R. & NGHIEM, L. D. 2017. Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes. *International Biodeterioration & Biodegradation,* 116**,** 191-197.

XIROS, C. & CHRISTAKOPOULOS, P. 2012. Biotechnological potential of brewers spent grain and its recent applications. *Waste and Biomass Valorization,* 3**,** 213-232.

XU, S., KONG, X., LIU, J., ZHAO, K., ZHAO, G. & BAHDOLLA, A. 2016. Effects of high-pressure extruding pretreatment on MSW upgrading and hydrolysis enhancement. Waste management, 58, 81-89

YE, J., LI, D., SUN, Y., WANG, G., YUAN, Z., ZHEN, F. & WANG, Y. 2013. Improved biogas production from rice straw by co-digestion with kitchen waste and pig manure. *Waste Management,* 33**,** 2653-2658.

YEN, H.-W. & BRUNE, D. E. 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource technology,* 98**,** 130-134.

YESSON, C., BUSH, L. E., DAVIES, A. J., MAGGS, C. A. & BRODIE, J. 2015. Large brown seaweeds of the British Isles: evidence of changes in abundance over four decades. *Estuarine, coastal and shelf science,* 155**,** 167-175.

YIN, X., HAN, P., LU, X. & WANG, Y. 2004. A review on the dewaterability of bio-sludge and ultrasound pretreatment. *Ultrasonics Sonochemistry,* 11**,** 337-348.

YIRONG, C., HEAVEN, S. & BANKS, C. 2015. Effect of a trace element addition strategy on volatile fatty acid accumulation in thermophilic anaerobic digestion of food waste. *Waste and Biomass Valorization,* 6**,** 1-12.

ZAMALLOA, C., VULSTEKE, E., ALBRECHT, J. & VERSTRAETE, W. 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. *Bioresource technology,* 102**,** 1149-1158.

ZHANG, C., SU, H., BAEYENS, J. & TAN, T. 2014. Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews,* 38**,** 383-392.

ZHANG, P., ZENG, G., ZHANG, G., LI, Y., ZHANG, B. & FAN, M. 2008. Anaerobic co-digestion of biosolids and organic fraction of municipal solid waste by sequencing batch process. *Fuel Processing Technology,* 89**,** 485-489.

ZHANG, Q., HU, J. & LEE, D.-J. 2016. Biogas from anaerobic digestion processes: Research updates. *Renewable Energy,* 98**,** 108-119.

ZHONG, W., ZHANG, Z., QIAO, W., FU, P. & LIU, M. 2011. RETRACTED: Comparison of chemical and biological pretreatment of corn straw for biogas production by anaerobic digestion. Elsevier.

ZINDER, S. H., CARDWELL, S. C., ANGUISH, T., LEE, M. & KOCH, M. 1984. METHANOGENESIS IN A THERMOPHILIC (58-DEGREES-C) ANAEROBIC DIGESTER - METHANOTHRIX SP AS AN IMPORTANT ACETICLASTIC METHANOGEN. *Applied and Environmental Microbiology,* 47**,** 796-807.

ZUBIA, M., PAYRI, C. & DESLANDES, E. 2008. Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, Sargassum mangarevense and Turbinaria ornata (Phaeophyta: Fucales), from Tahiti (French Polynesia). *Journal of applied phycology,* 20**,** 1033-1043.