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Review article

Anaerobic digestion of algae biomass: A review

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ABSTRACT

The anaerobic digestion of microalgae is a prospective environmentally feasible option for creating a renewable source of energy for industrial and domestic needs. Microalgae anaerobic digestion is a key unit process that integrates efficiency and beneficially into the production of microalgae derived biofuels. Anaerobic digestion culminating in methane fermentation improves the economic viability of microalgae liquid biofuel production and presents an opportunity for power generation from wastewater derived microalgae. However the anaerobic digestion of microalgae biomass is not straight forward due to several technical restraints including low concentration of digestible biodegradable substrate, recalcitrant substrate constituents, cell wall degradability, low carbon to nitrogen ratio, ammonia toxicity and effects from salinity and associated metal ions.

Current production methods for liquid biofuel production from microalgae produce approximately 60–70% residual biomass that is currently a byproduct. Anaerobic digestion provides biogas, but it can also provide essential nutrient recovery from lipid extracted microalgae biomass. The biogas produced from the anaerobic digestion process can be used to generate onsite electrical power or thermal heat to offset biomass processing and extraction processes. When both of these processes are integrated and operated simultaneously, the benefits to microalgae biofuel production and wastewater treatment derived energy production are increased significantly. To consider the integration of anaerobic digestion into a commercial-scale integrated microalgae production and biofuel refinery facility or wastewater treatment plant we present a review of the literature, the current state of the art and future directions for research.

Contents

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1. Introduction

1.1. Algae based biofuels

With the current increasing global population and the associated increase in fossil fuel use and demand, there has been an increased interest in renewable energy sources based on biomass transformation [1–3]. The use of agricultural derived biomass to produce biofuels has gained momentum [4,5]. This push for agricultural based biofuel production can lead to other less obvious problems for example eutrophication, resource depletion, reduced biodiversity due to current farming practices and the direct competition with current food crops [5,6].

Microalgae offer an interesting alternative feedstock for the production of biofuels. Microalgae have a high total yield and hence a lower land use footprint and can utilise land areas that are unsuitable for food production [1,7]. In addition microalgae production has the potential to utilise CO_2 emissions and offers the potential for a carbon neutral biofuel [8].

Biofuel production from microalgal feedstock has several challenges to overcome before it can become a mainstream industry capable of producing the quantity of biofuel required at a competitive price. Challenges faced by the industry include demand for fertiliser due to microalgae's significant utilisation of nutrients, high energy inputs required for harvesting and dewatering biomass and for the lipid extraction and conversion processes. Anaerobic digestion can offer a pathway to eliminate some of the overheads of the production cycle by producing biogas for utilisation in electricity production or thermal energy production. This benefit is highly dependent on the biofuel plant process and location, and the costs associated with natural gas prices and electricity prices will determine the efficiency improvement resulting from the biogas utilisation. In addition, the recovery of valuable nutrients from biomass via anaerobic digestion is essential for the sustainability of the algae biofuel industry. It is anticipated that the incorporation of anaerobic digestion in microalgae biofuel production and bio-refinery processes will increase the cost effectiveness of the production methods, helping it to become economically feasible and environmentally sustainable. Fig. 1 illustrates the conceptual implementation of anaerobic digestion into algal production processes. Three pathways have been defined: pathway 1 shows the direct anaerobic digestion after the biomass harvest and concentration step. Pathway 1 could be utilised in a wastewater process where the cell wall is degradable by bacterial activity within the digester. The second pathway illustrates the anaerobic digestion of biomass after cell wall disruption prior to conversion. The third pathway is the traditional biodiesel practice where lipid is extracted and residual algal biomass is converted to biogas by anaerobic digestion and methane fermentation.

1.2. Historical perspective of anaerobic digestion

Historically anaerobic digestion has been exploited for the stabilisation of raw, domestic sewage sludge that is typically removed from primary sedimentation basins [9]. However anaerobic digestion for bio-methane production has received renewed attention due to its viability as an alternative and renewable fuel source [10,11].

Municipal and Industrial anaerobic digestion of organic waste streams is widely practiced and recognised as a mature technology for producing biogas [12]. Anaerobic digestion has been included in the "first generation of biofuels" that have been developed in recent years. The "first generation biofuels" have focused largely on the production of biofuel from terrestrial plant crops [13]. In these systems solar energy is used to drive the photosynthetic fixation of carbon dioxide to organic matter. The energy crop is harvested and then used directly as a combustible fuel or converted to another form such as ethanol, hydrogen or methane [14–16]. These "first generation biofuels" have been highly criticised for their use of valuable food crops as feedstocks for fuel production. This criticism is due to the utilisation of valuable agricultural land and scarce water resources for feedstock production. With the upward pressure exerted on food prices, biofuel production from food crops has been deemed unsustainable [6,16].

2. Macroalgae and anaerobic digestion

Interest in the cultivation of microalgae for the production of bio energy, as one form of solar energy, was born in the 1950s, but when the global supply of oil was interrupted twice during the 1970s, interest in the cultivation of highly productive macroalgae for bio energy production was accelerated [11]. Macroalgae received a large amount of attention as a biofuel feedstock due to its prolific growth in eutrophic coastal water fouling beaches and coastal waterways. Anaerobic digestion has been used to dispose and process this material for the production of biogas [11,17]. Development of feedstocks for methane biogas production from macroalgae biomass led to significant plans for extensive marine farms [18,19], but these failed as the economic and geopolitical climate became favourable once again for fossil fuels.

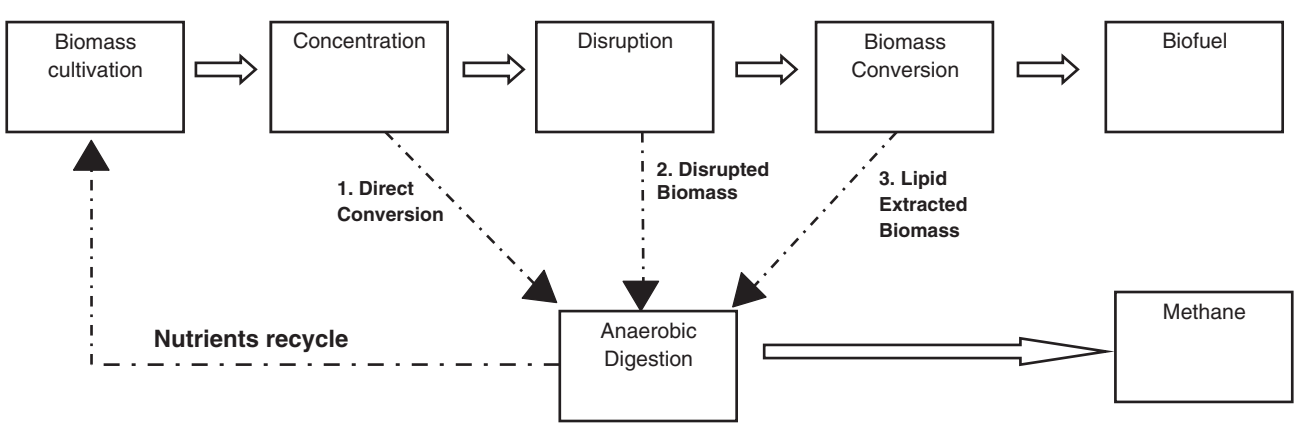

Fig. 1. Conceptual visualisation of anaerobic digestion incorporation into algal biofuel production.

Some of the problems that have been associated with anaerobic digestion of marine macroalgae include recalcitrant material such as polyphenols, cellulosic fibres and lignin type components resulting in the reduced biodegradability of the biomass by bacterial processes, hence limiting digestibility and gas production [20,11]. Also some high sulfide containing macroalgae species have been found to inhibit the anaerobic digestion process [17]. Other problems associated with the use of macroalgae for biofuel production include the seasonal growth associated with different types of macroalgae and hence variable feedstock for biogas production [11,17]. Macroalgae are again receiving attention as a substrate for anaerobic digestion, but macroalage are not discussed in the context of this review article.

3. Microalgae and anaerobic digestion

3.1. Historical and current perspectives

The focus on biofuel production is shifting towards what are known as second and third generation biofuels [10]. The second generation of biofuels utilise alternatives to food sourced biomass crops for feedstocks in biofuel production. The third generation or advanced biofuels are sourced from non-food crops, but the resulting fuel is indistinguishable from its petroleum counterparts [5]. A promising approach within the second and third generations of biofuels is the use of microalgae as a biofuel feedstock [10]. Microalgae are highly productive and are able to produce large quantities of biomass more efficiently than current cultivation practices for terrestrial crops. The photosynthetic efficiency of microalgae in engineered systems can reach 4–5% of the solar energy compared to 1–2% for terrestrial plants [21].

The first authors to report on the anaerobic digestion of microalgae biomass were Golueke et al. [22]. They investigated the anaerobic digestion of *Chlorella vulgaris* and *Scenedesmus*, microalgae species grown as part of a wastewater treatment process. Oswald, the co-author from the Golueke et al. [22,23] papers, continued research on anaerobic digestion of microalgae that is reported in a series of scientific publications describing the role of microalgae in sewage treatment using "Advanced Integrated Wastewater Pond Systems" [24–33].

This early work by Oswald and co-authors identified several key factors that could hinder the digestion of microalgae biomass; these factors are discussed later in this review article. The data shown in Table 1 provides a summary of the research that has been undertaken on several different microalgae species to date and highlights the gas potential from microalgae as a viable process for the production of biogas.

The lowest gas production recorded from freshwater microalgae biomass was ~70 mL g^{-1} VS (volatile solids) for untreated *Microcystis* sp. [34]. The gas production reported in this experiment was low due to the researcher's investigating inoculum start up volumes during the bio-methane potential assays rather than maximising gas productivity. The authors later recorded a gas production of 153 mL g^{-1} VS for the same microalgae species utilising an optimised inoculum ratio. The authors Lakaniemi et al. [35] reported a low production rate of 24 mL g^{-1} VS for the saline microalgae species *Dunaliella tertiolecta*. This low

Table 1
Methane production from the anaerobic digestion of microalgae biomass reported in scientific literature. (NR = Not reported).

Microalgae species	C/N Ratio	Methane yield	Loading rate	Reference
Arthrospira maxima	4.3–5.33	173 mL g^{-1} VS	500 mg/TS/L	[48]
Arthrospira platensis	N/R	481 mL g^{-1} VS	2000 mg/TS/L	[10]
Blue green algae	N/R	366 mL g^{-1} VS	281.96 mg/VS/L	[121]
Chlamydomonas reinhardtii	N/R	587 mL g^{-1} VS	2000 mg/TS/L	[10]
Chlorella kessleri	N/R	335 mL g^{-1} VS	2000 mg/TS/L	[10]
Chlorella sp., *Pseudokirchneriella* sp. *and Chlaqmydomas* sp.	N/R	0.28–0.60 m^3/kg/VS	402 mg VS	[8]
Chlorella sp., Scenedesmus, Euglena and Oscillatoria	N/R	300–800 mL g^{-1} VS	N/R	[23]
Chlorella sp., *Scenedesmus*	N/R	170–320 mL g^{-1} VS	1.44–2.89 g/VS/L	[22]
Chlorella sorokiniana	N/R	212 mL g^{-1} VS	N/A	[122]
Chlorella vulgaris	N/R	403 mL g^{-1} VS	2 g/VS/L	[66]
Chlorella vulgaris	N/R	286 mL g^{-1} VS	5000 mg/VS/L	[35]
Chlorella vulgaris	6	240 mL g^{-1} VS	1000 mg/VS/L	[6]
Chlorella vulgaris	N/R	189 mL g^{-1} VS	N/R	[122]
Chlorella vulgaris	N/R	0.40–0.45 L	2677–6714 mg (COD)	[43]
Dunaliella	N/R	440 mL g^{-1} VS	910 mg/VS/L	[123]
Dunaliella salina	N/R	505 mL g^{-1} TS	2000 mg/TS/L	[10]
Dunaliella tertiolecta	N/R	24 mL g^{-1} VS	5000 mg/VS/L	[35]
Durvillea Antarctica	N/R	492 mL g^{-1} VS	3000 mg/dry/TS/d	[72]
Euglena gracilis	N/R	485 mL g^{-1} VS	2000 mg/TS/L	[10]
Lake Chaohu natural population consortium	N/R	295 mL g^{-1} VS	N/R	[77]
Macroystis pyrifera and Durvillea Antartica(50% blend)	N/R	540 mL g^{-1} VS	3000 mg/dry/TS/d	[72]
Macroystis pyrifera	N/R	545 mL g^{-1} VS	3000 mg/dry/TS/day	[72]
Microcystis sp.	N/R	70.33–153.51 ml	1500–6000 mg/VS	[34]
Nannochloropsis oculata	N/R	204 mL g^{-1} VS	N/R	[110]
Nannochloropsis salina (lipid extracted biomass)	4.4	130 mL g^{-1} VS	2000 mg/l/VS	[80]
Phaeodactylum tricornutun	N/R	0.35 L g^{-1} COD	1.3 ± 0.4–5.8 ± 0.9	[47]
Scenedesmus obliquus	N/R	287 mL g^{-1} VS	2000 mg/TS/L	[10]
Scenedesmus obliquus	N/R	240 mL g^{-1} VS	2000 mg/VS/L	[47]
Scenedesmus sp.	N/R	170 mL g^{-1} COD	1000 mg/COD/L	[61]
Scenedesmus sp. (single stage)	N/R	290 mL g^{-1} VS	18,000 mg/VS/L	[107]
Scenedesmus sp. (two stage) Note: 46 mL/g/VS Hydrogen	N/R	354 mL g^{-1} VS	18,000 mg/VS/L	[107]
Scenedesmus sp. *and Chlorella* sp.	N/R	16.3–15.8 ft^3	7.8–9.2 ft^3/lb (VS)	[22]
Scenedesmus sp. *and Chlorella* sp.	6.7	143 mL g^{-1} VS	4000 mg/VS/L	[67]
Spirulina Leb 18	N/R	0.79 g/L	72,000 mg/L/TS	[124]
Spirulina maxima	4.16	0.35–0.80 m^3	20–100 kg/m^3 (VS)	[125]
Spirulina maxima	N/R	320 mL g^{-1} VS	910 mg/VS/L	[123]
Spirulina maxima	N/R	330 mL g^{-1} VS	22,500 mg/VS/L	[126]
Spirulina platensis UTEX1926	N/R	0.40 m^3 kg	N/R	[12]
Tetraselmis	7.82	0.25–0.31 L g^{-1} VS	2000 mg/VS	[109]
C/N ratio-[127]				
Waste water grown community	N/R	497 mL g^{-1} TS	2.16 g/L/TS	[81]
Zygogonium sp.	N/R	344 mL g^{-1} TS	N/R	[76]

production rate was attributed to the effects of salinity. The highest methane production recorded was by De Schamphelaire and Verstraete [8] who recorded a gas production of 600 mL g^{-1} VS for a mixed undetermined freshwater microalgae consortium. The data shown in Table 1 also highlights the difference in units and terminology used to report gas production from microalgae. Units range from gas production per grams of chemical oxygen demand (COD) destroyed, gas produced per gram of volatile solids loaded and gas produced per gram of total solids loaded. The standardisation of terminology and standard units to report biogas productivities are essential for comparing microalgae and other digestible substrates.

The methods used to determine volatile solids are also used to determine the ash free dry weight (AFDW) of microalgae [36,37]. Ash free dry weight is used extensively by phycologists to report quantities of microalgae biomass. When reporting microalgae biomass, the ash free dry weight or the volatile solids (digestible component) of the microalgae biomass is a percentage of the total solids and varies between species. The data in Table 2 includes the AFDW or VS of some common microalgae species. The variation in AFDW and VS can vary by up to 50% between species and can significantly affect predicting the theoretical biogas production potential for the anaerobic digestion of microalgae.

4. Problems with anaerobic digestion of microalgae

4.1. Low concentration of digestible substrate

The majority of authors listed in Table 1 conclude that the concentrating or harvesting of microalgae biomass presents a fundamental challenge to the financial viability of an energy system using microalgae biomass as a substrate for anaerobic digestion or alternative biofuel production.

Gouleke et al. [22] identified the low volatile solids loading rate that is associated with microalgae when used as a digestible substrate. The low VS rate is due to the low concentration of microalgae biomass present in large volume of water. Significant research has focussed on engineering issues associated with the, harvesting, dewatering, and further concentrating of the microalgae biomass energy. Engineering issues common in microalgae production for biofuel are discussed by Benemann et al. [26], Chen et al. [38], and Molina et al. [39–42]. Regarding the data presented in Table 1, all experiments except for the work published by Sanchez-Hernandez and Trvieso [43] and De Schamphelaire and Verstraete [8] were performed using concentrated microalgae. In the Sanchez-Hernandez and Trvieso [43] paper no concentrating step was reported and the chlorophyll *a* ranged from 2.87 mg/L to 9.62 mg/L. This higher chlorophyll *a* content would indicate that the microalgae were at a higher density, and the problem of low volatile solids may have not been evident in the experiment.

In the De Schamphelaire and Verstraete [8] experiment the authors came to the conclusion that a concentrating step would be required for optimal performance of the anaerobic digestion process. Results indicate that the digester completely failed once during the experimental period. The authors observed that the required volatile solids loading rate comprising of microalgae biomass was too dilute and contained excessive water, leading to the washout of the anaerobic bacteria community. Bacterial washout is due to a low digestible content of the wastewater or digestible feedstock. Hence when the subsequent hydraulic retention time within the digester is shortened to less than the bacterial generational time, the result is a decreased bacterial population [44,45].

McCarty [46] indicated that a settling tank could be utilised after the digester to allow bacteria and solids to settle via gravity. These solids could then be reintroduced to the digester for further processing. This step is essential to reduce bacterial washout when the hydraulic retention time (HRT) is lower than the solid retention time (SRT) of the substrate.

Bacterial washout can also be addressed by better anaerobic digester design. Zamalloa [47] used a laboratory scale membrane reactor to anaerobically digest *Phaeodactylum tricornutum*. The addition of the membrane to the reactor gave a hydraulic retention time of 2.5 days, while the solids retention time was increased to between 10 and 20 days depending on the solids loading rate. The decoupling of the hydraulic retention times and the solids retention time can also be achieved by utilising upflow anaerobic sludge blanket (USAB) reactors, anaerobic membrane reactors (AnMBR), anaerobic filters (AF) and anaerobic fluidised bed reactors (AFBR) [48] and by fermentation cells [24] or by in-pond digesters [27,28].

The paper by Collet et al. [1] reports a novel approach to concentrating microalgae. The authors first use a gravity settling-step to separate the microalgae before transferring it into a centrifuge for dewatering and concentrating to a higher percent biomass solid. The results indicated that by settling the culture for 1 h, 65% of the microalgae biomass was separated into slurry with a concentration 20 times higher than in the original culture stream. The authors then used centrifugation and reported a further concentration factor of five times. However this initial settling step was more effective with non-motile microalgae species.

Table 2
The volatile solids (VS) or ash free dry weights (AFDW) as a percentage of the total solids (TS) reported for different microalgae species.

Species	Fresh or saltwater	VS and AFDW as % of TS	Reference
Arthrospira maxima	Brackish	80–93%	[48]
Blue green algae	Fresh	94%	[121]
Chorella vulgaris	Fresh	93%	[128]
Chorella vulgaris	Fresh	90%	[6]
Chlorogloeopsis fritschii	Salt	92%	[128]
Dunaliella sp.	Saltwater	82%	[36]
Isochrysis galbana	Saltwater	86%	[36]
Nannochloropsis oculata	Saltwater	45%	[110]
Nannochloropsis salina	Fresh	77%	[80]
Nannochloropsis sp.	Saltwater	93%	[36]
Nitzschia closterium	Saltwater	78%	[36]
Phaeodactylum tricornutum	Fresh	82%	[47]
Porphyridium cruentum	Saltwater	91%	[36]
Scenedesmus dimorphus	Fresh	88%	[128]
Scenedesmus obliquus	Fresh	72%	[47]
Scenedesmus sp.	Fresh	60%	[61]
Spirulina maxima	Saltwater	86%	[58]
Spirulina platensis	Salt	92%	[128]
Spirulina platensis	Fresh	93%	[47]
Wastewater consortium	Fresh	88%	[91]
Wastewater consortium (lipid extracted)	Fresh	86%	[91]

The publications by Golueke et al. [29], Benamann et al. [26] and Harun et al. [49] investigated the use of chemical coagulation, flocculation and centrifugation as a means of harvesting, concentrating and dewatering microalgae. All three papers discuss the high energy costs associated with the use of centrifugation and flocculation harvesting techniques. Golueke and Oswald [33] identified that digester performance is unaffected by the centrifugation or by alum addition as a flocculant. Their work has shown that concentrations in sludge up to 4% aluminium have no effect on digester stability or gas production. Many new commercially formulated coagulants exist and are comprised of cationic and anionic poly-electrolytes, synthetic polyacrylamide polymers and starch-based polymer flocculants [50–52]. Most of these flocculants are currently utilised in the wastewater treatment industry, and their use has shown very few detrimental effects to digester stability or gas production [50,51].

The authors Kalyuzhnyi et al. [53] and Callander [54] have reported improved anaerobic digester performance when commercially available chemical coagulants have been utilised. The increased performance is due to better solid retention times of particulate matter, allowing more complete digestion of solids and resulting in higher conversions to biogas. Barford et al. [52] also noted that the use of the chemical flocculants resulted in an increased biomass concentration in the digester compared to the control that did not utilise a flocculant. The author noted that this higher concentration of particulate matter enabled considerably higher solids loadings per unit volume to be applied to the digester. However the authors also noted that the higher concentration of biomass could induce ammonia inhibition due to the much higher loading rates that could be applied to a digester with a flocculated biomass. Zhang et al. [55] reported that a high pH by chemical adjustment associated with struvite formation had no negative effect to the performance of anaerobic digestion.

With the high cost associated with these harvesting and dewatering steps many new cost efficient laboratory and pilot scale technologies are under development. Many of these technologies are still to be proven in full commercial scale settings, and their impact to anaerobic digestion is yet to be established.

4.2. Cell wall degradability and pre-treatment of microalgae biomass

Golueke et al. [22] demonstrated the ability of microalgae to pass through an anaerobic digester intact and remain undigested. The authors noted that microalgal cells are known to be able to effectively resist bacterial attack and found intact microalgae cells in digestate leaving a digester after a 30-day hydraulic retention time. Sanchez-Hernandez and Trvieso-Cordoba [43] observed that when *C. vulgaris* was added to a digester the chlorophyll *a* concentrations increased within the digester for the first two weeks of the experimental period but was still detectable 64 days after the start of the experiment. Zhou et al. [56] also found intact cells in digestate from a digester after 45 days. The longest duration reported for intact microalgae cells surviving within an anaerobic digester was reported by Mussgnung et al. [10]. They identified viable *Scenedesmus* cells after 6 months that had switched to mixotrophic growth.

Work by Mussgnung et al. [10] highlighted the role of the cell wall in the digestion process. Their results indicated that the higher gas production reported was due to the microalgae species that had either no cell wall or a cell wall made from protein. Gas production was observed to decrease for microalgal species that had a carbohydrate-based cell wall containing hemicellulose. The lowest gas production came from the species *S. obliquus* that has a particular rigid cell wall containing sporopollenin like biopolymers. Little or no cell wall degradation was detected in *S. obliquus* and very little gas was produced by the substrate. The authors concluded that the degradation of the cell wall was strongly correlated to the amount of gas produced during anaerobic digestion [10,57]. The results reported by Mussgnug et al. [10] also correlate to findings by Ras et al. [6] who noted changes in cell wall chemistry and its influence on substrate degradability. Their results indicate the need for a pre-treatment step to disrupt the cell wall and increase bacterial hydrolysis before addition to the anaerobic digester [10,23,25,47,57–60]. Cell lysis is also essential for solvent extraction of the lipid fraction in microalgae biomass [42], allowing solvents to react with internal cell lipids. Hence microalgal cell wall disruption processes are essential for both lipid-based biofuel applications and for optimal microalgae anaerobic digestion or co-digestion processes.

Golueke and Oswald [23] investigated a thermal pre-treatment step of microalgal biomass wherein the temperature was raised above the thermal limit of the microalgal species, resulting in cell disruption. Chen and Oswald [25] undertook experiments that investigated thermal pre-treatment combined with chemical pre-treatment using sodium hydroxide and variable exposure times. Their results demonstrated that all pre-treatments tested produced better results than untreated control comparisons. It was demonstrated that the most efficient pre-treatment for microalgal biomass required heating to 100 °C for 8 h without an increase in pH using the addition of sodium hydroxide. This treatment increased gas productivity by 33% as compared to untreated microalgae biomass. This study also indicated that up to 60% of the untreated microalgae biomass added to the anaerobic digester will remain undigested due to the cell wall remaining intact throughout the digestion process [25]. More recent studies by Gonzalez-Fernandez et al. [61] reported that thermal pre-treatment of *Scenedesmus* sp. increased methane potential. At 70 °C, a 9% increase in methane production was reported, which increased to 57% at 90 °C when compared to untreated microalgae biomass. Further work by Gonzalez-Fernandez et al. [62] investigated the effect of the organic loading rates and the thermal pre-treatment of biomass at 90 °C for 1 h. Results indicated that a 2.9 and 3.4 fold increase in methane production for organic loading rates of 1 and 2.5 kg COD m^{-3} day respectively. Research reported by Alzate et al. [63] showed an increase of 46% to 62% in methane productivity utilising thermal hydrolysis. However results from De Schamphelaire and Verstraete [8] reported no benefit when pre-treating a mixture of *Chlorella, Pseudokirchneriella* and *Chlamydomonas* microalgae species at 80 °C for 2.5 h.

Samson and Leduy [58] investigated thermo-chemical (heat and sodium hydroxide addition), mechanical and ultrasonic disintegration pre-treatment methods. The authors reported that at a temperature of 50 °C there was a 20% increase in substrate solubilisation and at 150 °C there was a 43% increase in substrate solubilisation. Their experiments indicated that the ultrasonic treatment gave similar results as the 150 °C heat treatment. The time taken for the ultrasonic treatment was relatively short and only took 10 min compared to 1 h for the thermal pre-treatment. Paris and Oswald [25] and Samson and Leduy [58] both indicated that the simple addition of sodium hydroxide was inefficient as a pre-treatment step for the anaerobic digestion of microalgae biomass or biosolids.

Gonzalez-Fernandez et al. [60] investigated sonic disruption pre-treatment of microalgae biomass. They utilised a frequency of 20 Hz but at varying power levels. All sonicated biomass exhibited higher methane production during the first days of digestion compared to untreated biomass. Overall the highest microalgae biodegradability of 44% was recorded for the longest sonication treatment as compared to 23% for un-sonicated biomass.

Samson and Leduy [58] reported a 26% increase in the solubilisation of microalgal substrates by freezing the biomass. This was due to the disruption of the microalgae cell wall by ice crystals. Keymer et al. [64] adapted high pressure thermal hydrolysis (HPTH), a commercially available technology used for the disruption of waste activated sludge biosolids for the purpose of pre-treating microalgae biomass. HPTH processes heat substrate to approximately 160 °C at a pressure of approximately 6 bars. After these conditions have been maintained for 20–30 min the contents are then reduced in pressure via a flash drum where the pressure change causes the cells to rupture and release the cell contents. Keymer et al. [64] reported that the process substantially

increased methane potential for lipid extracted and non-lipid extracted algae. The authors also reported an extraction method using a soxhlet apparatus with hexane to extract the lipid that increased the biomethane potential of the microalgae biomass. When both lipid extraction and HPTL were combined an increase in the digestibility of the lipid extracted and HPTL microalgae biomass of 110% was recorded compared to untreated microalgae biomass. However this process is energy intensive but energy balances demonstrate that HPTL coupled with anaerobic digestion can be energy positive due to the increased methane potential from the substrate [65].

The various mechanical, physical, thermal and chemical methods used to improve microalgae methane potential can have a high energy requirement. Several authors have found that the energy consumption for the pre-treatment of microalgae biomass is equal to or higher than the energy gained from the microalgal cell [42,59,66–69]. Due to this high energy demand alternative methods including enzymatic and bacterial methods have also been investigated. Lu et al. [66] cited results by Sander and Murthy [70] wherein they reported the cell walls of a mixed microalgae culture to be susceptible to degradation by lipase and cellulase. Results reported by Ehimen et al. [71] showed an increase in methane production by treating *Rhizoclonium* biomass with the addition of an enzymatic mixture. The greatest increase in gas production resulted from the addition of the single enzyme cellulase. Bacterial cell disruption has also been shown to increase methane production [66]. The authors Lu et al. [66] demonstrated an increase of 17–24% in biogas production by adding the bacterium *Clostridium thermocellum* to *C. vulgaris* biomass.

4.3. The carbon/nitrogen ratio associated with microalgae biomass

Vergara-Fernandez et al. [72], Sialve et al. [59] and Yen and Brune [67] identified further difficulties with the anaerobic digestion of microalgae biomass, due to the low carbon to nitrogen ratio present in microalgal species. Data reported in Table 1 shows that the carbon/nitrogen (C/N) ratio varies from 4.16 to 7.82 for microalgal species that have been investigated for anaerobic digestion. When the C/N ratio is below 20 there is an imbalance between carbon and nitrogen requirements for the anaerobic bacterial community or consortia [59]. This imbalance leads to nitrogen release in the form of ammonia during digestion, which can become inhibitory to methanogenic bacteria and result in volatile fatty acids accumulating within a digester [59]. Ammonia-nitrogen and volatile fatty acids (VFA) are important intermediates in anaerobic digestion processes but can also be potential inhibitors when allowed to accumulate [44].

To overcome problematically low C/N ratios, several researchers have investigated co-digestion, were microalgae has been co-digested with other waste streams or biomass to increase the C/N ratio. These studies include Gonzalez-Fernandez et al. [73] and Shouquan et al. [74] who investigated the addition of microalgae to pig manure prior to digestion. Saxena et al. [75] recorded increased methane production for the anaerobic co-digestion of green filamentous microalgae and water hyacinth supplemented with cow manure. Ramamoorthy and Sulochana [76] also investigated the addition of *Zygogonium* sp. with various quantities of cow manure. Shuchuan et al. [77] reported a significant increase in methane potential when blue green algae was co-digested with corn stalks. Samson and Leduy [78] undertook a co-digestion experiment wherein they blended sewage sludge with *Spirulina maxima* and observed a 2-fold increase in gas production when a mixture of 50% by weight of sewage sludge to microalgae ratio was used. Yaun et al. [79] reported increased methane potential in the co-digestion of municipal wastewater solids with *Chlorella* sp. and *Spirulina platensis* microalgae species respectively. Park and Li [80] investigated co-digestion using *Nannochloropsis salina* and lipid-rich fats, oil and grease. They observed higher methane production and were able to use an increased organic loading rate due to a more balanced C/N ratio with less inhibition and digester imbalance. An increase in gas production was also reported by Salerno et al. [81] by co-digesting domestic municipal wastewater grown microalgae and soybean oil.

Glycerol is a carbon-rich by-product of the trans-esterification conversion of lipids to biodiesel, and it can be used as a carbon source to maximise gas production in anaerobic co-digestion [82]. Ehimen et al. [83] investigated using glycerol produced from transesterified microalgae lipid. A slight increase in gas production rate was noted in this study; however a low application rate of glycerol was utilised. An increase in gas production was also observed by Salerno et al. [81] when co-digesting glycerol and domestic municipal wastewater derived microalgae biomass.

Yen and Brune [67] considered the addition of paper waste to improve the C/N ratio of the microalgae combination comprised of *Scenedesmus* sp. and *Chlorella* sp. Yen and Brune [67] illustrated that with the addition of waste paper there was an increase in the C/N ratio from 6.7 to 36.4. Results from this experiment showed that the best co-digestion ratio was 50% paper and 50% microalgae. The final C/N ratio of this combination was 18.0 with 1170 ± 75 mL/day biogas produced. In comparison the microalgae-only anaerobic digestion produced 573 ± 28 mL/day of biogas, which was about a 50% reduction in gas production compared to the more favourable C/N ratio treatment. Yen and Brune [67] concluded that the best C/N ratio for anaerobic digestion is in-between 20:1 and 25:1. This conclusion is similar to the C/N ratio discussed by the authors Parkin and Owen [44] wherein they indicated an optimum C/N ratio range of between 20:1 and 30:1.

Yen and Brune [67] indicated that as the C/N ratio increased the amount of total ammonia-nitrogen decreased as the C/N ratio became more favourable thus reducing ammonia-nitrogen inhibition effect. Ehimen et al. [83] suggested that a C/N ratio of 15 or below can result in a build up of free ammonia-nitrogen, which can be detrimental to anaerobic digestion processes. The high C/N ratio treatment of 36.4/1 used in the experiment was on the upper extreme for anaerobic digestion as high volatile fatty acid (VFA) concentrations can also become inhibitory to anaerobic digestion. At a high C/N ratio, the amount of total ammonia-nitrogen can be too low for the cellular needs of the anaerobic microorganisms. It has been shown that a minimum concentration of 50 to 200 mg/L of nitrogen as ammonia is essential for the requirements of the bacterial community associated with anaerobic digestion [44,84]. The co-digestion of two substrates may improve C/N ratio and VFA/alkalinity ratios and attenuate unfavourable ratios in a single substrate.

One problem that must be considered with the co-digestion of a second waste stream or biomass is the seasonal availability of the feedstock and location of production. This problem was highlighted previously where seasonal growth of macroalgae limited anaerobic digestion to only six months of the year [11,17].

4.4. Lipids and microalgae

Lipids are an attractive substrate for anaerobic digestion and have a higher theoretical methane potential compared to proteins and carbohydrates [85]. However due to their low alkalinity and buffering capacity, lipids can cause inhibition due to their intermediate products such as long chain fatty acids (LCFAs) and VFAs [80]. It has been suggested that the conversion of microalgal biomass to methane rich biogas is energetically more favourable than lipids removal from microalgae biomass with the total lipid content is lower than 40% [59]. However, the removal of lipids from microalgae biomass for liquid biofuel production prior to anaerobic digestion of the residual microalgae biomass can be beneficial to anaerobic digestion processes, as high lipid concentrations can be inhibitory [59,80,86].

Crine et al. [86] reported that there was no inhibition for lipid concentrations of 5, 10 and 18% respectively. However inhibition was observed for lipid concentrations of 31, 40 and 47%, where inhibition increased due to higher lipid fractions. It is anticipated that the lipid concentration for economically viable lipid-based biofuel microalgae

species generally exceeds 30%, which could have negative consequences for anaerobic digestion if the lipids are not extracted.

Lipid extraction methods used on microalgal biomass can affect the digestibility of residual microalgal biomass. Ehimen et al. [83] and Thiel [87] reported a significant decrease in gas production due to residual chloroform from the Bligh and Dyer extraction process even though it had been heat treated to remove residual entrained solvents after the extraction process [88]. Butanol, hexane and methanol have been shown to have no detrimental effects on anaerobic digestion when residual solvents are removed by heating [83].

5. Theoretical methane production

When the C, H, O and N composition of a wastewater or substrate is known, the stoichiometric relationship reported by Buswell and Boruff [89] can be used to estimate the theoretical gas composition on a percentage molar basis.

$$(C_aH_bO_cN_d) + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3 \quad (1)$$

where a, b, c and d equal the carbon content, hydrogen content, oxygen content and nitrogen molar composition respectively [59,89,90].

$$\text{Methane yield (litres/g (VS) destroyed)} = \left(\frac{4a+b-2c-3d}{12a+b+16c+14d}\right) * V_m \quad (2)$$

where V_m is the molar volume of methane or 22.14 L at 0 °C and 1 atm [59]. Eq. 2 is used to calculate the volume of methane gas depending on the amount of volatile solids (VS) available in the substrate being digested.

The data shown in Table 3 illustrates the theoretical methane potential for several microalgae species utilising Eq. 2 and values from literature. However Eq. 2 overestimates the gas production as it assumes 100% conversion of the volatile solids to biogas and also does not consider the needs for bacterial cell maintenance and anabolism [59,90]. When the theoretical methane potential was calculated for lipid extracted and non lipid extracted wastewater microalgae consortium reported in Chinnasamy et al. [91], a 13% decrease in the theoretical methane potential was reported highlighting the residual gas potential of lipid-extracted microalgae biomass.

6. Inhibition of anaerobic digestion

6.1. Ammonia-nitrogen toxicity

Ammonia-nitrogen is produced from the biological breakdown of nitrogenous matter, mostly in the form of proteins and urea [84]. The high nitrogen and protein levels found in microalgae can lead to significant release of ammonia-nitrogen during anaerobic digestion [59]. The equilibrium established between un-ionised ammonia- (NH_3-N) and ammonium- (NH_4-N) nitrogen can be affected by a change in pH or temperature within the anaerobic digester. An increase in temperature or pH can be very detrimental to the bacterial community as the equilibrium shifts to the more toxic un-ionised form of ammonia-nitrogen NH_3-N [44,59,92].

McCarty [93] indicated that ammonia gas within the digester is inhibitory at a much lower concentration than the aqueous ionised form of ammonium–nitrogen. Ammonia toxicity has been shown to affect methanogenic bacteria in two ways: (1) the ammonium ion may inhibit the methane synthesising enzyme directly, and (2) the hydrophobic ammonia-nitrogen molecule may diffuse passively into the cell, causing proton imbalance and/or potassium deficiency [59,94]. Ammonia-nitrogen is toxic at high levels and has a moderately inhibitive effect from 1500–3000 mg/L. Above 3000 mg/L there is a strong inhibitive effect associated with ammonium–nitrogen [44], which can lead to a drop in gas production. Inhibition of the methanogenic or acidogenic groups of anaerobic microbes was not quantified in this study.

There is a large amount of conflicting information in the literature relating to the ammonia-nitrogen tolerance of anaerobic microbes. Research based on methane production and growth rate comparisons indicate that inhibitory effects are greater for the acidogenic bacteria compared to the methanogenic bacteria [84,95–100]. It has been observed that acetate consuming methanogens have relatively high resistance to high ammonia-nitrogen concentrations [84,94,100–102].

This difference in opinion demonstrates the distinctive responses that can be associated with bacterial consortiums that are involved with anaerobic digestion. Among the methanogenic strains commonly found in digesters (for example: *Methanospirillum hungatei*, *Methanosarcina barkeri*, *Methanobacterium thermoautotrophicum*, *and Methanobacterium formicicum*) the species *M. hungatei* was found to be the most sensitive microbe to ammonia-nitrogen, with inhibition observed at 4200 mg/L compared to the other three strains where inhibition did not occur until ammonia-nitrogen levels were above 10,000 mg/L [103]. The inhibition of *M. hungatei* at 4200 mg/L is the only methanogenic strain out of the five methanogenic strains tested that corresponds to the findings of Parkin and Owen [44].

The utilisation of volatile fatty acids by methanogens must balance the production of volatile fatty acids by hydrolytic and acetogenic bacteria in order to maintain digestion stability. Efficient digester performance is therefore dependent upon maintaining the ammonia-nitrogen concentration below the inhibitory limits for all of the associated digestion bacteria [100].

A solution to the problem of digester stability and balancing the bacterial populations and end products is to separate the bacterial communities. This can be done by utilising a two-stage anaerobic digestion process [72]. The metabolic pathways of the two-stage anaerobic digestion processes are the same as those of single-stage anaerobic digestion. However the stages are physically separated with the hydrolytic and

Table 3
Biochemical analysis of microalgae species and their theoretical methane potentials calculated from the mean carbon, nitrogen, hydrogen and oxygen values.

Species	Carbon	Hydrogen	Nitrogen	Oxygen	Calculated methane potential (ml/g/VS)	Reference
Chlorogloeopsis fritschii	54.4 ± 2.1	6.9 ± 0.5	7.3 ± 0.3	31.4	309	[128]
Spirulina platensis	55.7 ± 0.4	6.8 ± 0.1	11.2 ± 0.1	26.4	319	[128]
Chlorella vulgaris	52.6 ± 0.8	7.1 ± 0.1	8.2 ± 0.2	32.2	283	[128]
Scenedesmus dimorphus	53.4 ± 0.6	7.8 ± 0.2	7.9 ± 0.1	31.0	260	[128]
Wastewater consortium	49.4 ± 0.1	6.7 ± 0.1	9.3 ± 0.2	21.6	347	[91]
Wastewater consortium (lipid extracted)	45.9 ± 1.9	6.2 ± 0.1	9.3 ± 0.9	23.6	303	[91]
Nannochlorospsis sp. (lipid extracted)	49.7	7.1	5.8	26.7	340	[3]
Nannochlorospsis sp.	47.6	6.6	5.5	21.7	383	[3]
Nannochlorospsis sp. (low lipid)	52.0	7.5	4.8	22.4	414	[3]
Nannochlorospsis sp. (high lipid)	51.5	7.3	4.5	22.4	414	[3]

acetogenic bacteria in the first stage and the methanogenic bacteria in the second phase [72]. Several authors have indicated that by using two-stage digestion there is increased degradation of organic matter, improved biogas production and better control over the conditions inside the digester limiting inhibition of the microbial populations [72,104–106].

Yang et al. [107] demonstrated that by utilising a two-stage digestion process an increased methane yield of 22% was achieved when anaerobically digesting *Scenedesmus* sp. The authors also recorded 46 ml g^{-1}VS of hydrogen production in the first stage of the two-stage digestion process. The hydrogen production was in addition to the methane production recorded for the second stage. A soluble COD reduction of 75% was recorded for the single stage digestion, whereas a soluble COD reduction of 81.8% was recorded for the two stage process.

Recent work by Inglesby and Fisher [48] has shown that the integration of microbial fuel cells can be beneficial in decreasing ammonia-nitrogen inhibition during anaerobic digestion. Improved performance is achieved by allowing the ammonium ion to migrate across the cation exchange membrane from the anode to the cathode. The use of the microbial fuel cells decreases the chance of free ammonia-nitrogen inhibition of methanogenic bacteria improving the stability of the anaerobic digestion process [48].

6.2. Saline microalgae and the effect of salinity

Some species of microalgae that have been identified for their potential as feedstock for liquid biofuels are grown in a saline environment. The use of saltwater for their production offers a sustainable alternative due to the ability to use non-arable land and seawater, hence reducing pressure on current agricultural land and scarce freshwater resources. Several scientific publications cited in Table 1 have dealt with saline species of microalgae. The marine species *Macrocystis pyrifera* and *Tetraselmis* sp. have been used as a substrate for anaerobic digestion. The cyanobacteria *Spirulina* sp. has also been identified for having the potential to be grown in saline waters [108].

Alkaline earth metal salts are needed in very low concentrations for cellular metabolism in bacteria, and higher concentrations can be extremely toxic to methanogenic bacteria [93]. Salinity and more specifically sodium divalent cations do pose a problem to bacteria associated with anaerobic digestion [44,93]. Vergara-Fernandez et al. [72] demonstrated that the digestion of marine microalgae is possible. They recorded a total biogas production of between 95 and 260 mL g^{-1} TS microalgae loaded using a two-stage digestion process to obtain these results. However this work did not quantify the salinity of the concentrated paste that was used to feed the digester, and the actual salinity of the substrate was less than that of seawater salinities.

Asinari Di San Marzano et al. [109] showed that a gas production of 310 ml and 440 mL g^{-1} VS for dry anaerobically digested *Tetraselmis* sp. of saline microalgae. Both methane production rates are for *Tetraselmis* sp. microalgae biomass that has a salinity of <1 g/L. However they also recorded 450 mL g^{-1} VS added with a substrate salinity of 35 g/L. The authors did not indicate whether this is for dry or wet *Tetraselmis* sp., and they did not indicate the source of inoculum used for this experiment. Buxy et al. [110] reported a methane potential of 204 mL g^{-1} VS for the marine microalgae *Nannochloropsis oculata*. This microalga was grown under seawater salinity conditions, harvested, concentrated to a paste and then used for the bio-methane potential experiments. Due to the use of concentrated paste and its addition to a freshwater anaerobic digester, the final salinities of the digestion vessel are not reported.

High salinity levels have been shown to be inhibitory as it can cause bacterial cells to dehydrate due to increased osmotic pressure [84]. Salinity is made up of multiple elements and can vary depending on the source of water and its associated environment [111]. The light metal ions including sodium, magnesium, calcium and aluminium can all be toxic at high levels [84,93].

The sodium ion is the most inhibitory to anaerobic digestion of these metal ions, and it makes up a larger percentage of the light metal ions found in seawater [59]. However inhibition due to sodium varies depending on the source of inoculum and overall elemental composition of the saline water and substrate being digested [111]. Rinzema [111] demonstrated that sodium concentrations of 5, 10 and 14 g/L caused 10, 50 and 100% inhibition in acetoclastic methanogen bacteria respectively. These measured sodium inhibition rates were also observed by Parkin and Owen [44] who reported a moderate inhibitory effect on anaerobic digestion at sodium concentrations ranging from 3.5 to 5.5 g/L. Above 8.0 g/L of sodium can be extremely inhibitive.

However, Parkin and Owen [44] noted that amelioration of sodium ions and potassium ions was possible. When this amelioration occurred it could reduce the toxicity caused by light metal ions during anaerobic digestion. In addition, the presence of sodium ions was found to be antagonistic to ammonia-nitrogen inhibition. Experiments by Kugelman [112] demonstrated that at an ammonia-nitrogen concentration of 0.15 mol/L, the methane production from acetic acid was reduced by 20%. The addition of 0.002–0.05 mol of sodium (Na^+) produced 5% more methane compared to that of the control. This research indicated a further increase of 10% in methane production was achieved by using a combination of sodium and potassium or sodium and magnesium cations [112].

Zhang et al. [113] identified that sodium induced build up of propionate to be problematic in their digester system, which became inhibitory and caused digester pH imbalance and digester failure. Zhang et al. [113] offered a solution to overcome the inhibition from sodium in syntrophic acetogenic bacteria. To help address high sodium inhibition, Zhang et al. [113] utilised an electrical current delivered to the digester via an iron anode and graphite cathode. They found by adding an electrical current to the digester enough free energy was produced to convert propionate acid to acetic acid allowing methanogens to further transform the acetic acid to methane [113].

6.3. Sulfur and its role in anaerobic digestion

Freshwater microalgal biomass contains low levels of sulfurated amino acids and their digestion releases lower amounts of hydrogen sulfide than other types of substrates [59]. However oxidised sulfur compounds can be present in saline waters and saline substrates. These sulfur compounds can act as electron acceptors for sulfate-reducing bacteria that convert organic compounds in an anaerobic reactor and produce hydrogen sulfide gas. Hydrogen sulfide when present in gas is corrosive and can cause damage to machinery, such as gas-engine power generators, and piping [90]. Except for sulfide, sulfur compounds are not harmful to anaerobic bacteria unless at high concentrations.

Sulfide is needed for cellular metabolism in low concentrations by bacteria, but concentrations higher than 200 mg/L become extremely toxic to methanogenic bacteria[44,114]. Also like un-ionised ammonia-nitrogen, un-ionised sulfide is much more toxic than ionised sulfide. The speciation between the two compounds is also dependent on temperature and pH [44]

Sulfate reducing bacteria compete with methanogenic bacteria for acetate and hydrogen. The sulfate reducing bacteria have a higher affinity for acetate than methanogens, outcompeting them under low acetate concentrations [114]. This competitive inhibition results in the shunting of electrons from methane generation to sulfate reduction. Sulfate reducers and methanogens are very competitive at COD/SO_4 ratios of 1.7 to 2.7. An increase of this ratio is favourable to methanogens, whereas a decrease is favourable to sulfate reducers [90,114].

The conclusion on the optimum COD/SO_4 ratio is supported by the paper by Aspe et al. [115] wherein they found methane fermentation inhibition at a COD/SO_4 ratio lower than 0.5. Aspe et al. [115] highlighted the difference between inoculums used for seeding anaerobic digesters. Their research focused on two inoculums: one was sourced from

piggery effluent and the other was sourced from a marine sediment inoculum. The authors indicated that the sulfate reducing bacteria did not grow as well in the marine sediment sourced inoculum as compared to the piggery effluent sourced inoculum.

7. Bacterial consortium and its role in anaerobic digestion

Aspe et al. [115] highlighted the different results that can be obtained from the source of the bacterial inoculum. Little work has been done on bacterial communities within the microalgal anaerobic digester, and there is a lack of information in the scientific literature on this topic.

The authors Zhang et al. [113] and Patil et al. [116] used polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) to profile the microbial communities associated with anaerobic digestion. This analysis revealed the presence of unknown and as yet uncultured microorganisms associated with the digestion process. Unknown organisms and differences within anaerobic communities are problematic when comparing the variety of results from similar substrates for anaerobic digestion. The inoculum source and the associated bacterial species can change over geographical and environmental locations.

The ability of bacteria to utilise environmental plasticity to change and adapt to different substrates and environmental conditions over time is reported by Buxy et al. [110], who reported an adaptation of bacteria to saline conditions when undertaking bio-methane potential experiments shown by an increase in gas potential and a decrease in the initial lag phase of the digestion process. Further investigation of the microbial community's adaptation and environmental plasticity could potentially offer improvements in the bacterial metabolism and the anaerobic digestion process under adverse conditions.

8. Anaerobic digestion and nutrient recycling

Nutrients are a large and expensive input into the mass production of microalgal biomass. Large amounts of nitrogen and phosphorous are needed for algae biomass production. With the proposed expansion of the commercial algal biomass production industry, the competition with current agricultural industries for organic fertilisers is expected to increase [5], which could increase fertiliser prices. Historically fertiliser prices are closely related to the cost of fossil fuel, and as fossil fuels becomes more expensive fertiliser prices will also increase [5,117]. This increase in oil prices combined with greater fertiliser and energy demand from the agricultural sector could result in increased oil prices making inorganic fertiliser un-competitive for algal biofuel production [5]. Due to these compounding factors, nutrient recovery from processed biomass via anaerobic digestion is highly desirable for the sustainable growth of the algal biomass.

Lyovo et al. [118] indicated that 45 kg of nitrogen is needed for one ton of algal biomass based on a composition of $CO_{0\cdot48}H_{1\cdot83}N_{0\cdot11}P_{0\cdot01}$ [118]. When analysing this stoichiometric relationship, an 11:1 ratio of nitrogen to phosphorus can be determined. This nitrogen to phosphorus ratio indicates that approximately 4 kg of phosphorous is required for every one tonne of algal biomass grown. This ratio further highlights the role that anaerobic digestion provides in the recycling of nutrient that is vital to the sustainability and economic feasibility of commercial-scale algal biofuel industries [1,59,64,80].

Phosphorus as a nutrient has had very little research attention when compared to other nutrients such as carbon and nitrogen [119]. Vaccari [119] stated, “the on-going supply of phosphorus has become one the most significant sustainability issues facing our future” [119].

Several authors have highlighted that there are finite reserves of rock phosphorus available and human civilisation is heading towards a similar scenario as peak oil except with dwindling phosphorus reserves. However the exact quantity of reserves and timing of the shortage is still highly disputed [117,119,120]. This future decline in available phosphorus reserves must be addressed for the sustainable growth of algae for commodity products such as biofuel [2].

Anaerobic digestion of algal biomass produces a nutrient-rich digestate containing both nitrogen and phosphorus nutrients. Digestate nutrient values of 2940 mg/L ammonia-nitrogen, 390 mg/L of total phosphorous, and 320 mg/L of potassium have been reported by Collet et al. [1]. However, these values are in the moderate to high inhibition ranges recorded for ammonia-nitrogen by Parkin and Owen [44]. In contrast, studies conducted by Zamalloa [47] indicated a clear liquid digestate was produced with a total ammonia-nitrogen (TAN) concentration of 546 ± 48 mg/L and a phosphate concentration of 141 ± 41 mg/L from anaerobically digested *P. tricornutum*. These results demonstrate the high strength nutrient-rich digestate that can be produced from the anaerobic digestion of microalgae biomass. Results from De Schamphelaire and Verstraete [8] also indicate that the high strength, nutrient-rich digestate nitrogen to phosphorous ratio from several digestion experiments was in the range of 10 to 17:1 that is ideal for the cultivation of algal species.

The gross chemical composition of microalgae can be highly dependent upon environmental conditions such as light intensity, temperature and nutrient availability. Generally microalgae contain varying proportions of proteins, lipids, carbohydrates, nucleic acids, pigments and vitamins [85]. The mineral composition of microalgae also meets the nutrient and mineral requirements for the microbial micro-flora that are associated with the anaerobic digestion process [59].

The use of digestate from digested microalgae biomass is highlighted in the research by Asinari Di San Marzano et al. [109], Benemann et al. [26], De Schamphelaire and Verstraete [8], Gonzalez-Fernandez [60] but first by Golueke and Oswald [23]. Golueke and Oswald [23] and Ras et al. [6] both setup a closed looped system where microalgae was grown and then harvested and immediately digested to produce biogas. The digestate from the anaerobic digester was then fed back into the high rate pond or photo-bioreactor and used as a nutrient source for further microalgae growth.

A further synergistic benefit of integrating anaerobic digestion with a algal biofuel program is the ability to utilise the microalgae cultures for purifying the methane content of the biogas [12,59,85]. The concentration of carbon dioxide derived biogas from anaerobically digested microalgae is in the range of 30 to 50% [59]. From an energy recovery perspective the biogas CH_4/CO_2 ratio needs to be above 1 [72] indicating that a gas purification step is required for microalgae derived biogas. Due to the low solubility of methane and high solubility of carbon dioxide, uptake of carbon dioxide is high leaving high concentrations of methane after the purification step [1]. Also during this process other impurities such as hydrogen sulfide are removed from the biogas [1]. Biogas CO2 bio-fixation by microalgae is discussed by Green et al. [28].

Research undertaken by Converti et al. [12] also highlighted an increased methane percentage in the biogas produced by utilising the microalgae culture to strip carbon dioxide gas from the biogas as it is produced. This is similar to the high methane gas composition recorded with the advanced integrated wastewater pond system utilising in pond fermentation pits [24]. Methane has been shown to be non-detrimental to microalgae growth and the upgrading of biogas via high density microalgae cultures would be beneficial due to the supply of carbon dioxide to microalgae cultures. The carbon dioxide that is stripped from the biogas would be utilised as a nutrient source by the microalgae [59].

9. Conclusions

Early and more recent research have provided greater understanding of the complexity of individual algal species as a substrate for anaerobic digestion or co-digestion. This knowledge will be extremely beneficial to the anaerobic digestion of algae and will allow the methane production rates from individual algae species to be increased and optimised. Each individual species of algae must be treated differently and processed specifically to optimise biogas yields. Anaerobic digestion of algal biomass is a key unit process that integrates efficiently and beneficially into the production of algae-based biofuels and algae-based

wastewater treatment. The integration of algae-based biofuel production with the anaerobic digestion of algae residuals is the most applicable scenario for the maximisation of methane-rich biogas.

Several technical issues including the low concentration of digestible (biodegradable) algal substrates and cell wall disruption can be overcome by the pre-treatment methods used to process algae for liquid or gaseous biofuels. The integration of anaerobic digestion into proposed algae-derived biodiesel operations has the benefit of being able to utilise glycerol, a by-product, in a co-digestion with microalgae improving the carbon to nitrogen ratio and thus increasing gas production. Gas production can also be utilised for electrical or thermal energy production, while algal cultures can also be utilised for biogas upgrading.

The resulting digestate can improve efficiency as it has been shown to be an ideal nutrient source for the production of algal biomass. The utilisation of this digestate for regrowth of additional algal biomass will help to close the nutrient loop associated with large scale algal biomass production and to achieve more widespread environmental sustainability.

With a greater understanding of algal species and their growth and biological characteristics, the anaerobic digestion of macroalgae and microalgae, and their residues, can be optimised to play a promising role in the sustainable future of clean energy derived from algal biomass.

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