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.
1. 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,b; Misselbrook et al., 2010), affecting water and air quality (Withers and 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 and 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 CO2 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 CO2 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 and 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 economic opportunities o
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 microalgal 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. 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. Fig. 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/floculants 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 NH₃) and P (primarily PO₄), 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 (Fig. 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 and 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 h 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 and 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 et al., 2011; Medina and Neis, 2007; Gonçalves et al., 2017). In this symbiosis, the O₂ released by algal photosynthesis is utilized by aerobic-heterotrophic bacteria to mineralize organic compounds, and bacterial respiration provides CO₂ 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; Lahej 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 (Rang 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 (Lulacat 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 et al., 2010) was used to model growth potential in C. vulgaris, iCZ843 (a standard model organism – Zuñiga et al., 2016), using different dilutions of swine with crop fed digestate (Fig. 2a), with a key focus on docosahexaenoic acid (DHA) production (Fig. 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 et al., 2013).

The constituents of swine and arable crop digestate streams at various dilutions have been measured elsewhere (ammonia and acetic acid – Zuliani et al., 2016; phosphate, nitrate, magnesium, and iron – Levine et al., 2011). These values were used to model microalgae growth rates under mixotrophic, phototrophic and heterotrophic growth conditions for different dilution factors (Fig. 2a). As per Orth et al. (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 (Fig. 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 Fig. 2b, iCZ843 predicted that heterotrophic growth on digestate diluted 30 times would result in optimal production of DHA (1.49 x 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 (Fig. 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 Fig. 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 biomass (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 > 35 mmol gDW$^{-1}$ hr$^{-1}$, was explained by N limitation. To test this hypothesis, a robustness analysis was performed to predict the biomass of heterotrophic cells grown in conditions of 30-fold digestate dilution, with acetate constrained to an optimal uptake of 35 mmol gDW$^{-1}$ hr$^{-1}$, as determined by the above analysis.

The optimised heterotrophic growth rate was revealed to be a function of acetate and NH$_4$ uptake. Optimal uptake bounds of NH$_4$ are determined at 15 mmol gDW$^{-1}$ hr$^{-1}$ and any excess beyond this inhibits cell growth, confirming the need to dilute digestate. Furthermore, at an uptake rate of 35 and 15 mmol gDW$^{-1}$ hr$^{-1}$ for acetate and NH$_4$ respectively, algal cells were shown to more than double their production of DHA from 0.149 x 10$^{-3}$ gDW$^{-1}$ hr$^{-1}$ to 1.106 x 10$^{-3}$ gDW$^{-1}$ hr$^{-1}$. To achieve this optimised production of DHA, using a metabolic reconstruction of C. vulgaris, model predictions suggest digestate diluted 30 times should be supplemented with acetate to a final concentration of 35 g L$^{-1}$ and NH$_4$ should be reduced to 15 g L$^{-1}$. All other nutrients can be kept at 30 fold dilutions.

### 2.5. Implementation

Commercial scale algae cultivation is currently a relatively immature sector and the techno-economic challenges of integrating this process with AD have to be addressed. However, in order to catalyse wider adoption of these systems we also need a better understanding of the scope and scale of potential market opportunities from a bioremediation perspective as well as from the perspective of high value products. This requires a foundation of knowledge and data/information from across the whole value chain, which can be translated and transferred to stakeholders (particularly project developers and investors). This information may be complex technical, economic and regulatory information or tacit knowledge (experience and ‘know how’ of expert and non-expert stakeholders). Current research around implementation 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. 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 (Mulbry et al., 2005). 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. 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 heterotrophic Schizochytrium 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 shrimp 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

<table>
<thead>
<tr>
<th>% Crude Protein</th>
<th>% Crude Lipid</th>
<th>% Crude Carbohydrate</th>
<th>% Ash</th>
<th>Gross Energy MJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>63.0</td>
<td>11.0</td>
<td>7.6</td>
<td>15.8</td>
</tr>
<tr>
<td>Poultry meal</td>
<td>58.0</td>
<td>11.3</td>
<td>3.1</td>
<td>18.9</td>
</tr>
<tr>
<td>Cen gluten</td>
<td>62.0</td>
<td>5.0</td>
<td>18.5</td>
<td>4.8</td>
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<tr>
<td>Wheat gluten</td>
<td>82.0</td>
<td>1.4</td>
<td>15.2</td>
<td>1.4</td>
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<tr>
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<td>44.0</td>
<td>2.2</td>
<td>39.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Spirulina</td>
<td>58.0</td>
<td>11.6</td>
<td>10.8</td>
<td>13.4</td>
</tr>
<tr>
<td>Chlorella</td>
<td>52.0</td>
<td>7.5</td>
<td>24.3</td>
<td>8.2</td>
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<tr>
<td>Tetraselmis</td>
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<td>14.0</td>
<td>45.4</td>
<td>11.5</td>
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<tr>
<td>Nannochloropsis</td>
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<td>16.6</td>
<td>33.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Schizochytrium</td>
<td>12.5</td>
<td>40.2</td>
<td>38.9</td>
<td>8.4</td>
</tr>
</tbody>
</table>
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. Janczyk et al. (2007) tested the digestibility of *Chlorella* biomass in rats using three treatments such as spray-dried, electro- 
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) (*Cryptococcus, 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 affected when fish were fed a mixture of *Isochrysis* *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 (Cardinalletti 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 EPA 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 approximation 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 polysaturated 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 1 MW<sub>e</sub> costs in the region of £3.5 M to construct. In the UK, a biomethane AD plant would also typically include a 499 kW<sub>e</sub> Combined Heat and Power (CHP) plant, with the remaining biogas, a little over 5000 m<sup>3</sup> day<sup>-1</sup> or approximately 22.1 GWh year<sup>-1</sup>, 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 Fig. 1c. A 499 kW<sub>e</sub> CHP plant operating for 8100 h year<sup>-1</sup> (92.5% load factor), at 40% electrical efficiency and 56% thermal efficiency, could produce 4.04 GWh year<sup>-1</sup> of electricity and 5.7 GWh year<sup>-1</sup> 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<sub>e</sub> 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<sub>2</sub>O<sub>5</sub>, Triple Super Phosphate (TSP) and Murate of Potash have been derived from AHDB (2018), respectively and converted to a value per kg. The two digestate values of £9.53 t<sup>-1</sup> and £5.52 t<sup>-1</sup> 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% and 90% respectively for N, P and K availability. Valuing digestate based on this nutrient availability would reduce the value to £7.07 t<sup>-1</sup> using ADAT nutrient levels and £4.14 t<sup>-1</sup> using RB209 nutrient levels – these figures, however, are not comparable with fossil fuel fertilisers, which are valued on nutrient levels and not nutrient availability.

If the whole digestate is separated into a liquid and fibre fraction, the nutrient level and value in each fraction will be dependent upon the

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Quantity (t yr&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>DM (% of W/W)</th>
<th>VS (% of DM)</th>
<th>BMP (m&lt;sup&gt;3&lt;/sup&gt; t&lt;sup&gt;-1&lt;/sup&gt; VS)</th>
<th>CH4 (m&lt;sup&gt;3&lt;/sup&gt; yr&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>N (g kg&lt;sup&gt;-1&lt;/sup&gt; TS)</th>
<th>P (g kg&lt;sup&gt;-1&lt;/sup&gt; TS)</th>
<th>K (g kg&lt;sup&gt;-1&lt;/sup&gt; TS)</th>
<th>N kg year&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>P&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt; kg year&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>K&lt;sub&gt;2&lt;/sub&gt;O kg year&lt;sup&gt;-1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slurry</td>
<td>48,180</td>
<td>9.0%</td>
<td>83.0%</td>
<td>185</td>
<td>665,824</td>
<td>57</td>
<td>10</td>
<td>48</td>
<td>247,163</td>
<td>99,299</td>
<td>249,765</td>
</tr>
<tr>
<td>FYM</td>
<td>26,499</td>
<td>25.0%</td>
<td>80.0%</td>
<td>190</td>
<td>1,006,962</td>
<td>24</td>
<td>6</td>
<td>27</td>
<td>158,994</td>
<td>91,024</td>
<td>214,642</td>
</tr>
<tr>
<td>Poultry litter</td>
<td>7,468</td>
<td>30.0%</td>
<td>75.0%</td>
<td>325</td>
<td>546,090</td>
<td>53</td>
<td>8</td>
<td>21</td>
<td>118,740</td>
<td>41,044</td>
<td>56,457</td>
</tr>
<tr>
<td>TOTAL</td>
<td>82,145</td>
<td>25.0%</td>
<td>80.0%</td>
<td>340</td>
<td>1,007,572</td>
<td>76</td>
<td>16</td>
<td>75</td>
<td>436,907</td>
<td>140,363</td>
<td>470,109</td>
</tr>
</tbody>
</table>

**Table 3**

Typical farm waste feedstock characteristics and nutrient values for an example 1 MW<sub>e</sub> equivalent farm waste digester fed on agricultural feedstocks – values derived from ADAT (BORRG, 2015).

FYM – Farmyard manure; DM – dry matter; VS – volatile solids; BMP – best management practice.
type of separator (Lukehurst et al., 2010), and be dictated by the requirements for the other biofinery processes.

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

3.4. Environmental potential of nutrient recycling technologies

The manure-to-digestate-to-microalgae-to-animal-feed value chain proposed in this paper involves multiple diversions of waste streams and product substitutions compared with business-as-usual (BAU). Assessing the net environmental outcomes, e.g. GHG emission abatement, of such value chains requires a life cycle approach. Life cycle assessment (LCA) is the evaluation of inputs, outputs and potential environmental impacts of systems, expressed in relation to a unit of product or service (“functional unit”) delivered by those systems (Finkbeiner et al., 2006). The delivery of multiple products through a circular value chain requires careful definition of goal, scope and system boundaries prior to any LCA study.

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

The first stage in the digestate-microalgae value chain is the production of biogas and digestate in an AD plant (Fig. 1a). If the AD and microalgae production systems are part of an integrated biofurnery, then the AD stage may be included in the LCA, accounting for, inter alia, fossil energy replaced by biomethane (Budzianowski, 2016). If, however, microalgae production is regarded as an add-on to an existing AD system, then evaluation of the environmental consequences of microalgae production begins with an assessment of conventional (pre-existing) management of the liquid digestate (LD) fraction after digestion and separation (stage 2 in Fig. 1c). Taking an expanded boundary approach, products and processes involved in this stage are considered to be avoided, leading to environmental “credits”. These credits may be substantial, given that LD storage and spreading can give rise to large emissions of ammonia (NH₃), nitrous oxide (N₂O) and methane (CH₄) (Nicholson et al., 2013; Mivelbrook et al., 2010; Rodhe et al., 2015), alongside leaching of N and P, contributing towards global warming, acidification and eutrophication burdens (Rehl and Müller, 2011; Styles et al., 2016). Microalgae may be produced directly from heavily diluted LD, or from liquid effluent arising from the chemical extraction of biofertilisers (Rehl and Müller, 2011; Vázquez-Rowe et al., 2015), in each case avoiding emissions arising from the storage and spreading of digestate. Biofertiliser extraction processes include struvite precipitation and ammonia stripping (stage 3 of Fig. 1c), generating process effluent containing almost 60% of the K, 30% of the total N and 8% of the NH₄-N contained in the original LD (Styles et al., 2018). Microalgae may be used to treat such effluent, at considerably reduced dilution factors compared with unprocessed LD, avoiding burdens and costs associated with treatment e.g. in an integrated constructed wetland (Fig. 1c).

Liquid digestate is a valuable bio-fertilizer, rich in readily available nutrients (Vaneecchaut et al., 2013). Therefore, in addition to the aforementioned burdens, agronomic use of LD can generate significant environmental credits through the avoidance of fertiliser manufacture and spreading (stage 4 in Fig. 1c). These credits will no longer arise if microalgae are used to directly treat diluted LD. However, the economic propensity for larger AD plants and short-distance transport of LD (FNR, 2012) can lead to over-application of LD close to large AD plants (Fedomljak, 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 (Fig. 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 (Fig. 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 (Fig. 1c). Calculation of credits arising from microalgae value chains may be complicated by the wide range of products and production pathways substituted by microalgae (Mulby et al., 2005; 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 NH3 emissions in the context of sustainability (Steffen et al., 2015), external pollution costs (Sutton et al., 2011), and phosphorous cycling in the context 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. 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. NH3 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.

Acknowledgements

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