Anaero Technology in collaboration with the Department of Plant Sciences, University of Cambridge

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Project title: Lab- scale evaluation of biogas potential from microalgae

Abstract

While food and growth are priorities in some developing nations, the expansion of "high living standards" is seen as a pre-requisite for "sustainability" in more developed nations. In both cases, energy security is a fundamental factor for economic performance and environmental impact. The concerns of these two "worlds" come into apparent conflict as developed economies search for "sustainable" sources of energy in the form of energy crops where agriculture land is diverted to growing fuel rather than food.

The search for alternatives to energy crops (or "purpose grown crops") for sustainable power generation includes the potential use of algae biomass as a carbon sink and energy source that could potentially avoid the use of good agricultural land to grow energy. The results achieved suggest that the processes such as algae-based wastewater treatment could be sources of biomass for energy generation in anaerobic digestion (AD) plants as well as capturing nutrients, which can be returned to agricultural soils in the form of digestate.

The Biomethane Potential equipment developed by Anaero Technology was used to evaluate three types of algae and maize, the most widespread energy crop in AD. The relative biogas yield over a period of 30 days achieved was *Phaeodactylum tricornutum* (719.9 $l.kg^{-1}$ VS), *Scenedesmus obliquus* (401.27 $l.kg^{-1}$ VS) and *Tetraselmis suecica* (372.7 $l.kg^{-1}$ VS), while Maize produced 750$l.kg^{-1}$VS.

The tests confirm that the anaerobic digestion of algae biomass is a possible route of utilisation of algae biomass. However, the high biomass yields required for energy-specific algae growth are still not being achieved even in warmer countries. Further, development work on efficient harvesting of microalgae is scarce, limiting the potential of algae for energy generation. Complementing environmental applications, such as use of algae for wastewater treatment, with energy generation and nutrient recovery through anaerobic digestion could be the best route forward while improvements on biomass yield and harvesting methods that make the process self-sustaining are developed. It is apparent, from the literature search on micro algae for AD, that biogas potentials and energy content are being evaluated in isolation from data on growth and harvesting rates for the algae species used. Future evaluations of energy and resource potential from algae should include data on rates of algae biomass production (t ds/ha.y) in order to enable consistent evaluation and comparison with established alternatives for land use, such as energy crops. This misrepresentation could be creating an "algae bubble" that could damage the long term potential of this technology.

1. Introduction

Algae is a potential sustainable feedstock to produce biodiesel, bioethanol, bio-methane and bio-hydrogen. Advantages of algae include short growth cycles, possible cultivation on non-arable land, year-round harvesting and possible growth as part of wastewater treatment processes. Although algae have been widely studied for energy or biochemical production, full-scale applications are limited, or non-existent. Uncertainty remains on actual production rates of algae per hectare of surface use, seasonal production patterns, product yields (biogas, fats, organic chemicals) per kg of volatile solids produced, methods and efficiency of harvesting, optimum reactor design and energy use for the full production cycle.

Integrating anaerobic digestion of algae with other applications, such as wastewater nutrient removal, production of hydrogen, or lipid extraction could improve the economic viability of algae (Park et al. 2011). Mussgnug et al. 2010 suggested that hydrogen production from the green algae *Chlamydomonas reinhardtii* prior to anaerobic digestion increased final biogas yield. However, Harun et al. 2011, concluded that more energy could be recovered from algae through anaerobic digestion than converting it to biodiesel.

This project used novel biomethane potential (BMP) equipment as a tool to evaluate potential of selected algae as feedstock for anaerobic digestion. The BMP values obtained were complemented with biomass yields for the species used to evaluate the energy potential in the context of full-scale operation. Maize silage from a nearby full-scale operation was tested for BMP, in parallel to the algae trials, in order to produce a baseline for the evaluation of energy potential ignoring the practicalities of growing and harvesting these feedstocks.

Growth, harvest and productivity of microalgae

Algae can be grown using different cultivation conditions, such as phototrophic, heterotrophic, mixotrophic and photoheterotrophic. Phototrophic cultivation is the most common and simple to scale up. The technologies used for bulk harvesting of algae include flotation, gravity sedimentation, flocculation, centrifugation, and filtration.

Most published literature fails to report the water depth and the reactor geometry, limiting the possibility of calculating the equivalent area productivity. The productivity would allow yield comparisons with alternative crops, such as maize, and would help evaluate the volumes of water to be processed in order to separate microalgae biomass. It is likely that most yield estimates are based on the top 20 cm of water for deeper ponds. Shallow ponds, especially in warmer weather, require continuous water replenishment and treatment in order to compensate for evaporation losses and salinity rise.

The highest algal biomass productivity rates reported in the literature were for *P. carterae* (128.29 tds/ha.y) and *D. salina* when used outdoor raceway ponds in Australia (137.532 tds/ha.y) respectively (Moheimani et al. 2006). A similar study conducted in Tokyo on *Scenedesmus* spp. and *Chlorella* using open circulation method resulted in an areal productivity of around 49.5 tonnes dry weight/ha.y for both species. Productivity of 114 tds/ha.y was reported for *Tetraselmis suecica* grown in outdoor flumes in Hawaii (Laws et al. 1988).

Phaeodactylum is a marine diatom. It is a well-known species in aquaculture and is also considered to a potential feedstock for biodiesel production due to its lipid profile. The highest productivity found in the literature for *Phaeodactylum tricornutum*, grown in PBRs, is 73 ton/ha.y. in Spain, followed by that obtained by Benavides et al. 2013 of 47.815 ton/ha.y. However, it is not clear from the papers if these figures correspond to wet or dry weight, again highlighting the need for consistency in the reporting of growth figures for algae. *Phaeodactylum* is a common marine diatom considered to be a potential feedstock for biodiesel production due to its high lipid content. However, growing *Phaeodactylum tricornutum* has difficulties. The culture has high temperature sensitivity (20-25^{o}C) and it grows better in controlled environments, such as closed photo bioreactors.

Studies in Almeria using *Scenesmuss* spp reported a productivity of 91 tds/ha.y (Tran et al. 2014). Personal communication with Professor Molina Grima confirmed that these yields are still being obtained. Conversely, an equivalent 0.511 ton ds/ha.y was obtained for the same species (Aitken & Antizar Ladislao 2012).

The lack of consistency in the reporting of algal productivity and energy potential in published literature create uncertainty for the decision-making process and could limit investor confidence. Studies on the potential use of algae for energy and biomass should include, in all cases, data on biomass yield rates. Results should include figures expressed in terms of mass, or energy, generated per hectare per year (ton ds/ha.y; or MWh/ha.y, etc.)

Table 1 presents the biomass yields found in the literature for some algal species used in our study.

Table 1 Areal productivity of different species from literature

Algal Species	**Areal Productivity of biomass ($g/m^2/d$)***	**References**
Phaeodactylum tricornutum	20	(Fernandez et al. 2001)
	2.4-11.3	(Laws et al. 1983)
Scenedesmus spp.	2.43-13.52 (DW)	(Kanazawa et al. 1958)
Tetraselmis suecica	37.5	(Nrel 1998)
	31.3	(Laws et al. 1988)

*wet weight unless mentioned, DW- dry weight

Anaerobic digestion of algae

The anaerobic degradability of algae varies with species due mainly to differences in cell structure, cell wall composition, and metabolite production. Operational temperature (mesophilic-thermophilic) could also have an impact on degradability of algae, as well as potential pre-treatments, such as pre-pasteurisation. However, it is thought that the rigidity of its cell wall is a determining factor for the biodegradability of algae. Marsolek et al. 2014, reported that thermal pre-treatment at 90 ^{o}C increased the biogas production from *Nanochloropsis oculata* while the methane content remained the same. On the other hand, Mussgnug et. al. (2010) suggested that the energy demand of pre-treatment can be higher than any potential increase in methane yield achieved.

Anaerobic digestion has been studied for both fresh water species and marine species. In general, the biomethane potentials of algae species range between 200 $l.CH_4.kg^{-1}VS$ and 360 $l.CH_4.kg^{-1}VS$, table 2. Co-digestion experiments have been reported to enhance the methane production and increase the digestibility of carbohydrates and proteins. In one case, co-digestion of *N. salina* with lipid waste resulted on methane yield of 540 $l.kg^{-1}VS$ d for the mixture (Park & Li 2012).

In order to produce a baseline for interpretation of the BMP test that were conducted at the Anero equipment at Waterbeach, Cambridgeshire, UK, the value of the areal yield for a selection of fresh and salt water microalgae species reported in the literature was paired with published BMP data, and compared with maize, table 3.

Table 2 Methane yields of different algal species from literature

Algal species	Methane ($l.CH_4\ kg^{-1}$ VS)	References
Phaeodactylum tricornutum	350 ± 0.03	(Zamalloa et al. 2012)
	362 ± 5	(Frigon et al. 2014)
Tetraselmis suecica	250–310	(Marzano et al. 1982)
Scenedesmus obliquus	287	(Mussgnug et al. 2010)
	240	(Zamalloa et al. 2012)
	210 ± 0.03	
	130-140	(Tran et al. 2014)
Chlorella vulgaris	240	(Ras et al. 2011)

Table 3 Productivity values of different algal species compared with Maize

Feed	Tonnes/hectare. Year	Tonnes volatile solids/hectare. Year *	$m^3\ CH_4$ /tonne volatile solids	$m^3\ CH_4$ /hectare. year	References
Maize	42	11.34	397-618	4501-7008	
Phaeodactylum tricornutum	8.76-76.6	0.5-4.3	364.5	182.25-1567.35	(Laws et al. 1983)
	47.815	2.739	350	958.65	(Silva Benavides et al. 2013)
	73	4.1	380		(Fernandez et al. 2001)
Tetraselmis suecica	69.35	3.321	280	929.88	(Mata et al. 2010)
	114 (DW)	5.46	310	1692	(Laws et al. 1988)
	136	6.556			(Nrel 1998)
Chlorella vulgaris	75.65	7.580	240	1819.2	(Park et al.2011)
	5.9-60	0.58-5.9	286	165.8-1687.4	(Mata et al. 2010)
Scenedesmus spp.	8.8-49.3	7.52-41.86	210	1579.2-8790.6	(Kanazawa et al. 1958)
	0.511	0.433	170	73.61	(Aitken & Antizar Ladislao 2012)
	23.944	20	287	5829	(Su et al. 2012)
	91 (DW)	77	127	9779	(Tran et al. 2014)

*calculated using the volatile solid content (% wet weight) value in table 4

1.1. Materials and methods

Inoculum

Digestate obtained from a food waste AD plant in the South West of England was used as inoculum in the BMP tests. The digestate was collected fresh from active digesters and stored at 20 °C for three days before the start of the tests.

Algae

Phaeodactylum tricornutum was grown in a photo bioreactor (PBR) at the University of Cambridge Botanic Garden, University of Cambridge. The PBR was 6 m in length and had an inner diameter of 0.2m with 94.26 litres of working volume. The growth cycle of *Phaeodactylum tricornutum* was 15 days. Growing of the biomass took place in October 2014. The algae was harvested by centrifugation and the separated paste was stored at -80^{o} C.

Figure 1 300L Photobioreactor containing Phaeodactylum tricornutum at the University of Cambridge

Scenedesmus obliquus was grown in raceway ponds at the University of Bath. The working volume of the pond was 500 litres, operated in a semi-continuous process, with a retention time of 25-30 days. The algae was gravity separated and freeze-dried. *Tetraselmis suecica* was provided by the University of Swansea.

Bio-methane potential (BMP) test

The BMP tests were carried out using equipment developed by Anaero Technology (Patent) Ltd. The set consisted of 15 1-litre HDPE reactor bottles submerged in a water bath maintained at 35ºC, figure 2, until biogas production flattened. The working volume in the reactors was around 700 mL. An inoculum to substrate ratio of 8 g VS inoculum to 1 g VS substrate was used for the batch study. All reactors in the set were mixed at 45 rpm by a single motor that drove all internal paddle mixer in the reactors at the same speed. Gas flow was monitored and logged continuously and converted automatically to standard temperature and pressure (STP) by continuous monitoring of temperature and atmospheric pressure.

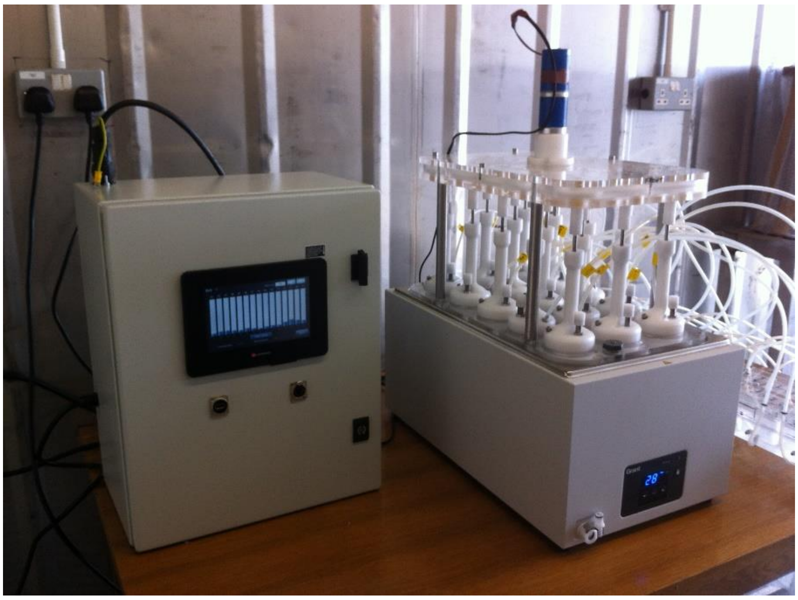

Figure 2 Bio methane potential test Kit (Source: Anaero Technology Ltd) 1. PLC device 2. Reactor bottles 3. Water Bath 4. Motor 5. Glass flow meter 6. Sampling port.

Analytical methods and calculations

Total and volatile solids were determined according to the standard methods (APHA, 1998). Samples were processed for dry solids (DS) in a Memmert oven at 105°C for 6 hours. Volatile solids were heated overnight at 550°C.

The methane content was measured by collecting the biogas produced in the reactors in teddlar gas bags that were connected to the discharge from each gas flow meter. The biogas was analysed for methane, carbon dioxide and hydrogen sulphide using a Geotech BIOGAS 5000 gas monitor (Appendix A).

The biogas potential of substrates was calculated as a cumulative measure of the average daily biogas yields in $l.kg^{-1}$ VS_{added}, minus the biogas potential of the inoculum and was represented graphically.

2. Results

2.1. Physical composition of the algal species

The characteristics of inoculum, maize, *Scenedesmus obliquus, Tetraselmis suecica* and *Phaeodactylum tricornutum* are presented in table 4. The inoculum used to for the BMP tests had an average total solids (TS) 3.92 % and Volatile solids (% of TS) of 60.30 %. The TS of the three algal species used varied between 6.69 % for *Phaeodactylum tricornutum* and 92.43 % for *Scenedesmus obliquus* (freeze dried), while the VS ranged between 69.03 %, for *Tetraselmis suecica*, and 91.78 % for *Scenedesmus obliquus*.

Table 4 Characteristics of the inoculum and substrates used in the tests

	Total Solids % w/w	**Volatile Solids % w/w**	**VS (% of TS)**
Inoculum	3.92	2.37	60.30
Maize silage	29.40	27.82	94.61
Algal species			
Scenedesmus obliquus (freeze-dried)	92.43	84.83	91.78
Tetraselmis suecica	6.94	4.79	69.03
Phaeodactylum tricornutum	6.69	5.73	85.77
*Chlorella vulgaris**	11.57	9.89	85.57

*analysis done in October (2014) on *Chlorella vulgaris* sourced from University of Cambridge

2.2. Productivity of algal species evaluated

Phaeodactylum tricornutum grown in PBRs at the Botanic garden, University of Cambridge, yielded 2.02 g (dry weight).$m^{-2}d^{-1}$, which corresponded to the lower values in table 5. The low algal yield could be explained by the time of the year when the growth took place. Algal productivity of 8.08 g (dry weight).$m^{-2}d^{-1}$ has been achieved for the same species at the botanic garden in peak summer time (Personal communication-Brenda Parker, University of Cambridge).

Table 5 Productivity of the algae used in the tests

	Media	**Growth**	**$g/m^2/d$ (October 2014)**
Scenedesmus obliquus	Wastewater	Open race	Not known
Tetraselmis suecica	Nutrient media	Photo-bioreactor	Not known
Phaeodactylum tricornutum	Nutrient media	Photo-bioreactor	2.02

2.3. Bio-methane potential

The biogas yield of the three algal species tested *Scenedesmus obliquus, Tetraselmis* and *Phaeodactylum tricornutum* ranged from 786.51 to 393.26 l kg^{-1} VS (figure 3). *Phaeodactylum tricornutum* produced the highest biogas yield of 786.51 l.kg^{-1} VS after day 50, followed by *Tetraselmis suecica* (423.69 l.kg^{-1} VS), and *Scenedesmus obliquus* (393.26 l.kg^{-1} VS). There was an initial inhibition lag observed for the biogas production of *Phaeodactylum tricornutum* from day 1 to day 7. Biogas yield started to rise from day 7 and a sharp increase took place from day 10 to 19. Biogas production reached a relatively stable rate until day 25 when biogas production started to decline. *Tetraselmis suecica* produced 46 % less biogas yield when compared to *Phaeodactylum tricornutum*. It presented a similar pattern of biogas yield to that of *Scenedesmus obliquus* from day 1 to day 7 and from day 21 to day 28, after which it increased gradually and reached a value of 423.69 l.kg^{-1} VS on day 50. *Scenedesmus obliquus* produced 50% less biogas than *Phaeodactylum tricornutum* and 7 % less than *Tetraselmis suecica*. *Scenedesmus* had a sharp increase in biogas yield from day 1 to day 7 after which the biogas yield decreased slightly and reached 393.26 l.kg^{-1} VS on day 50.

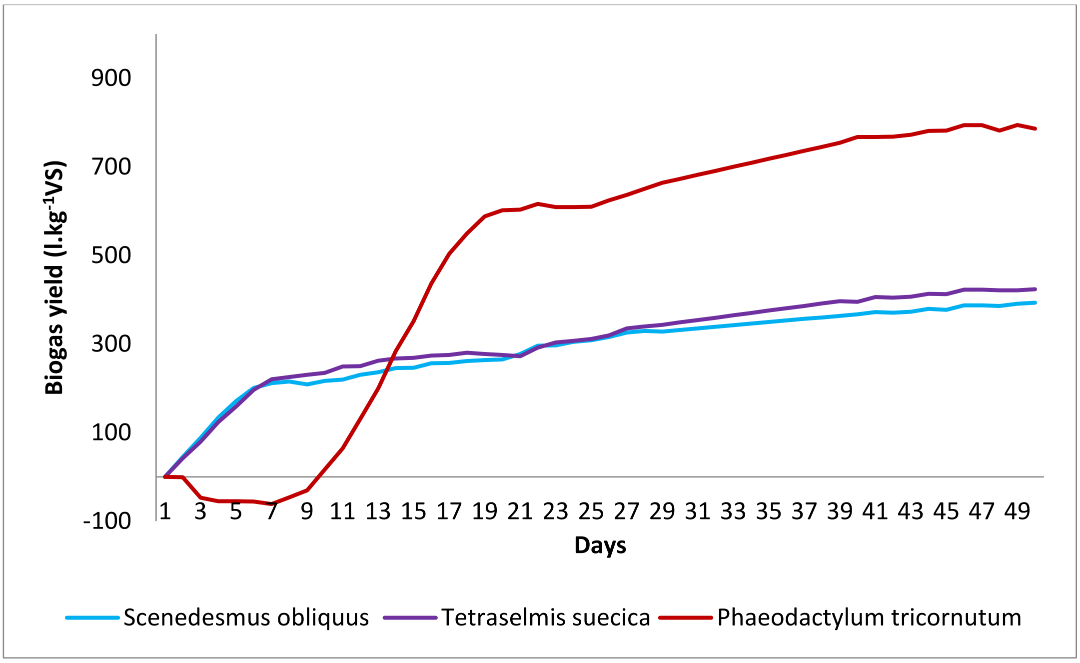

Figure 3 Biogas yield from 50 day BMP tests of 3 different algal species

Phaeodactylum had a very high methane content of 70.9%, which could explain its high methane yield of 557.63 $l.kg^{-1}$ VS (Table 6). The initial lag in biogas generation together with its high final biogas yield could be due to chemical composition of the algae, such as a higher fat content that the other species. *Tetraselmis suecica* reached a methane yield of 233$l.kg^{-1}$ VS for biogas at 55% methane content. *Scenedesmus obliquus* and *Tetraselmis* had a 37 % and 41 % lower methane yield than maize.

Table 6 Biogas yield and methane yield obtained from three different algal species and maize.

Substrate	Biogas yield $l.kg^{-1}$VS	Methane %	Methane yield $l.kg^{-1}$ VS	KgVS required/MWh electric
Scenedesmus obliquus	393	55.2	217	1340
Tetraselmis suecica	424	55	233	1244
Phaeodactylum tricornutum	786	70.9	557	520
Maize silage	650	54	351	829

3. Discussion

The literature review identified a potentially significant flaw of most algae studies: the biomass yield (g DS per m^3, and per m^2 of reactor) is not reported, or measured at all. Data on biomass yield is fundamental to evaluate the economic and environmental potential of algae as sources of energy. For example, if the biogas yield (per kg VS) of algae and maize was similar; but a hectare of algae yielded half the VS that growing maize would; it would still be impossible to argue the case to flood an area to grow algae, and harvest it continuously when maize could produce twice as much energy. Growing maize would also require significantly less handling and energy to grow. For this reason, BMP evaluations of algae, and other biomass applications, such as biodiesel and bulk biochemicals, should be accompanied by biomass yield figures and reported in terms of MWh, or litre of biodiesel, produced per hectare per year. If possible, estimates for energy use of growing, processing and separation should also be included.

The equipment used to measure BMP was simple to operate and produced consistent results, as indicated by the low standard deviation of the data sets (<1% STD). The results obtained were comparable with published literature for some of the species evaluated with the advantage that the equipment produced better kinetic data that could give insights into the effect of the chemical composition of different algae species on biogas production dynamics.

The methane yield of algae volatile solids was comparable with that of maize silage, table 6. However, only if the high biomass yield obtained by Tran et al. (2014) were achieved for all algae types could the energy yield of the biomass produced in a hectare of area compete with that achieved growing maize. Compared with maize, biomass algae have significant disadvantages: while maize requires one harvesting, maximum two, per year, the harvesting of algae is a continuous operation. In addition, algae cultivation requires the continuous treatment of wastewater, compensation for evaporation losses, and can pose serious decanting challenges.

The methane yield obtained for maize silage (351 $l.kg^{-1}$ VS) was very similar to the values reported by Amon et al. (2007) for different maize varieties. *Phaeodactylum tricornutum* had the highest methane yield of 557.62 $l.kg^{-1}$ VS on day 50, although slightly lower than its theoretical methane yield of 629 $l.kg^{-1}$ VS calculated by Zhao et al. (2014). Inhibition was apparent early in the test (day 1 to day 7). This could be caused by the release of inhibitory compounds after lysis of the cell walls as suggested by Frigon et al. (2014). Frigon et al. (2014) also found that early on during anaerobic digestion of *Phaeodactylum tricornutum* there was high production of palmitoleic acid which caused transient inhibition of the process. It is also possible that *Phaeodactylum tricornutum*, being a marine species, could have carried salt into the test resulting in short term inhibition as suggested by (Sialve et al. 2009). After day 25 there was a rise in biogas yield that could have been due to the slow degradation of the long chain fatty acids produced during the first few days. Of the algae species evaluated, *Phaeodactylum* shows the best promise in terms of relative yield.

Further tests on *Phaeodactylum tricornutum* should be conducted to evaluate methane production without the risk of inhibition during continuous anaerobic digestion. The curve in the graph for *Phaeodactylum tricornutum* towards day 50 indicates that not all potentially available carbon had been digested yet, which would further enhance its potential. On the other hand, if high LCFA residual was confirmed in further tests, this could open an opportunity use *Phaeodactylum* for either lipid extraction and/or anaerobic (Sialve et al. 2009).

The methane yield obtained by *Scenedesmus obliquus* (218.73 $l.kg^{-1}$ VS) was higher than previously published work (Tran et al. 2014). Biogas production for *Scenedesmus obliquus* had not flatten out by day 30, suggesting that longer retention time is required to fully digest the substrate. A positive factor regarding *Scenedesmus* is that the biomass used in the study was grown as part domestic wastewater treatment work. Growing biomass in conjunction with environmental services, such as wastewater treatment could improve the viability of such algae as source of energy and resource recovery, as suggested Park et al. 2011. A possible flow diagram for one such application is presented in figure 4.

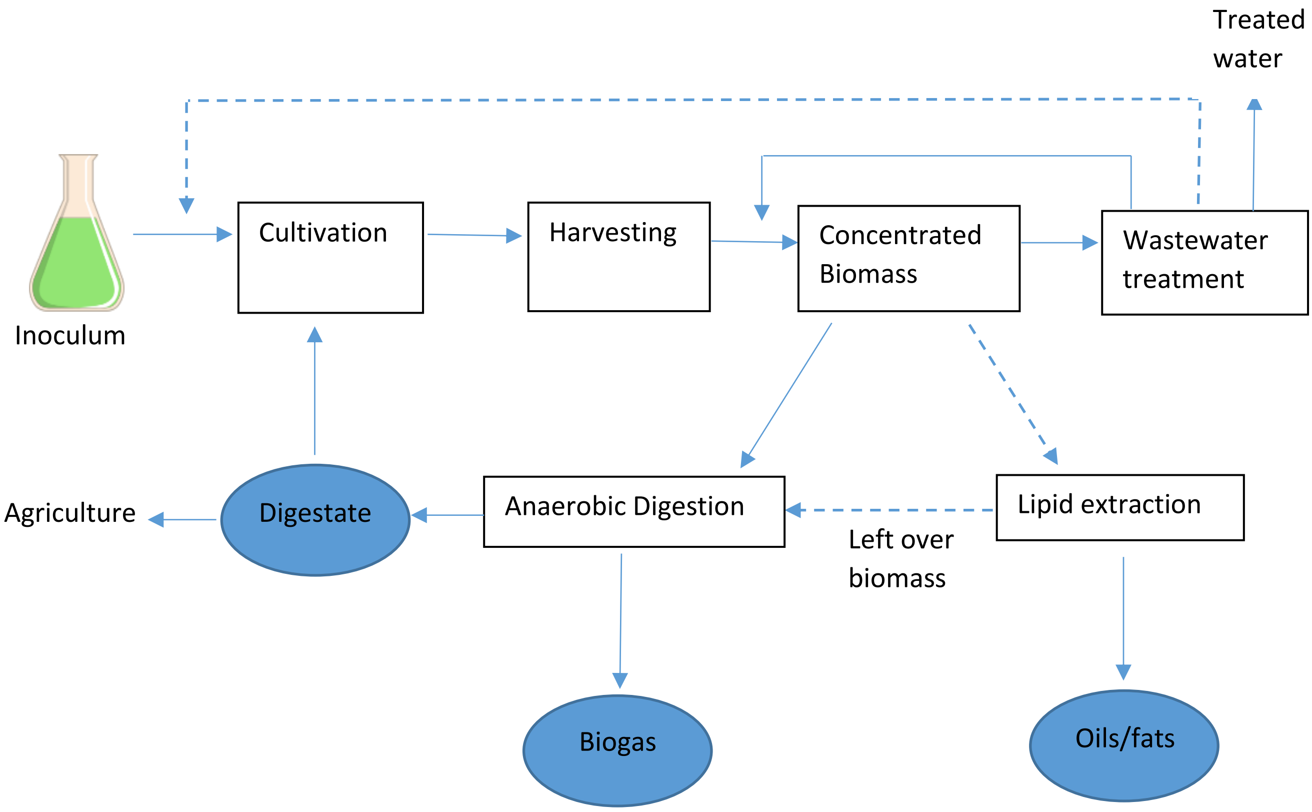

Figure 4 Potential integration of Anaerobic digestion with environmental applications for algae

Based on currently available data, algae cultivation for biofuel production alone is not economically viable. To improve the potential of algae for energy uses, consistently higher biomass yields which far exceed the yield of maize must be achieved, unless other benefits, such as wastewater treatment are realised. Strains with high energy content, robust enough to grow all year round, and self-flocculate would be ideal. Algae growing operations, such as those reported to be producing 90 t ds.$(ha.y)^{-1}$, e.g., (Tran et al. 2014), should be reviewed and replicated accounting for seasonality and in conjunction with evaluation of energy and resource use for the whole production cycle (e.g., electricity use, algae separation, wastewater treatment)

The recent launch of a novel algae harvester by an algal sector company serves to highlight the current challenges in the use of algae as biomass for energy generation. However, the energy use of the algae harvester is far more than the potential energy that could be generated by the algae by AD as calculated below:

> Using the technical figures published by the unnamed algal harvester company - the machine uses 0.7KWh of electricity per m^3 of water treated, and the daily treatment capacity is $36m^3/d$. Operating the machine 24 hours a day would use 25.2KWh/d. Using high harvesting figures for microalgae, $36m^3$ of water containing algae would be expected to yield around 0.8KgVS/day. Using the high biogas production rates achieved for *Phaeodactylum*, table 6, 520kg of algae VS would be required to generate 1MWh electric. Since For 36m3/d only 0.8kgVS can be separated per day, the separator would need to be operated at full capacity, and uninterrupted, for 650 days in order to produce enough volatile solids to generate 1MWh electric. For that period of time, using energy use figures for the machine, the algae separator would use: 650days x 0.0252MWh/day = 16.38MWh. In other words, 16 times more energy would be used just for algae separation, than that generated from the algae processed.

The example above highlights the importance of not limiting evaluations of the potential of algae for energy generation to isolated factors like biomethane potential ($m^3.g^{-1}$VS) alone, without consideration of key elements, such as biomass yield per hectare, and of the technical, financial and energy costs of harvesting and separation of algae.

From the biogas production results obtained, together with biomass yield data, the potential use of algae as a source of energy biomass is not currently viable. However, there could be scope for use of algae in energy and nutrient recovery if algae could be used to provide other services, such as wastewater treatment.

4. Conclusions

- The methane yield measured for *Phaeodactylum tricornutum* was 503 $l.kg^{-1}$ VS, followed by maize (351 $l.kg^{-1}$ VS), *Scenedesmus obliquus* (221.5 $l.kg^{-1}$ VS) and *Tetraselmis suecica* (204.98 $l.kg^{-1}$ VS)
- Data on areal and volumetric algae yield is scarce. Absence of this data seriously limits proper evaluation of the potential use of algae for energy or bioresource recovery.
- From results obtained in our study, the cultivation of algae for energy generation alone is not currently economically feasible.
- The high biogas yield obtained for *Phaeodactylum tricornutum* points to the potential of this species for energy recovery and further tests to confirm the yield obtained in this study should be considered.
- Integrating biomass growth with nutrient removal in wastewater could enhance the viability of algae for energy and resource recovery for ecosystem service industries.

4.1. Further work

- Further BMP evaluation of high yielding species accompanied by lipid profiling and microscopy studies should be considered.
- Emphasis should be given to the development of large-scale, energy efficient methods of separation of algae.
- Additional work should be carried out focusing on algae used in wastewater treatment, and should also include nutrient characterisation of digestate. Producing digestate for nutrient evaluation requires continuous feed tests in order to establish steady state conditions and to produce digestate that would represent what would be produced at full-scale.
- The effect of thermal pre-treatment on the biogas potential and kinetics of algae digestion should be considered. This would be especially relevant for biomass produced as part of wastewater treatment.

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Appendix A

Substrate	CH_4 %	CO_2 %	O_2 %	Balance
Phaeodactylum tricornutum	70.9	10.2	3.3	15.7
Scenedesmus obliquus	55.2	6.5	5.9	32.4