Using microalgae in the circular economy to valorise anaerobic digestate: challenges and opportunities

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Abstract:

Managing organic waste streams is a major challenge for the agricultural industry. Anaerobic digestion (AD) of organic wastes is a preferred option in the waste management hierarchy, as this process can generate renewable energy, reduce emissions from waste storage, and produce fertiliser material. However, Nitrate Vulnerable Zone legislation and seasonal restrictions can limit the use of digestate on agricultural land. In this paper we demonstrate the potential of cultivating microalgae on digestate as a feedstock, either directly after dilution, or indirectly from effluent remaining after biofertiliser extraction. Resultant microalgal biomass can then be used to produce livestock feed, biofuel or for higher value bio-products. The approach could mitigate for possible regional excesses, and substitute conventional high-impact products with bio-resources, enhancing sustainability within a circular economy. Recycling nutrients from digestate with algal technology is at an early stage. We present and discuss challenges and opportunities associated with developing this new technology.

Keywords:

Anaerobic digestion, algae, nutrient recycling, livestock feed, circular economy

1.0 Introduction:

Current agricultural approaches to organic waste management can result in large losses of nutrients, particularly nitrogen (N) and phosphorus (P), to the atmosphere and local aquatic ecosystems (Carpenter et al. 1998; Smith et al. 2001a & 2001b; Misselbrook et al. 2010), affecting water and air quality (Withers & Lord, 2002; Erisman et al. 2008). Current agricultural activities also result in the emission of greenhouse gases (GHGs), both directly as a result of organic waste management approaches (Chadwick et al. 2011), and indirectly as a consequence of land use change, driven by changing patterns in animal product consumption (Tilman & Clark, 2014).

By 2050, consumption rates of meat and livestock products are predicted to double (Steinfeld et al. 2007). The global increase in demand for meat products will result in a rise in demand for protein for animal feed, particularly soya, which is likely to drive land-use change in the form of deforestation (Gasparri et al. 2013). This activity is a major contributor to global anthropogenic GHG emissions, and has been estimated to account for ~20% of global CO_2 emissions (Van der Werf et al. 2009). European dependence on the import of protein for animal feed also has implications for food security, due to large potential for future supply chain volatility (de Visser et al. 2014). Increased global demand and competition, coupled with reductions in supply as a consequence of climate change, are likely to drive price increases and reduce availability (Osborne et al. 2013).

Reducing GHG emissions from agriculture is an essential component in the UKs national strategy for CO_2 equivalent emission reduction, necessary in order to meet the obligations of the Paris climate agreement (Wollenberg et al. 2016). Managing GHG emissions from manure can be achieved through improved infrastructure, such as covered slurry lagoons, or with technology such as anaerobic digesters. These harvest the produced methane in a controlled environment for the purposes of energy production (Hopkins & Del Prado, 2007).

Due to the financial opportunities offered by energy production, food and farm waste is increasingly being converted to biomethane via anaerobic digestion (AD). Recognised for its potential pollution abatement qualities, the AD process also yields a typically nutrient rich digestate. Digestates, when applied onto agricultural land, can provide benefits such as waste stabilisation and reduction in GHG emissions, odour reduction and the provision of low carbon nutrients and biostimulants that support crop growth (e.g. Möller & Müller, 2012; Walsh et al. 2012; WRAP, 2012; Scaglia et al. 2017; Sigurnjak et al. 2017). Digestates can be rich in a number of macro nutrients (e.g. N, P, K, S, Mg, Ca, Fe, and Na) and may contain a number of trace elements (e.g. Co, Fe, Se, Mo and Ni) either as a result of the original feedstock used (Marcato et al. 2008), or due to supplementation as part of a trace element addition for improved digester performance (Williams et al. 2013). Digestate can be separated into solid and liquid fractions. Liquid digestate typically has a high nutrient status, intermediate in strength between livestock manures and inorganic fertiliser (Nkoa, 2014). Digestate contains significantly more available N than cattle slurry (80 – 90 % of N in whole or liquor digestate – AHDB, 2017). Whilst the compound form of N in digestate is more readily available for uptake by plants, environmental losses can occur after land application, posing particular risks in regions where N is in excess.

Under the EC Nitrates Directive (91/676/EEC) and Nitrate Vulnerable Zone (NVZ) legislation, the amount of N that can be returned to land is restricted. Phosphate land

overloads are now also significant in numerous European regions and land usage restrictions are being implemented (Sigurnjak et al. 2017). Regional and seasonal restrictions on the use of digestates, either due to crop non-growth periods or limitations on nutrient loadings to agricultural land in particular for N and P, the resulting long periods of storage required and the restricted local farm land availability, are becoming significant barriers to AD deployment and for digestate use (Passanha et al. 2013). In order to support a continual growth in AD technology deployment and mitigate for overloads of nutrients potentially causing a negative environmental impact, new markets and novel uses for digestates are required.

Alternative uses for digestates have started to be investigated and results seem promising in particular within biorefining platforms, such as enhancing ethanol production by using digestate effluents instead of freshwater and nutrients (Gao and Li, 2011); enhancing polyhydroxyalkanoate production by using digestate as fermentation nutrient media (Passanha et al. 2013), and for increasing the yields of carboxylic acids from acid phase anaerobic fermentations when thermally treated and filtered digestate was used as bacterial stimulant (Kumi et al. 2016). Another option to valorise digestate is to establish a microalgae biorefining platform and further mitigate environmental impacts in terms of avoiding excess nutrient loads discharged onto environmental receptors and at the same time drive a low carbon protein production industry.

Microalgae are increasingly being researched and used globally to remediate nutrients in organic waste, and as a source of biomass, products and energy (Sivakumar et al. 2012; Abinandan et al. 2015). Microalgae need a source of nutrients to grow and can therefore be used to recycle nutrients in digestate (Wang et al. 2010; Uggetti et al. 2014). The resultant microalgae crops, which are high in protein, can be used as a feed source for livestock or aquaculture industries (Becker, 2007; Yaakob et al. 2014). This system presents an

opportunity to establish a circular economy solution for organic waste streams, which would limit the impact of agriculture and organic waste management on the environment, by reducing nutrient pollution, GHG emissions, and the requirement for land use change to enable animal feed production, and increase the potential for food security in the European Union and beyond.

1.1 Background

The potential of using algae to remediate waste, including nutrients, metal, carbon dioxide and organic pollutants, has been recognised over many decades. Pioneering work in the 1950s by William Oswald established the potential of microalgae in domestic sewage treatment and, in particular, that consortia rather than unicellular culture were the most effective (Oswald et al. 1953). The drive for secure energy in the US led to the National Renewable Energy Laboratory's Biofuel Program and the Aquatic Species Program (Sheeman et al. 1998). This program undertook screening of microalgae for lipids and cultivation, which established the foundation for further studies. This coupled with a renewed drive for renewable energy production in the early 2000s, culminated in a series of Roadmaps (Fishman et al. 2010; Parker and Schlarb-Ridley, 2013; Barry et al. 2016). More recently, improved 'omics techniques and better understanding on algal genomes has re-invigorated algal biotechnology research. In addition, there is now an increasing recognition that we need to reduce and recycle waste and reduce consumption of finite resources including nutrients working towards a circular economy approach. The ability to cultivate algal biomass from waste nutrients, of which digestate is an excellent source, and then use biomass either whole or fractionated as a commodity is an attractive proposition. Algae are a rich source of protein and lipids and many other useful compounds with bioactive properties. In addition to the food, feed and fuel industries, algal bioactives have proven application in the pharmaceutical

and cosmetics industry (Singh et al. 2017). Algae, particularly cyanobacteria, can also be applied as a soil treatment and a slow release fertiliser (Sharma et al. 2012).

It has been suggested at a global level that the contribution of microalgae protein to human nutrition is limited due to the small scale of production. Within the EU, factors including current legislation, unfavourable climatic conditions for growth, and insufficient consumer demand, are the cause of this adverse effect on production (Vigani et al. 2015). Nevertheless, the growing need for a stable and reliable domestic supply of protein for animal feed from within the EU (de Visser et al. 2014) makes this a key area for research. In addition, the production of microalgae has the potential to generate essential nutritional compounds, such as omega-3, where the current source of supply (fish-oils) is becoming increasingly costly and rare (Vigani et al. 2015). This may have significant implications for human nutrition globally. Thus, the global market application for microalgae products is increasing. The EU has the potential to become a market leader in the next decade due to its dominant position in the global agri-food markets.

2.0 Challenges

2.1 AD Technology Infrastructure and Digestate Separation

AD technology infrastructure differs depending on the plant design, which is influenced by feedstock characteristics, their processing and temporary storage of feedstocks, types of digesters, the level of processing and use of the biogas and also according to the level of processing and storage of digestates. Figure 1a shows a schematic of typical AD technology infrastructure.

Detailed schematics of a variety of AD plants have been previously presented in Monson et al. (2007). Digestates can be utilised without any further processing directly after digestion,

or they can go through a number of separation and processing techniques. Whilst the majority of digestate from digesters is currently applied to land as whole digestate, some digestates are separated into the solid or fibre fraction and the liquor fraction. In the case of crop-based digestates including animal slurries, separation is used to ensure that the liquor fraction can be applied to land using precision equipment (digestate shallow injection) without blockages. Separation or 'dewatering' is the preliminary step in a host of digestate enhancement techniques, which include ammonia stripping, micro, ultra, nanofiltration and reverse osmosis. Dewatering tends to represent a substantial investment with potentially high operational costs, but can dramatically reduce transport costs if a chosen outlet can be found for the liquor fraction. Dewatering can be achieved by the use of centrifuges and belt filter presses. The efficiency of dewatering depends upon the nature of the digestate and the characteristics of residual particles digestates' chemical and microbial matrices following the AD process. For example, the presence of polysaccharides or cellular intracellular water typically provides difficulties in dewatering and coagulant/flocculants are used to support the task (e.g. Oliveira et al. 2016). The ability to sterilise digestates, recover, separate and concentrate various nutrients residual in digestate utilising membrane systems for further utilisation is receiving considerable attention. Recent developments in membrane separation technologies have made it possible to separate and recover products from digestates, with these technologies being more cost efficient (Fuchs and Drosg, 2010).

2.2 Challenges of applying anaerobic digestate as a feedstock

Digestates are typically rich in two essential nutrients, N (primarily NH_3) and P (primarily PO_4), which are essential for the growth of photosynthetic organisms such as microalgae. However, digestate may also contain other potentially toxic elements (PTEs) or compounds such as lead (Pb), zinc (Zn) and copper (Cu) (Coelho et al. 2018). Essential nutrients and PTE

concentrations present in the digestate vary depending on feedstock composition in AD plants.

Metals and phosphates bind strongly to solids during the digestion process, but this will be affected by digestate sludge pH, as solubilisation will happen at low pH statuses. Thus, acidifying the digestate sludge can release metals and P into a soluble form. Microfiltration coupled with acidification can then be applied to remove metals and produce a material of different N:P compositions (from 34 to 8), by varying the P component (Gerardo et al. 2013).

In order to optimise the digestate and prepare the medium that will be used during the microalgae biomass production process, a suitable system must be established (Figure 1b). Here, the flow of the digestate is presented in two main parts: upstream and downstream. During the upstream process, the digested liquor (digestate) is collected from the main digester and put in the settling tank. This is necessary because digestates collected from AD plants have typically mesophilic temperatures ranging from 27 to 42°C, and pH mainly in the alkaline region (typically between 7.4 – 8.2) (Coelho et al. 2018). Both these abiotic parameters are above the optimal values for the common microalgal strains such as *Chlorella* or *Scenedesmus* (e.g. 25°C and neutral pH).

After a Hydraulic Retention Time (HRT) of >8 hours in a settling tank to allow solid matter precipitation, the upper layer of the digestate from the tank should be passed through microfiltration (0.2 μm) in order to retain the remaining solids in the digestate. Membrane technology (micro/ultrafiltration) is a well known technology that recently has been applied to the upstream and downstream process in microalgae production (Gerardo et al. 2014; Mayhead et al. 2018).

It is highly advisable to use the same technology to perform the digestate pre-treatment during the upstream process. Using this technology will allow mechanical sterilisation of the

digestate, avoiding the inclusion in the microalgae culture of the main pathogens present in digestates, such as *Eschericia coli* (0.5 -2.0 *μm*) and *Salmonella spp*. (2.0-5.0 *μm*). Also, using micro/ultrafiltration (filtration with a low molecular weight cut off) can help to adjust N:P ratio of the digestate to an optimum level, as suggested above. This will be different for each strain of microalgae, but a ratio of 7:1 for N:P has been suggested as suitable for balanced nutrients in algae (Fenton & O´hUallachain, 2012). Managing the digestate to achieve an optimum ratio for N:P is vital for a successful microalgae culture. This is necessary because high ammonia concentrations (> 2.3 μM) can inhibit microalgae growth (Cho et al. 2013). Furthermore, the presence of solid matter will have a direct impact on microalgae growth, by reducing the potential for light availability, resulting in a lower growth rate (Mayhead et al. 2018). Further research is necessary in order to improve the potential of ultra/diafiltration technology for the removal of PTEs that potentially can inhibit microalgae growth. Special attention should be paid to Cu, since it is one of the most toxic elements for photosynthetic organisms.

2.3 Algal species selection

Amongst the many thousands of microalgal species present in nature, there are only a few commonly occurring species currently studied and known to be robust survivors in wastewater or in digestate. These include species belonging to the genera *Chlorella*, *Scenedesmus*, and *Desmodesmus*, with key species being *Chlorella vulgaris* and *Scenedesmus obliquus*. Algal consortia and algal-bacteria consortia are more suitable for large-scale cultivation on wastewater than unicellular culture, by acting symbiotically, especially in terms of preventing contamination and enabling long-term cultivation (González-Fernández, 2011; Medina and Neis, 2007; Gonçalves et al. 2017). In this symbiosis, the O_2 released by algal photosynthesis is utilized by aerobic-heterotrophic bacteria to mineralize organic compounds, and bacterial respiration provides CO_2 as a carbon (C) source to the algae.

Uptake of nutrients from digestate has been shown to be more efficient in mixed algal and bacterial consortia systems than for unicellular systems (Kerckhof et al. 2014; Mahapatra et al. 2014; Lahel et al. 2016; Vulsteke et al. 2017). In mixed algal-bacterial consortia systems, growth increases the pH and allows precipitation of phosphorus, promoting the remediation process (Kang et al. 2018). Furthermore, cultures cultivated under mixotrophic conditions, have been shown to have higher growth rates compared to when cultivated under heterotrophic or autotrophic conditions (Lalucat et al. 1984).

There are a number of challenges in large-scale cultivation of algae on digestate. A key challenge in mixed consortia and mixotrophic systems, especially where there is a source of dissolved C present (e.g. glycerol or organic acids), is to ensure that bacteria do not dominate the consortia system causing the algal cells to crash. Another challenge in large-scale algal cultivation on digestate is the dynamic nature of the algal-bacterial consortia. Successful large-scale cultivation of algae particularly on wastewater and digestate requires close monitoring and regulation of biotic and abiotic conditions (Van Den Hende et al. 2014; Silkina et al. 2017). The ability to maintain a functional and reproducible stock culture of a mixed algal consortia is beneficial and has been demonstrated through cryopreservation (Silkina et al. 2017).

2.4 Optimising digestate feedstock for algal growth

To understand the influence of digestate on algal metabolic processes, flux balance analysis (FBA) (Orth, 2010) was used to model growth potential in *C. vulgaris*, *i*CZ843 (a standard model organism – Zuñiga et al. 2016), using different dilutions of swine with crop fed digestate (Figure 2a), with a key focus on docosahexaenoic acid (DHA) production (Figure 2b). Robustness analyses were then performed to identify optimal conditions for growth and DHA production. The model was first validated with experimentally measured growth rates

(Table 1). All simulations were conducted using the COBRApy toolbox using Python and Gurobi solver, version 7.5.2 (Ebrahim, A., 2013).

The constituents of swine and arable crop digestate streams at various dilutions have been measured elsewhere (ammonia and acetic acid - Zulini et al. 2016; phosphate, nitrate, magnesium, and iron - Levine et al. 2010). These values were used to model microalgae growth rates under mixotrophic, phototrophic and heterotrophic growth conditions for different dilution factors (Figure 2a). As per Orth (2010), growth rate is expressed as hr^{-1} and metabolite fluxes, such as that of DHA, is expressed as mmol per gram of dry weight growth (mmol gDW^{-1} hr^{-1}).

Thirty-fold dilutions of digestate resulted in the highest rate of predicted growth for each growth regime (Figure 2a), which is in agreement with the results presented by Zuliani et al. (2016). The highest growth rate was observed with a 30-fold dilution with heterotrophic metabolism (0.111 hr^{-1}) followed by mixotrophic growth and phototrophic growth (both predictions were 0.042 hr^{-1}). This trend was consistent across all dilutions bar the 200-fold digestate dilution, where the mixotrophic regime yielded the highest growth rate.

Heterotrophic growth of microalgae to produce biotechnologically important metabolites is cheaper and simpler than mixotrophic growth (Perez-Garcia, et al. 2011). The capacity of potential production of DHA was therefore explored for each growth regime and dilution using Flux Variability Analysis (FVA).

As seen in Figure 2b, *i*CZ843 predicted that heterotrophic growth on digestate diluted 30 times would result in optimal production of DHA (1.49×10^{-4} mmol gDW^{-1} hr^{-1}). At each dilution factor tested, heterotrophic metabolism resulted in more DHA production than mixotrophic and phototrophic growth. At a 200-fold dilution, *C. vulgaris* cells grown mixotrophically are predicted to be completely incapable of synthesising DHA. Thus, these

simulations suggest that optimal production of DHA can be obtained from heterotrophic growth on digestate diluted 30 times.

Biomass and DHA production were predicted with the model (Figure 2a & b), and used to investigate which nutrients limit or increase biomass. Robustness analyses were also conducted for acetate, NH_4 and NO_3. For NH_4 uptake, an optimal growth rate of 0.103 hr^{-1} was achieved with uptake of 2 mmol gDW^{-1} hr^{-1}, after this, biomass decreased. For NO_3, a detrimental effect on biomass was observed with increasing uptake, suggesting NH_4 alone can provide almost all of the N requirements to sustain a heterotrophic algal cell grown on digestate diluted 30-fold (original growth rate of heterotrophic grown cell on 30-fold diluted digestate sample was predicted to be 0.111 hr^{-1}).

Since heterotrophically grown cells rely on an inorganic C source to grow, a robustness analysis was performed to investigate how acetate uptake affects growth rate. Increasing acetate uptake resulted in greater heterotrophic growth rates, even beyond the predicted flux presented in Figure 2a (0.111 hr^{-1}), to a high of 0.837 hr^{-1}. This result indicates the optimal acetate uptake rate is 35 mmol gDW^{-1} hr^{-1}, which corresponds with an 8-fold increase in algal biomass. After this point, any increase in acetate has an adverse effect on cell biomass.

Digestate diluted 30 times contains 3.33 mg L^{-1} of acetate. The analysis conducted suggests the acetate concentration of digestate can be increased by a factor of 10 when acid anaerobic fermentations are targeted, with other conditions remaining the same for optimised cell growth. The ratio of C:N is accepted to be a key factor governing plant and microalgae growth (Commichau et al. 2006; Zheng, 2009; Fait et al. 2018). This was also explored further in the analysis. The reduction in the growth rate that was observed when NH_4 uptake exceeds 2 mmol gDW^{-1} hr^{-1} can be explained by the impact of C limitation. In the same respect, the reduction in growth rate observed when acetate uptake was greater than 35 mmol

311 gDW^{-1} hr^{-1}, was explained by N limitation. To test this hypothesis, a robustness analysis was
312 performed to predict the biomass of heterotrophic cells grown in conditions of 30-fold
313 digestate dilution, with acetate constrained to an optimal uptake of 35 mmol gDW^{-1} hr^{-1}, as
314 determined by the above analysis.

315 The optimised heterotrophic growth rate was revealed to be a function of acetate and NH_4
316 uptake. Optimal uptake bounds of NH_4 are determined at 15 mmol gDW^{-1} hr^{-1} and any excess
317 beyond this inhibits cell growth, confirming the need to dilute digestate. Furthermore, at an
318 uptake rate of 35 and 15 mmol gDW^{-1} hr^{-1} for acetate and NH_4 respectively, algal cells were
319 shown to more than double their production of DHA from 0.149 $x10^{-3}$ gDW^{-1} hr^{-1} to 1.106
320 $x10^{-3}$ gDW^{-1} hr^{-1}. To achieve this optimised production of DHA, using a metabolic
321 reconstruction of *C. vulgaris*, model predictions suggest digestate diluted 30 times should be
322 supplemented with acetate to a final concentration of 35 g L^{-1} and NH_4 should be reduced to
323 15 g L^{-1}. All other nutrients can be kept at 30 fold dilutions.

324 **2.5 Implementation**

325 Commercial scale algae cultivation is currently a relatively immature sector and the techno-
326 economic challenges of integrating this process with AD have to be addressed. However, in
327 order to catalyse wider adoption of these systems we also need a better understanding of the
328 scope and scale of potential market opportunities from a bioremediation perspective as well
329 as from the perspective of high value products. This requires a foundation of knowledge and
330 data/information from across the whole value chain, which can be translated and transferred
331 to stakeholders (particularly project developers and investors). This information may be
332 complex technical, economic and regulatory information or tacit knowledge (experience and
333 'know how' of expert and non-expert stakeholders). Current research around implementation
334 of Algal-AD systems is delivered by multi-disciplinary teams working transnationally with a

wide range of stakeholder groups. In order to provide coherent and consistent support to stakeholders the data and information generated through research needs to be synergised and harmonised.

Standard methodologies from knowledge based engineering can be utilised to collate and integrate data and information from a wide range of sources and translate and represent it via user friendly online decision support tools. These tools can then be used to explore aspects such as technical feasibility, economic viability, and environmental sustainability. Traditionally, knowledge based engineering has been applied to mature sectors such as aerospace and automotive where data and information is explicit and can be stored easily as facts and rules, however, research across the biobased industries is still evolving and this can make knowledge capture, integration and representation far more challenging. Translating tacit knowledge into machine-readable data enables greater accessibility, consistency and less error (Farazi et al. 2018). This can enable project developers to reduce the risk of a project earlier in the project life cycle. For example, one of the challenges of implementing AD projects is the security and consistency of biomass supply. Tools have been developed which integrate geographical data (identifying the location of bioresources) and local infrastructure (roads, rail etc.) with supplier information relating to availability of supply and biomass characteristics. This enables project developers to undertake a bioresource assessment prior to project implementation. This technique can also be used to identify current land use (e.g. agricultural), existing facilities (e.g. AD plants) as well as protected areas such as Nitrate Vulnerable Zones (NVZs).

These map based applications represent complex data in a more accessible way. They enable stakeholders to evaluate potential opportunities and connect with other stakeholders thereby improving supply chain integration.

Tools have also been developed that enable end users to understand process performance for a given technology and explore multiple valorisation pathways according to their specific resources or requirements. This would have traditionally required consultation with various experts; however, by capturing this knowledge within an online tool, users can conduct preliminary feasibility assessments. For example, growth modelling tools can be used to explore the potential of a given technology based on design or on process inputs (e.g. light, nutrients, water, etc.).

The methodologies for developing these tools are continually being developed. Working closely with stakeholders (across the value chain and also data providers) enables knowledge engineers to understand requirements and optimise the tools' design and functionality. The architecture of these tools is modular and therefore flexible and adaptable. This means they can be expanded and updated as further data is generated over time.

3.0 Opportunities

3.1 Commercial Applications

The production of microalgae has been demonstrated for numerous applications, including the production of cosmetics (Spolaore et al. 2006), biofuels (Suganya et al. 2016), human or animal feed (Becker, 2007), or as a soil treatment and slow release fertiliser (Mulbury et al. 2004). Of key interest here is the potential for this material to provide a solution to the burgeoning problem of protein production for livestock feed (de Visser et al. 2014).

Protein and lipid substitutes for the animal feed sector represent the most obvious use of the cultivated biomass, either used as a whole biomass or fractionated into bulk constituents. Further refinement of the biomass to produce higher value products including pigments, niche fatty acids and peptides present a more convincing economic LCA. A key challenge

here is the regulatory and legislative requirement associated with the use of algae in feed and food and with the use of a waste to produce feed. Currently only a handful of species are generally recognised as safe (GRAS). Although the commercial scale algal industry has been active for several decades, there are still only a handful of species cultivated on a large scale and for only a small range of products. Wider acceptance of algae across more species, and for a wider range of products, requires a shift in legislation and regulation on the use of these valuable organisms.

3.2 Microalgae for animal or aquaculture feed

Cultivated microalgae play an important role in the early rearing of farmed marine shellfish and finfish. In intensive hatcheries, individual strains of microalgae are cultivated in separate reactors and administered regularly to the farmed species. Algae biomass is also incorporated in formulated animal feeds, both for aquaculture species and terrestrial livestock. To date, feed formulators have mainly focused on algae as a supplement to provide specific functional benefits rather than gross nutrients such as protein. Algae have been credited with improving the immune system (Turner et al. 2002), lipid metabolism (Nakagawa, 1997), improved gut function (Michiels et al. 2011) and stress resistance (Nath et al. 2012; Sheikhzadeh et al. 2012), as well as providing an organic source of carotenoids (Gouveia et al. 2002; Choubert and Heinrich 1993). The reason only a few studies evaluate algae as a major feed ingredient for farmed animals is typically due to the large amounts of biomass needed.

Nevertheless, the demand for meat and fish is rising worldwide and so is the need for animal feeds and ingredients. Historically, aquaculture has depended heavily on fishmeal, and fish oil as the main source of protein and lipids, but these sources are finite. Consequently, there is a growing interest in partial or complete replacement of fishmeal by alternative protein sources of either animal or plant origin. The main challenge in reducing fishmeal use is to

find alternatives that maintain acceptable growth rates, and support animal health and quality of the final product. Furthermore, alternative feed sources must have nutritional characteristics such as a medium to high protein level, a balanced amino acid profile, high digestibility, palatability as well as low levels of antinutritional factors.

Several suitable protein substitutes are commercially available such as soybean meal, pea seed meal, corn gluten, poultry by-product meal (Table 2). However, none of them contains the long chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Without DHA and EPA in the aquafeed, the end product would also lack these long chain omega 3 fatty acids, which are an important nutritional element of fish and seafood for humans. Freshwater algae such as *Chlorella* and *Spirulina* lack DHA and EPA but may still have good potential as protein sources (Table 2), whereas marine microalgae such as *Nannochloropsis, Tetraselmis, Pavlova* or the heterotroph *Schitzochytrium* are the fundamental sources of EPA and DHA. As fish oil supply is limited, marine lipid rich algal biomass is being considered as an alternative ingredient especially in aqua feeds.

In order to evaluate the suitability of a novel feed ingredient, determination of the digestibility is crucial in order to assess the overall nutritional value. In a digestibility trial using mink (*Mustela vison*), reported by Skrede et al. (2011), three algal species *Nannochloropsis oceanica*, *Phaeodactylum tricornutum* and *Isochrysis galbana* were included at graded levels up to 24% (dry weight) in the feed. The protein digestibility determined for *N. oceanica*, *P. tricornutum* and *I. galbana* were found to be 35.5%, 79.9% and 18.8%, respectively, which is rather low. The authors hypothesized that the cell wall of the diatom *P. tricornutum* may be have been more easily broken down by digestive processes than the others, thus resulting in higher digestibility. Other authors have noted the negative effects of a tough algal cell wall on digestibility. Jarynsk et al. (2007) tested the digestibility of *Chlorella* biomass in rats using three treatments such as spray-dried, electroporated and

ultrasonicated. Ultrasonication was found to increase the protein digestibility of *Chlorella* from 53% (spray-dried) to 63%. In another study by Blake and Lupatsch (2012), using spray-dried and freeze-dried *Chlorella* in tilapia, the process of freeze drying improved protein digestibility from 63% to 69%. Digestibility coefficient of solar dried *Spirulina* biomass has also been tested for Arctic char and Atlantic salmon at 30% dietary inclusion level (Burr et al. 2011). Protein digestibility ranged between 82% and 84.7% for the two fish species respectively. These relatively high digestibility coefficients compare favourably with terrestrial plant ingredients, confirming the high potential of *Spirulina* as a protein source for farmed fish.

Unlike terrestrial crops, marine algae can directly produce HUFA such as arachidonic acid (AA, 20:4n-6) (*Porphyridium*), eicosapentaenoic acid (EPA, 20:5n-3) (*Nannochloropsis*, *Phaeodactylum*, *Nitzschia*, *Isochrysis*, *Diacronema*) and docosahexaenoic acid (DHA, 22:6n-3) (*Crypthecodinium*, *Schizochytrium*). Whilst most of these algae are not suitable for direct human consumption, they might indirectly boost the nutritional value for humans if added to animal feeds.

According to a recent study by Gbadamosi and Lupatsch (2018), *Nannochloropsis* added as the sole protein and lipid source in the diet outperformed a soybean only based diet. In addition, feeding tilapia the EPA rich algae resulted in a considerable boost of the EPA levels in the fish. The growth performance and feed conversion efficiency of European seabass (*Dicentrarchus labrax*) were also unaffected when fish were fed a mixture of *Tisochrysis lutea* and *Tetraselmis suecica* freeze-dried biomass, which replaced 45% crude protein and 36% lipid in the diet. Moreover, including the dried microalgae in the diet resulted in a higher nutritive value than that of a high-soybean meal control feed (Cardinaletti et al. 2018).

Several studies evaluated the DHA-rich algal meal derived from *Schizochytrium,* as a replacement for fish oil in Atlantic salmon. Salmon fed 11% algal biomass in their diet had similar DHA levels in their filet compared to fish oil fed fish (Sprague et al. 2015). Including 5% of *Schizochytrium* in salmon feed can successfully replace fish oil as source of n-3 LC-PUFA without compromising fish growth rate, feed conversion efficiency and flesh quality (Kousoulaki et al. 2016). The replacement of fish oil with a DHA-rich *Schizochytrium* also significantly decreased both dietary and flesh fillet organic pollutants levels such as dioxin and PCBs compared to fish oil based treatments (Sprague et al. 2015).

In order for algal biomass to become a readily available ingredient, algae producers and feed manufacturers will need to take into account the potentially large variations in approximate composition (proteins, lipids, fatty acids, minerals, etc.) and digestibility encountered among different algal strains and growing conditions. Effort is needed to ensure a more consistent composition of algal biomass, a consistent supply so that manufacturers can readily incorporate this new feedstuff in formulated feeds. Possible means of increasing the nutritional value of some algal species would be to break down the cell wall fragments by mechanical treatment or even removal of most of the fibre, although such additional processing steps would add further to their cost. As several suitable protein sources are available, marine algae would be most attractive as a source of long chain polyunsaturated fatty acids such as EPA and DHA.

3.3 Economic potential of nutrient recycling technologies

The profitability of an AD plant of any size depends on a combination of the organic waste disposal/utilisation cost, current local renewable energy incentives, and fossil fuel energy prices. An AD plant running on selected farm wastes and sized to produce at least a $1MW_e$ costs in the region of £3.5M to construct. In the UK, a biomethane AD plant would also

typically include a 499 kW_e Combined Heat and Power (CHP) plant, with the remaining biogas, a little over 5000 m^3 day^{-1} or approximately 22.1 GWh $year^{-1}$, diverted to biomethane upgrading.

The CHP plant would provide heat to the AD plant/algal production system, as well as electricity to carry out necessary biorefinery processes, such as those outlined in Figure 1c. A 499 kW_e CHP plant operating for 8100 hours $year^{-1}$ (92.5% load factor), at 40% electrical efficiency and 56% thermal efficiency, could produce 4.04 GWh $year^{-1}$ of electricity and 5.7 GWh $year^{-1}$ of heat for on-site utilisation. Thus, the economics of the system can be improved by maximising the on-site utilisation of CHP heat and electricity; this would also mitigate some environmental burdens associated with algal production.

Biogas production and digestate nutrient levels vary considerably, depending upon the quality and quantity of the feedstock input into the digester. Feedstocks and biogas production figures were derived from the BORRG AD Assessment Tool (ADAT, 2015) for a potential 1 MW_e equivalent digester configuration are shown in Table 3. These three agricultural feedstocks are considered typical for the purpose of this study, due to wide availability. However, many AD suppliers prefer to limit the inclusion of poultry litter to less than 10% of total feedstock, due to its propensity to produce ammonia within the process, which can potentially inhibit biogas production.

The value of whole digestate is shown in Table 4. The value of ammonium N, P_2O_5 Triple Super Phosphate (TSP) and Muriate of Potash have been derived from AHDB (2018), respectively and converted to a value per kg. The two digestate values of £9.53 t^{-1} and £5.52 t^{-1} were derived from these specific AD feedstocks using the ADAT nutrient levels from Table 3 above and standard 'agricultural AD' RB209 values (AHDB, 2017). The NNFCC model (NNFCC, 2010) values digestate on the availability of the nutrients, using 70%, 60%

503 and 90% respectively for N, P and K availability. Valuing digestate based on this nutrient
504 availability would reduce the value to £7.07 t^{-1} using ADAT nutrient levels and £4.14 t^{-1}
505 using RB209 nutrient levels – these figures, however, are not comparable with fossil fuel
506 fertilisers, which are valued on nutrient levels and not nutrient availability.

507 If the whole digestate is separated into a liquid and fibre fraction, the nutrient level and value
508 in each fraction will be dependent upon the type of separator (Lukehurst et al. 2010), and be
509 dictated by the requirements for the other biorefinery processes.

510 The use of digestate as a biofertiliser is often compared against the economic cost of applying
511 manufactured fertiliser. Table 4 demonstrates manufactured fertilisers are much more
512 concentrated (34.5% ammonium N), compared with digestate (~0.3% - RB209) and other
513 organic fertilisers. Therefore, the cost of transportation of these materials to farm or field can
514 be high, offsetting the savings against manufactured fertilisers. Upstream processing of
515 digestate utilised in algal technology, using membranes and de-nitrification technology,
516 separates both solid and liquid fractions, and further processing of the liquid removes N via
517 volatilisation of gaseous ammonia. Capturing this ammonia as ammonium can allow it to be
518 reintroduced to the solid fraction sludge to produce a dewatered digestate. Increasing the
519 concentration of the digestate nutrient value increases the distance which digestate can be
520 utilised as a biofertiliser, before the cost of fuel in transportation outweighs the cost of
521 manufactured fertiliser equivalents. For some digestates, the dewatering and modest removal
522 of N also has the potential to create a favourable balance of NPK for crops such as grass
523 silage, by increasing the proportion of phosphate and potassium applied per unit of applied N.

524 **3.4 Environmental potential of nutrient recycling technologies**

525 The manure-to-digestate-to-microalgae-to-animal-feed value chain proposed in this paper
526 involves multiple diversions of waste streams and product substitutions compared with

527 business-as-usual (BAU). Assessing the net environmental outcomes, e.g. GHG emission
528 abatement, of such value chains requires a life cycle approach. Life cycle assessment (LCA)
529 is the evaluation of inputs, outputs and potential environmental impacts of systems, expressed
530 in relation to a unit of product or service ("functional unit") delivered by those systems
531 (Finkbeiner et al. 2006). The delivery of multiple products through a circular value chain
532 requires careful definition of goal, scope and system boundaries prior to any LCA study.

533 Full evaluation of the environmental effects of manure-to-animal feed value chains may
534 require application of expanded system boundaries to account for environmental "credits"
535 associated with product substitution. Alternatively, consequential LCA (Weidema, 2000;
536 Weidema and Schmidt, 2010) may be applied to account for significant indirect
537 consequences incurred in other systems as microalgae value chains develop. This approach
538 requires prospective evaluation of changes associated with the deployment of new microalgae
539 value chains, usually informed by economic models or trade data to predict indirect changes
540 in marginal production and consumption driven by market signals (Ekvall and Weidema,
541 2004). Consequential LCA is associated with higher levels of uncertainty compared with
542 standard "attributional" LCA (Zamagni et al. 2012), but can potentially highlight unintended
543 consequences associated with deployment of new innovations and management practises
544 (Weidema and Schmidt, 2010; Tonini et al. 2012; Styles et al. 2018) by capturing (some)
545 system interactions within the market economy. In Figure 1c and the text below, an indicative
546 approach for evaluating the environmental balance of the digestate-micro-algae value chain is
547 described.

548 The first stage in the digestate-to-microalgae value chain is the production of biogas and
549 digestate in an AD plant (Figure 1a). If the AD and microalgae production systems are part of
550 an integrated biorefinery, then the AD stage may be included in the LCA, accounting for,
551 *inter alia*, fossil energy replaced by biomethane (Budzianowski, 2016). If, however,

552 microalgae production is regarded as an add-on to an existing AD system, then evaluation of
553 the environmental consequences of microalgae production begins with an assessment of
554 conventional (pre-existing) management of the liquid digestate (LD) fraction after digestion
555 and separation (stage 2 in Figure 1c). Taking an expanded boundary approach, products and
556 processes involved in this stage are considered to be avoided, leading to environmental
557 "credits". These credits may be substantial, given that LD storage and spreading can give rise
558 to large emissions of ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4) (Nicholson et
559 al. 2013; Misselbrook et al. 2015; Rodhe et al. 2015), alongside leaching of N and P,
560 contributing towards global warming, acidification and eutrophication burdens (Rehl &
561 Müller, 2011; Styles et al. 2016). Microalgae may be produced directly from heavily diluted
562 LD, or from liquid effluent arising from the chemical extraction of biofertilisers (Rehl &
563 Müller, 2011; Vázquez-Rowe et al. 2015), in each case avoiding emissions arising from the
564 storage and spreading of digestate. Biofertiliser extraction processes include struvite
565 precipitation and ammonia stripping (stage 3 of Figure 1c), generating process effluent
566 containing almost 60% of the K, 30% of the total N and 8% of the NH_4-N contained in the
567 original LD (Styles et al. 2018). Microalgae may be used to treat such effluent, at
568 considerably reduced dilution factors compared with unprocessed LD, avoiding burdens and
569 costs associated with treatment e.g. in an integrated constructed wetland (Figure 1c).

570 Liquid digestate is a valuable bio-fertilizer, rich in readily available nutrients (Vaneeckhaute
571 et al. 2013). Therefore, in addition to the aforementioned burdens, agronomic use of LD can
572 generate significant environmental credits through the avoidance of fertiliser manufacture and
573 spreading (stage 4 in Figure 1c). These credits will no longer arise if microalgae are used to
574 directly treat diluted LD. However, the economic propensity for larger AD plants and short-
575 distance transport of LD (FNR, 2012) can lead to over-application of LD close to large AD
576 plants (Fedorniak, 2017), asynchronously to plant uptake, leading to low nutrient use

efficiency (Nkoa, 2014; AHDB, 2017) and a poor environmental balance (Styles et al. 2016). The extraction of biofertilisers from LD can avoid most of the emissions associated with LD handling in stage 2, whilst considerably enhancing synthetic fertiliser substitution credits in stage 4 (Figure 1b), although at the expense of heat, electricity and chemical (e.g. sodium hydroxide and potassium chloride) inputs – overall helping to close nutrient loops and improve the environmental balance of LD management (Styles et al. 2018). Microalgae could help to further close nutrient loops and improve the environmental balance of LD management by mopping up surplus nutrients contained in process effluent from stage 3.

Microalgae production requires considerable inputs of infrastructure, energy and water for processes including cultivation in photoreactors, filtration and centrifuging algae, and fractionation into valuable constituent products (Figure 1c) (Xu et al. 2015), leading to significant global warming, abiotic and fossil resource depletion burdens (Mata et al. 2010). The key question to be answered in future LCA studies is whether these burdens are outweighed by the environmental credits associated with substitution of high-value products including aquaculture feed, pharmaceutical and cosmetic ingredients, and the avoidance of LD or biofertiliser effluent management (Figure 1c). Calculation of credits arising from microalgae value chains may be complicated by the wide range of products and production pathways substituted by microalgae (Mulbury et al. 2004; Spolaore et al. 2006; Becker, 2007; de Visser et al. 2014; Suganya et al. 2016). There may be trade-offs across impact categories, given the significant eutrophication and acidification credits likely to arise from closing nutrient loops. The latter credits are becoming increasingly highly weighted (implicitly or explicitly) owing to the increasing attention being paid to nutrient leakage and NH_3 emissions in the context of sustainability (Steffen et al. 2015), external pollution costs (Sutton et al. 2011; Sutton et al. 2013), and phosphorous cycling in the context of finite resource depletion

(Cordell et al. 2009; Schipper, 2014). Closing nutrient cycles and minimising losses is imperative if the bioeconomy is to be sustainably expanded.

3.5 Agronomic nutrient and feed efficiency

During the digestion process about 20 – 95% of the feedstock organic matter (OM) is degraded (Möller & Müller, 2012). Nitrogen is converted to NH_4, but the majority of both N and P are conserved so that the N & P content of the resultant digestate is typically comparable to that of the feedstock material (Provenzano et al. 2011). As such, digestate has the potential to offer an organic option for agricultural fertiliser, which could replace some of the demand for inorganic fertiliser (Nkoa, 2014), avoiding burdens associated with energy-intensive fertiliser manufacture (Walsh et al. 2012). However, in comparison to undigested animal manures, anaerobic digestates have higher rates of NH_3 emission, which presents the potential for comparatively higher rates of pollution. Using direct injection, which is considered best practice for spreading digestate, will reduce gaseous emissions to the atmosphere. Nevertheless, whilst this material is readily available for plant uptake, should the digestate be spread at times other than when optimum for crop usage, then environmental losses have the potential to be high, particularly with regard to the pollution of watercourses and/or groundwater (Nkoa, 2014; Möller, 2015.).

The production of anaerobic digestate in regions dominated by pastoral agriculture, where organic manure options are often widely available, can lead to a surplus of nutrients in a geographic location least suited for effective use (Hanserud et al. 2017). Farms and regions of intensive livestock production often import animal feeds from predominantly arable areas, but the transfer of these nutrients back to arable areas in the form of slurry or liquid digestate is costly and therefore unlikely to occur. Recycling excess nutrients in such scenarios, to create animal feed products, can reduce the inappropriate land application of anaerobic

digestate, and help to close nutrient cycles in livestock areas, thus curtailing environmental impact. In addition, the generation of protein for animal feed through this approach may reduce reliance on soybean imports from tropical regions (de Visser et al. 2014), currently needed to meet demand for high protein animal feed. This will in turn reduce deforestation and land-use change as a consequence (Gasparri et al. 2013), which is a major cause of GHG emissions (Van der Werf et al. 2009).

4.0 Conclusion

A circular economy solution for organic waste management through the application of microalgae to remediate excess nutrients from anaerobic digestate and create alternative valuable products has real potential. Here it has been demonstrated that an effective system should include mixed algal and bacterial consortia and should optimise digestate feedstock for algal growth by diluting 30 times and supplementing with acetate (to a concentration of 35 g L^{-1}) to avoid C limitation. NH_4 should also be reduced to 15 g L^{-1}. This can be achieved through membrane filtration technology to establish a favourable C:N:P ratio.

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Figure/table captions:

Figure 1. Microalgae biorefining system. (a) Typical AD Technology infrastructure (b) diagrammatic representation of proposed system for the upstream/downstream process of digestates used during microalgae production in closed photo reactors. (c) Products and processes incurred or avoided (green) along the digestate-to-microalgae value chain. DBF = digestate biofertilizer; ICW = integrated constructed wetland; HVCs = high-value chemicals.

Figure 2. Modelling results: (a) iCZ8473 predictions of *C. vulgaris* growth rate and (b) DHA flux when grown under mixotrophic, phototrophic, and heterotropic conditions on different digestate dilutions.

Table 1. *i*CZ843 was able to accurately predict experimentally measured growth rates for phototrophic, mixotrophic and heterotrophic growth regimes.

Table 2. Typical composition of commercially available feed ingredients and selected algal species (per dry matter)

Table 3. Typical farm waste feedstock characteristics and nutrient values for an example 1 MW_e equivalent farm waste digester fed on agricultural feedstocks – values derived from ADAT (BORRG, 2015).

Table 4. Value of nutrient based on ADAT and RB209 nutrient levels and AHDB fertiliser prices

1023 **Figure 1**

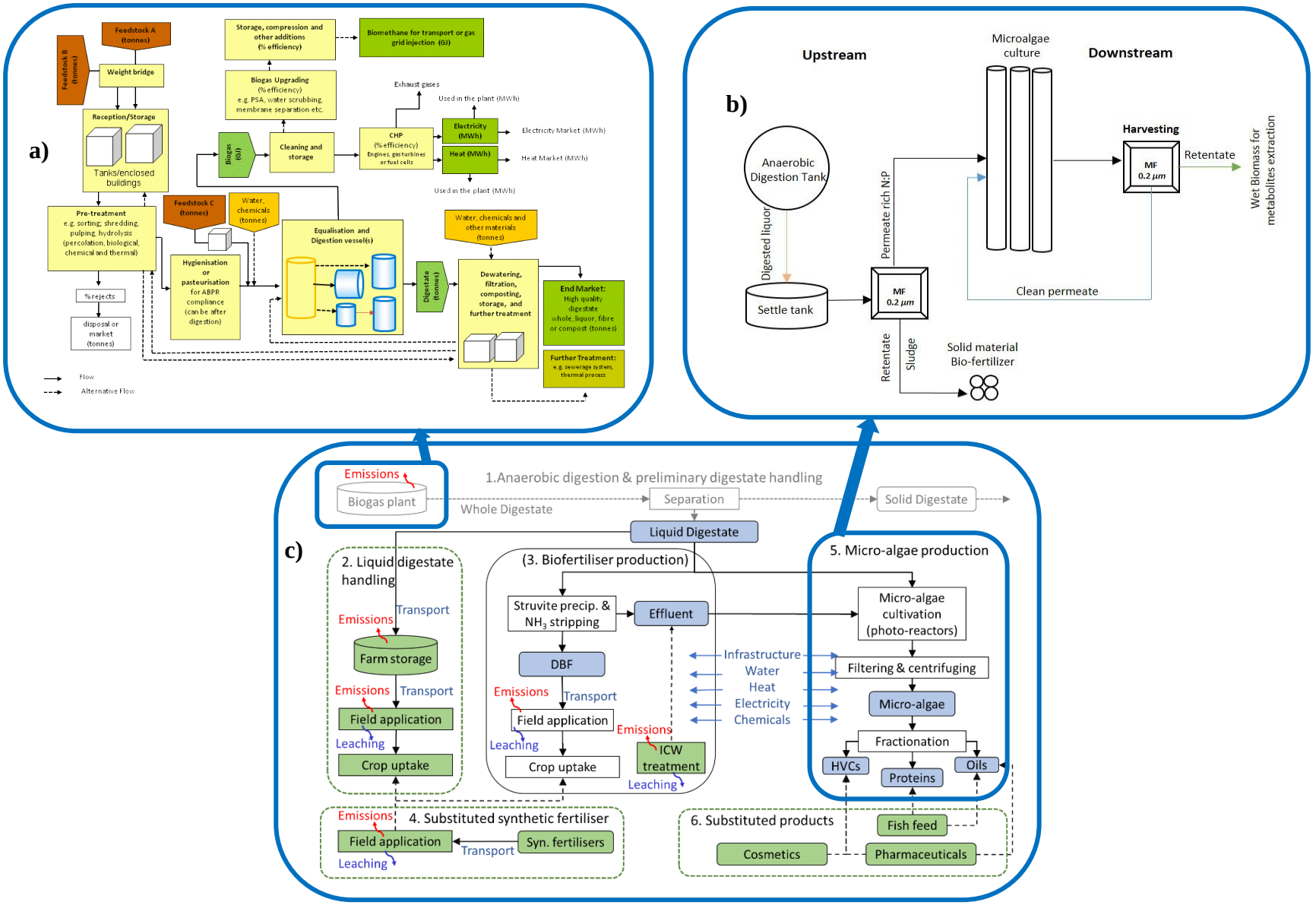

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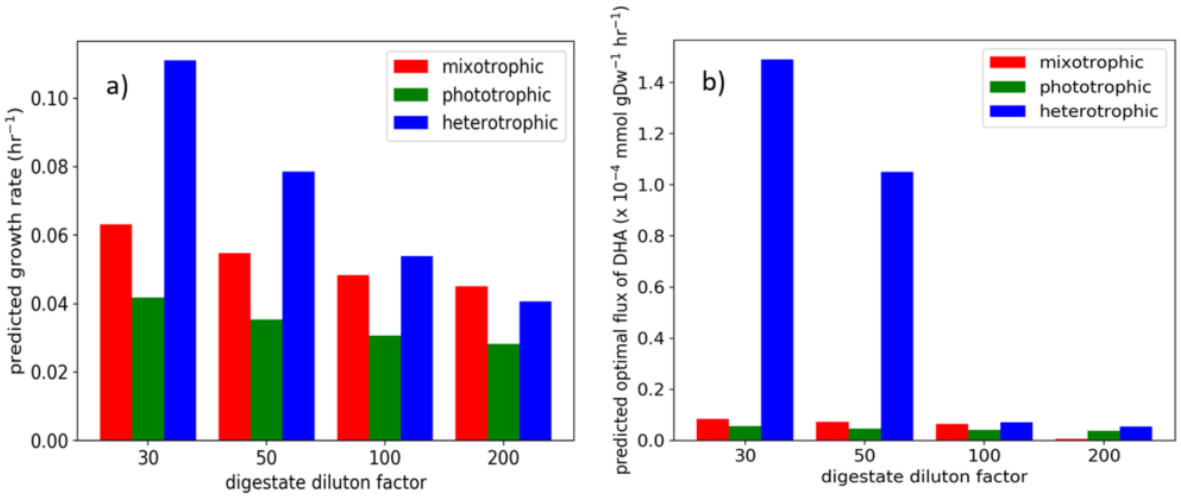
Figure 2:


a)
mixotrophic
phototrophic
heterotrophic
predicted growth rate (hr⁻¹)
0.00
0.02
0.04
0.06
0.08
0.10
30
50
100
200
digestate diluton factor
b)
mixotrophic
phototrophic
heterotrophic
predicted optimal flux of DHA (x 10⁻⁴ mmol gDw⁻¹ hr⁻¹)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
30
50
100
200
digestate diluton factor

1029 **Table 1.**

Growth regime	Predicted growth rate (hr^{-1})	Experimentally measured growth rate (hr^{-1})
Phototrophic	0.0248	0.014-0.025 (Zuliani et al. 2016)
Mixotrophic	0.0402	0.02-0.06 (Mezzari et al. 2013)
Heterotrophic	0.0168	0.018-0.025 (Zuliani et al. 2016)

1030

1031 **Table 2**.

	% Crude Protein	% Crude Lipid	% Crude Carbohydrate*	% Ash	Gross Energy MJ/kg
Fish meal	63.0	11.0	-	15.8	20.1
Poultry meal	58.0	11.3	-	18.9	19.1
Corn gluten	62.0	5.0	18.5	4.8	21.3
Wheat gluten	82.0	1.4	15.2	1.4	22.5
Soybean meal	44.0	2.2	39.0	6.1	18.2
Spirulina	58.0	11.6	10.8	13.4	20.1
Chlorella	52.0	7.5	24.3	8.2	19.3
Tetraselmis	27.2	14.0	45.4	11.5	18.0
Nannochloropsis	42.8	16.6	33.9	6.7	22.6
Schizochytrium	12.5	40.2	38.9	8.4	25.6

1032

Table 3.

Feedstock	Quantity ($t\ yr^{-1}$)	DM (% of W/W)	VS (% of DM)	BMP ($m^3\ t^{-1}$ VS)	CH_4 ($m^3\ yr^{-1}$)	N ($g\ kg^{-1}$ TS)	P ($g\ kg^{-1}$ TS)	K ($g\ kg^{-1}$ TS)	N kg $year^{-1}$	P_20_5 kg $year^{-1}$	K_2O kg $year^{-1}$
Slurry	48,180	9.0%	83.0%	185	665,824	57	10	48	247,163	99,299	249,765
FYM	26,499	25.0%	80.0%	190	1,006,962	24	6	27	158,994	91,024	214,642
Poultry litter	7,468	30.0%	75.0%	325	546,090	53	8	21	118,740	41,044	56,457
TOTAL									524,897	231,367	520,864

FYM – Farmyard manure; DM – dry matter; VS – volatile solids; BMP – best management practice.

Table 4.

Nutrient	Nutrient £ t^{-1}	Nutrient £ kg^{-1}	ADAT kg t^{-1}	Value £ t^{-1} digestate	RB209 kg t^{-1}	Value £ t^{-1} digestate
34.5% ammonium N	242.00	0.70	6.75	4.73	3.6	2.53
46% P_2O_5 Triple Super Phosphate	287.00	0.62	2.97	1.86	1.7	1.06
60% Muriate of Potash (MOP)	263.00	0.44	6.70	2.94	4.4	1.93
Nutrient value of digestate				9.53		5.52