



Politecnico
di Bari

Repository Istituzionale dei Prodotti della Ricerca del Politecnico di Bari

Technologies and developments of third generation biofuel production

This is a pre-print of the following article

Original Citation:

Technologies and developments of third generation biofuel production / Alaswad, A., Dassisti, M., Prescott, T., Olabi, A.G.. - In: RENEWABLE & SUSTAINABLE ENERGY REVIEWS. - ISSN 1364-0321. - 51:(2015), pp. 1446-1460. [10.1016/j.rser.2015.07.058]

Availability:

This version is available at <http://hdl.handle.net/11589/60915> since: 2021-03-18

Published version

DOI:10.1016/j.rser.2015.07.058

Publisher:

Terms of use:

(Article begins on next page)

Technologies and Developments of Third Generation Biofuel Production

A.Alaswad, M.Dassisti, T.Prescott, A.G.Olabi

Abstract:

Many authorities have reached the conclusion that using fossil fuels, as the main source of energy to satisfy the increasing global energy demand, is ultimately unsustainable. This is because of depleting resources, and also because, if this approach is followed, emissions of carbon dioxide would continue to build up. Consequently, research on biofuel, that is fuel made from biomass, derived from materials that were recently living, has attracted attention. In this context, the generation of biofuels from algae shows promise for the following reasons. The algae can be grown quickly, all the materials produced are non-toxic and biodegradable, and during this growth there is an opportunity to cause greenhouse gas fixation. Also, since growing algae does not need arable land, it can be grown without competing with food or feed crops, the fuel derived from it being a “third generation” biofuel.

This paper describes different types of algae (microalgae and seaweeds), and presents different technologies employed in making biofuels (biogas and biodiesel) from them.

Keywords: Renewable, Biomass, Algae, Third generation.

1. Introduction

Many now acknowledge that fossil fuels (coal, oil and natural gas) which are the main sources of energy for the world today are ultimately unsustainable, and related directly to air pollution, land and water degradation and climate changes. Furthermore, many fossil fuels are being depleted (e.g. liquid fuel is expected to end by the middle of the century) (1, 2) and therefore, the world faces huge challenges in order to find suitable, sustainable and clean replacements. On the other hand, the use of renewable energy is already growing. Of the 300 gigawatts of new electricity generation capacity built globally between 2008 and 2009, about 140GW is a capacity to generate from renewable sources (3). In 2005, renewables produced 16.5% of world primary energy (4, 5). According to the special report on renewable energy sources and climate

change mitigation (SRREN), renewable energy could account for almost 80% of the world's energy supply within four decades (6).

Biofuel is fuel that is produced from, or by, recently living organisms, and most often it has reference to plants or plant-derived materials (7). The energy in biofuel is stabilised during a process of biological carbon fixation in which carbon dioxide (CO₂) is converted into sugar that is found only in living organisms, plants (8). In contrast with fossil fuel, biofuel is produced by, or derived from, living organisms in a relatively short period of time rather than being derived by the decomposition of organic matter over several million years (9). The benefits of using biofuels over fossil fuels can be summarized as follows (10, 11, 12, and 13):

- Emissions. One of the more serious problems involved with using fossil fuels is the effects of the emissions (greenhouse gases) which are produced. The gases trap the sun's rays inside the earth's atmosphere and this causes global warming. By contrast, biofuels are carbon neutral, because all of the carbon contained in a biofuel has been already absorbed from the atmosphere by photosynthesis in plants, and is therefore not contributing to the problem of the build-up of these gases.
- Renewability. Many fossil fuels will no longer be available in the next few decades at convenient prices. Biomass materials which can produce biofuels can be produced continuously, being part of a cycle of production, energy usage, emissions absorption and production again.
- Safety. Extracting some fossil fuels from the earth can be a dangerous process. It may be much safer to produce biofuels by farming. Moreover, biofuels are derived from organic substances which are biodegradable, and so they are far less hazardous in the event of fuel spills that may occur. Oil spills are made worse due to the fact that the oil is not biodegradable. If these spills were of biofuels, they could be broken down, and absorbed naturally, and the effect on the environment would not be so serious.
- Economic Enhancement. Developing a Biofuel industry has the potential to decrease fossil fuels imports, create more jobs and keep the economy independent of adverse international developments.

So-called "First generation" biofuels are produced directly from food crops like corn, wheat, sugar cane and soybeans. The biofuel is derived from the starch, sugar, and vegetable oil that these crops provide (14). The most controversial issue with "first generation" biofuels is that it is necessary to choose one, or other of the 'fuel vs food' alternatives (15). Producing biofuels

from food crops inevitably leads to an increase in the volumes of crops being diverted away from the global food market. This may have caused some of the increases in food prices over recent years. In order to avoid that issue, so-called “second generation” biofuels have been introduced. These are made from non-food crops such as grass, wood, food crop waste, animal fats and other organic wastes (16). However, the need for large areas of land with moist soil is a disadvantage for many 2nd generation biofuels. There is still the problem of competition with food production in the use of land and freshwater resources. This difficulty is overcome totally with the so-called “third generation” of biofuels which are derived from marine biomasses (17, 18). It is important to note that the characteristics of the biofuel itself may not change between these so-called “generations”, but rather, the source from which the fuel is derived changes (14).

In this paper, a description of seaweeds and microalgae types is given, and their chemical compositions, properties, and production systems are outlined. Details of the different technologies used in the production of biogas and biodiesel from both types of algae are included. Algal Biofuel is compared to biofuel types from other feed stocks.

2. Algae as a biofuel source

Algae are a diverse group of uni- and multicellular photoautotrophs. They may be considered as biological solar panels that fix CO₂ from the atmosphere, to absorb energy from sun light, for growth, and for the production of intracellular storage compounds (19, 20). They are plant-like organisms in that they always use photosynthesis, and they are usually aquatic. The efficiency of their photosynthesis is higher than that of other plants, and some species are considered to be among the fastest growing plants in the world (21). It has been reported that photosynthetic efficiencies for algae range from 3% to 8%, compared with 0.5% for many terrestrial crops (22). Algae can be classified into two categories: microalgae and seaweeds.

2.1. Seaweeds:

Macro-algae or “seaweeds” are multicellular, exhibit differentiated cell structure and function and are generally more plant like. They do not have true roots, stems, leaves or vascular tissue and have simple reproductive structures. They can grow in either salt water or fresh water. They are often fast-growing and can reach sizes of up to 60 m in length (23). Most of them can be classified into three broad groups, based on their pigmentation: i) brown seaweed (Phaeophyceae); ii) red seaweed (Rhodophyceae) and iii) green seaweed (Chlorophyceae). At present the few uses of seaweeds include the production of specialty foods and the extraction

of hydrocolloids. In their natural environment, macro-algae grow on rocky substrates although it seems that in some cases they can be found attached to sand particles. They form stable, multi-layered, vegetation capturing as many available photons of light as possible (Figure 1). Major advantages in the cultivation of seaweeds over that of their terrestrial counterparts are their shorter life cycles, making the cultivation easier, more cost-effective, and often involving environment-friendly methods. The marine seaweeds don't need any fresh water or fertilizers to grow and so their cultivation does not cause any change in land use. They produce thus a biomass which is more sustainable in comparison to first- and even second-generation biomass sources, in the sense that there is almost no competition for resources with the food industry (24).

Care is needed when cultivating seaweeds. The uncontrolled growth of macro-algae can have unfavourable consequences. When these living organisms die their remains decompose as a result of both aerobic and anaerobic processes, resulting in the production of ingredients which may disturb the biological balance of the local ecosystem. Large quantities of this biomass when allowed to decompose in this way can produce toxic substances and pollutants which can have an effect on both flora and fauna, and become a nuisance for local residents and even cause a reduction in tourism (25).



Figure.1: Seaweeds in Cummington coast, Moray, Scotland.

In comparison with microalgae, seaweeds have higher volumetric production rates (biomass per volume per time) and produce greater biomass densities. In contrast with microalgae, macroalgae do not produce significant quantities of lipid. Seaweeds also require relatively mild

conditions for processing compared to lignocellulosic biomass. Lower temperatures, less severe acid conditions, and shorter reaction times are generally needed (26). Another significant advantage is that seaweeds can be collected (by hand) without costs payable to landowners.

Laminaria Japonica is the most cultivated alga in the world, with 5.14 million tonnes produced in 2010. This is the result of a programme following the adoption of a Chinese policy that has established China as the most important producer of seaweed throughout the world [90]. (Table. 1) describes most public seaweeds families based on the literature in (27- 38).

Table 1: Public species of seaweed

Species List	Description	Image
Ulva	A small green alga (up to 30 cm across) with a broad, wrinkled leaf that is tough, translucent and membranous. It is attached to rock via a small hold-fast.	
Enteromorpha	Green seaweeds, with tubular and elongate leaves that may be branched, flattened or inflated. They are bright green in colour and may occasionally be bleached white, particularly around rock pools.	
Monostroma	As the name suggests, algae of this genus are monostromatic (single cell layered). Gametophyte thallus sac-like when young, becoming sheet-like, light green seaweeds, delicate, attached by a small disk, with some trapped oxygen bubbles can arise on.	
Laminaria	A genus of brown algae, all sharing the common name "kelp". It is characterized by long, leathery and relatively large size. It can grow up to 2.5 m long and 60 cm across the frond. The frond is large and flat, smooth and rubbery with finger like sections.	

Alaria	A genus of highly variable brown algae. It has mature sporophytes as small as 15 centimetres and as large as 15 metres in length. Alaria species consist of a ramified holdfast, an unbranched stipe, and a blade with a percurrent and cartilaginous midrib.	
Sargassum	Brown or dark green algae which consists of a holdfast, a stipe, and a frond. Some species have berrylike gas-filled bladders which help keep the fronds afloat to promote photosynthesis. Many have a rough sticky texture, which together with a robust but flexible body helps it to withstand strong water currents.	
Padina	Brown algae that is fan-shaped, becomes funnelled when growing old, to 120 mm long and 10- 100 mm board; fronds thin and translucent, olive to yellow- brown, darker near the base. It is abundant on shallow reef flat and tends to attach to rocks, shells and hard subtracts.	
Porphyra	Red algae that is growing in the intertidal zone in cold waters of temperate oceans. The thallus of the erect frond of Propyhra species is in the form of a flat, lanceolate or broadly elliptical blade. The fronds are composed entirely of either small rectangular or rounded cells.	
Rhodymenia	A genus of red algae that is flattened, fan-shaped, rather stiff, rose-red fronds, to 100 mm high, with long or short stipes arising from a discoidal base. Fronds repeatedly dichotomously lobed, axils wide, apices rounded, margin smooth.	
Gracilaria	A genus of red algae that is semi-transparent tropical marine macro algae that comes in shades of red. Plants of the largest species can reach 60 cm in length.	

Due to the fact that seaweeds are attached to rocks, values of maximum biomass density may be 10 times higher for an individual seaweed colony attached to an individual rock than for a microalgae population (39). The density of microalgae is much lower because most of the photons are absorbed or scattered by abiotic particles, and so to obtain sufficient light to grow the algae have to be thinly distributed.

The criteria for the selection of species for biofuel production generally include the following: (1) the species must be available in large quantities in many areas of the country, so that the requirements of production can be met; (2) the species should be available throughout the year or for most of the year; and, (3) sustainable cropping (or cultivation) can be conducted without adverse effects on the environment (40). Compositional values for the seaweed families which have been described in (Table. 1) have been compiled and are shown in (Table. 2) (41). It can be seen that, compared with Microalgae, the lipid content of all the seaweed species is very small (less than 5% of dry weight) while there are larger amounts of carbohydrates and protein.

Table 2: Compositional data for some seaweed families (41)

Seaweed family	Protein	Lipid	Carbohydrates	Ash
Ulva	26.1	2.1	42.0	7.8
Enteromorpha	19.5	0.3	64.9	15.2
Monostroma	20.0	1.2	63.9	14.9
Laminaria	16.1	2.4	39.3	19.6
Alaria	17.1	3.6	39.8	14.9
Sargassum	19.0	2.9	33.0	16.2
Padina	18.81	1.7	31.6	10.3
Porphyra	28.4	4.5	45.1	6.9
Rhodomenia	21.5	1.7	44.6	5.3
Gracilaria	24.37	1.8	61.75	11.3

In the past, for a long time, the algal biomass, which was used at the time in a wide array of applications, could be obtained from natural stocks. However, by the early 1970s it had become clear that many seaweed species had been collected for these uses, at such a high rate, that the plants did not have sufficient time to recover, and therefore they had disappeared from the natural beds where they had been found traditionally. It was considered that the controlled cultivation of certain species was the only way to increase production. [42, 43]. At the same time it was also found that intensive and uncontrolled harvesting had led to a significant drop

in native populations, particularly those with economic potential. This led to a movement to outlaw some techniques used in mechanical harvesting, and bans still exist in some countries, because of the environmental damage caused by harvesting tools. At that time the opinion was held that the only way to harvest algae was by hand. Obviously this approach to harvesting is cheaper, but it was clear enough that it would be impossible to extend this process to an industrial scale. Thus, seaweed cultivation came to be seen not only as the only economic option, but it was also realised that the harvesting had to be carried out in an ecologically feasible way to conserve natural resources.

Seaweed cultivation requires less investment compared with that required for other modes of aquaculture such as the production of shrimp or fish. It can also be developed in association with these other types of aquaculture, thereby increasing profits without the need for many new facilities. A further advantage is that the seaweed can make use of many of the organic wastes produced by these other aquacultures, and so the discharge of wastewater pollutants can be minimised [42].

Inshore cultivation is today considered to be the most feasible means of production of macroalgae biomass from an economical point of view, taking into account transport costs and greenhouse gas emissions. However the sites for inshore cultivation need to be selected carefully. The site should have low risks because its rocky or sheltered features should protect crops from damage due to strong tidal water movements, storms or strong currents [44]. Inshore cultivation should always be established in natural water areas such as bays or tidal pools in positions where there is low tidal activity. To be economical the cultivation must use only naturally-available light, heat, water motion energy, and nutrients [42]. Seaweeds may be cultivated on lines or ropes, in nets or grown in seabeds. The productivity of cultivation depends also upon many other factors, in addition to the hydrological and hydrochemical features of a site. These features include a favorable temperature regime, a high concentration of nutrients in the seawater with a balanced ratio of nitrogen to phosphorus, sufficient radiation from the sun, moderate currents and suitable wave action. The inherent productivity of the plants is also very important for obtaining large crops, and new highly-productive strains can be selected. These are being constantly improved [45].

An alternative to this approach is offshore cultivation. In this case, there are no problems about occupying valuable parts of the coastline, which would be the case for inshore cultivation. There would also be less of a problem with the visual impact of offshore farms, whereas in the

case of inshore cultivation, public opinion about visual impact can cause difficulties. An offshore farm can be built as a free-floating system with the algae attached to buoys or the seabed [46]. In some cases cultivation takes place in tanks using natural or artificial light, with the supply of nutrients and phytohormones. There could also be the possibility of applying agronomic techniques such as the removal of unwanted species (weeding), and reducing epiphyte growth. In cases where artificial light is used, the light can also be regulated to make the whole system more productive. A possible means of reducing cost to enhance offshore cultivation profitability would be to operate the system using the wind turbine towers as structures to anchor algal farms, programming the maintenance of both the wind farm and the algae farm to occur at the same time.

In experimental trials effluents from installations where marine animals are cultivated were analysed. Effluents from both intensive and semi-intensive mariculture installations were studied. The effluents were found to contain large amounts of ammonium nitrate and phosphorus compounds, and the mixture of these chemicals proved to be a good medium for the cultivation of seaweeds. The seaweeds studied were capable of removing almost 100% of these compounds [47]. This approach suggests a valid strategy for the bioremediation by algae of wastes produced by fish and other marine animals. However the approach must include elements to provide careful control. If left without suitable controls over a long period [48], exuberant growth could lead to concentrations of oxygen so low that no life is possible. However, if managed adequately higher incomes could be obtained using this integrated cultivation approach. In the experimental trials macroalgae growth rates increased [49] using this approach, with higher quantities of *Palmaria Palmate* (48%) and *Saccharina Latissima* (61%) being produced when in close proximity to salmon farm cages. Integrated seaweed mariculture cultivation can take place in either ponds, pools or tanks with effluents being pumped from reservoirs containing fish or invertebrates. Other arrangements include the seaweed growing together with fish, crustaceans or mollusks. It is also possible to grow the algae in the open sea near cages with these marine animals. The approach looks promising, but the primary objective in the integration with mariculture is to achieve high yields of the marine animals with high quality animal production and to reduce environmental pollution, and the high growth rate and higher quantities of macroalgae would have to remain secondary in importance.

The seasonal variation in the amount of carbohydrates in seaweeds is large. In contrast to the analysis of starch and lignocellulose-based biomass, there is no reliable standard analytical

protocol for the analysis of carbohydrates in seaweeds. Determining the biochemical composition of seaweeds, and thereby estimating their economic potential, is thus difficult (26).

2.2. Microalgae:

It is now necessary to turn from macro-algae in order to provide a description of the other type of algae. A microscope is required in order to observe Microalgae because of their size, being either unicellular or multicellular but always smaller than 0.4 mm in diameter. The category includes groups such as the diatoms and cyanobacteria that have high growth rates. Their photosynthetic mechanism is similar to land-based plants but due to a simple cellular structure, and being submerged in an aqueous environment where they have efficient access to water, CO₂ and other nutrients, they are generally more efficient in converting solar energy into biomass (20, 23, 50). The three most important classes of micro-algae in terms of abundance are the diatoms (Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae (Chrysophyceae). Compared with terrestrial crops, microalgae seem to be able to grow in aqueous solutions with a wide range of salinities and chemical compositions; growth can occur in ponds or pools in both arable and non-arable land without the need for herbicides or pesticides; and the high specific production yields and photosynthetic efficiency [51- 55]. Residual biomass, which is poor in lignin and very rich in proteins and other compounds of commercial interest, can then be used in animal feed production and in the synthesis of different high-valued compounds, such as nutritional supplements, cosmetics and pharmaceuticals in a biorefinery-based production.

The lipid content of microalgae varies in accordance with the culture conditions. It is possible to increase the lipid concentration by almost 80 % above natural levels, by optimizing growth determining factors (56, 57). The oil contents of various microalgae species, as found in natural environments, in relation to their dry weights were determined by different researchers (20, 23, 58, 59, 60, and 61) and are summarized in Table 3. However, there are clearly some differences between the values determined by these researchers, particularly when a range is given for values of all the species in a given family.

Table 3: Oil content of selected micro-algal species

Name of microalgae species	(as %age of dry wt.)	Name of microalgae species	(as %age of dry wt.)

Ankistrodesmus TR-87	28-40	Isochrysis sp.	7-33
Botryococcus braunii	25-75	Monallanthus salina	20
Chaetoceros calcitrans	15- 40	Nannochloris	6- 63
Chlorella emersonii	25- 63	Nannochloris sp.	20- 56
Chlorella sp	10- 57	Nannochloropsis	31-68
Chlorella sorokiniana	19 – 22	Nannochloropsis oculata	23- 30
Chlorella protothecoides	15-58	Nannochloropsis sp.	12- 53
Chlorella vulgaris	5 – 58	Neochloris oleoabundans	29- 65
Cryptocodinium cohnii	20	Nitzschia TR-114	28-50
Cyclotella DI-35	42	Nitzschia sp	45-47
Cylindrotheca sp.	16-37	Pavlova lutheri	36
Dunaliella primolecta	23	Pavlova salina	31
Dunaliella tertiolecta	36-42	Phaeodactylum tricornutum	18- 57
Dunaliella salina	6 – 25	Phaeodactylum tricornutum	20- 31
Dunaliella primolecta	23	Scenedesmus TR-84	45
Dunaliella tertiolecta	18 -71	Scenedesmus obliquus	11- 55
Dunaliella sp.	18 – 67	Scenedesmus quadricauda	2- 19
Dunaliella salina	6 – 25	Scenedesmus sp.	20- 21
Dunaliella primolecta	23	Schizochytrium sp	50-77
Ellipsoidion sp	28	Stichococcus	9-59
Haematococcus pluvialis	25	Spirulina platensis	4- 17
Hantzschia DI-160	66	Tetraselmis suecica	15-32
Isochrysis galbana	7- 40	Thalassiosira pseudonana	21-31

Harvesting microalga biomass is a major challenge because of their small size and their low concentration in the culture medium. In the overall production process, microalgae are initially grown to reach a maximum biomass concentration of 0.02–0.5%. From the culture medium, the biomass is concentrated to 15- 20%, either in a single step or in a series of concentration steps, before they can be processed further via drying, extraction or other downstream processing steps (20, 62, 63, 64). Different technologies have been developed for microalgae cultivation using open ponds, closed ponds, or PBRs (Photobioreactors). Open pond systems that are most often used are shallow ponds or tanks with large surface areas [65, 66, 53, and 54]. The major advantages of open ponds are that they are easier to construct and operate compared with other systems [67]. However, poor light utilization, evaporative losses, losses

of CO₂ to the atmosphere, contamination by wild animals and other living organisms and the requirement for large areas of land are major disadvantages with these systems [68, 69]. Closed or artificial ponds are more efficient in producing a microalgal crop because control over the production environment is much greater than when open ponds are used [67]. However, the cost of closed pond systems is higher than for open pond systems but it is still lower than when PBRs are used [69]. Photobioreactors can have either flat-plates or tubes. They are the most efficient and improve the algal cultivation yields when compared to both open and closed ponds; the reason for this is that the algae are protected from contamination, pathogens, and wildlife [20, 67, 68, and 69]. PBRs are most suitable after a specific strain of algae has been selected for biomass production. The selectivity is carried out either to increase the lipid productivity (mg/L/day) or the biofuel productivity (Kg per day), or a combination of both. Other benefits of photobioreactors are greater temperature control, better control of gas (CO₂) transfer and protection from climate-related impacts [20, 69]. A tubular photobioreactor, designated PBR 4000 G IGV Biotech, is shown in Fig. 2.



Figure 2: A tubular photobioreactor designated PBR 4000 G IGV Biotech.

In order to make microalgae cultivation more cost-effective, the cultivation equipment can be located where it can be combined with other industrial installations such as where flue gases are being discharged to the atmosphere, or where there are wastewater effluents which need to be treated before they can be discharged. The combination of microalgal cultivation with both waste exhaust CO₂ in flue gases being discharged and wastewater streams is recommended [70- 73], because, at the same time as reduction in costs can be made, environmental benefits can also be achieved.

The reason why micro-algal cultivation can be of benefit in the treatment of wastewater is explained below, but in the first place, it is necessary to provide the context why wastewater streams need to be treated. Global annual freshwater consumption was estimated at 3,908.3 billion m³ [74] during 2009, and most of the water consumed was turned into wastewater. Total nitrogen and phosphorus concentrations in wastewater can be as high as 10–100 mg/L in municipal wastewater and even more than 1000 mg/L in agricultural effluents. Without adequate treatment, the release of N and P would lead to eutrophication and ecosystem damage in downstream watersheds [75– 77]. However, wastewater can be used as a low cost source of nutrients for microalgae cultivation. In fact microalgae have the potential to remove large quantities of these unwanted chemicals from wastewater using them as nutrients for growth and so biomass can be accumulated for biofuel production [70]. The effect of combining microalgal cultivation with wastewater treatment facilities is two-fold. Firstly, as far as algal cultivation is concerned, the costs of supplying nutrients is reduced, and secondly as far as wastewater treatment is concerned, algal treatment of wastewater offers a cheaper and more efficient means to remove some chemicals and even some metals from wastewater than conventional tertiary treatment [78– 81]. However, obviously, it may not always be possible to follow this approach. Sometimes wastewater contains toxic substances which would have a negative effect on algal growth.

Similar benefits can also be achieved if the micro-algal cultivation facility can be located where there is access to exhaust flue gases from combustion equipment being discharged to the atmosphere. Again the environmental context needs to be given. It is already recognised that the concentration of CO₂ in the atmosphere has risen due to industrialization where there is fossil fuel combustion. Now elevated levels of CO₂ may be a cause of climate change. Therefore the reduction in the concentration of CO₂ is an issue which must be solved urgently, and is being supported by much of present-day research. The absorption of CO₂ from the atmosphere can be achieved by plants and other organisms including microalgae, which utilize CO₂ by converting it to biomass. The biomass can then be further used for biofuel production or other beneficial purposes. In fact microalgae are particularly effective in removing CO₂ because they can carry out photosynthesis very efficiently. Adapting microalgae for CO₂ sequestration also has the potential to produce other useful by-products, and if these have uses which last for a period of time, the sequestration of CO₂ accomplished will also last for a period of time.

A number of industrial facilities release large quantities of CO₂ to the atmosphere, and are therefore of major concern, because of the long-term damage to the environment. Among these are facilities where fossil fuel is burnt to produce energy as heat, and the CO₂ is released in the exhaust flue gases.

While such installations at present are in the position of causing the greatest damage to the environment, they could also be chosen strategically as offering the greatest possible opportunity for CO₂ reduction, by means of the cultivation of microalgae, which can absorb the CO₂ from the rest of the flue gases [70, 82].

The microalgae actually need the CO₂ in order to grow. For them, this gas is a vital part of the way they are able to build up biomass, using the carbon in CO₂ [83]. It is therefore very convenient that when used in this way, the costs of supplying carbon-containing nutrients for the microalgae can be reduced at the same time as the damaging effects of the release of CO₂ into the atmosphere can be reduced.

While the basic technological idea here is simple, the actual technology to be developed has many problems to overcome. Flue gases are released into the atmosphere at high temperatures, and microalgae would not be able to survive in such environments. The problem is made a little easier if the flue gases come from a facility where Combined Heat and Power systems are used, because it is then very probable that the flue gases have been cooled to some extent. However, it would still be true that the temperature of these flue gases would be much too high for direct contact with the microalgae to take place. There would have to be a way of cooling the exhaust gases to a greater extent. Alternatively the CO₂ could be absorbed selectively from the other flue gases using an intermediary chemical. This intermediary compound could then be cooled and treated in such a way that it would release the CO₂, to allow the micro-organisms to absorb it. Then the intermediary compound could be recycled back to absorb fresh quantities of CO₂ from the flue gases.

An objection to this idea might be raised because the microalgae which absorb the CO₂ grow as a result and eventually biofuels will be made from their biomass. The biofuel is then used to generate energy and when this is done CO₂ would be released into the atmosphere. However, although it is true that CO₂ may still eventually be released into the atmosphere when the fuels derived from algal biomass are consumed, the combination of microalgae farms with CO₂ capture from the flue gases has the potential to approximately double the amount of energy produced per unit of CO₂ released. It is therefore a worthwhile approach, and should be

followed. This is particularly so since flue gas has the potential to provide sufficient quantities of CO₂ for large-scale microalgae farms [84, 85].

3. Producing Biofuel from Algae.

Algae may serve as potential sources of many types of biofuels, including biogas produced in the processes of anaerobic degradation of biomass, and biodiesel produced from lipids accumulating in the cells of the algae. Biogas and biodiesel can be used to replace natural gas and Petroleum diesel respectively, and are the most common types of biofuel which can be produced from algae. While microalgae tend to produce large amounts of lipid (oil), seaweeds tend to produce sugars and other carbohydrates rather than lipids. The oil content of macro-algae is very small being less than 5% of dry weight, and therefore it is generally accepted that macro-algae would not be an economically viable source of biodiesel (86, 87).

On the other hand, methane production from microalgae biomass, as a stand-alone process, has been shown to be uneconomic in the light of the energy costs associated with the cultivation and harvesting of unicellular organisms which includes microalgae species. However, producing biodiesel from microalgae has been proved to be much more feasible and sustainable (88). While it may be true that the harvesting of microalgae may be expensive, the use of filamentous algae species could potentially improve the economics of energy generation (i.e. as CH₄) from algae (66, 89). This is because filamentous algae, although unicellular tend to form long chains of cells where each cell forms a bond with its neighbours, making the harvesting easier in comparison with harvesting unicellular micro-organisms where the cells remain physically isolated from each other.

In summary therefore, macroalgae tend to be used to produce biogas using anaerobic digestion, while microalgae, in the first instance, tend to be used to make biodiesel.

3.1 Deriving Biogas from Algae

3.1.1. Introduction to biogas:

Biogas consists of a mixture of gases, the major components being methane and carbon dioxide, but there are also other trace gases. The ratio of the major components varies depending on the starting materials, but in general the gas has about 60% methane (CH₄), ~ and about 40% carbon dioxide (CO₂). By removing the CO₂ a sample of biogas can be

upgraded to increase its methane content up to the same level as that of natural gas, giving the same performance when used as fuel in internal combustion engines or as a gas for household cooking.

Methane is a particularly valuable fuel. Compared with other hydrocarbon fuels, the burning of methane produces less carbon dioxide for each unit of heat released. Furthermore, methane, being the simplest hydrocarbon, produces more heat per unit of mass (55.7 kJ/g) than other complex hydrocarbons. The most common use of the methane produced in biogas, is where the gas fuels an internal combustion gas engine in a Combined Heat and Power (CHP) unit to produce electricity and heat (90).

3.1.2. Anaerobic Digestion Process:

It is claimed that among technologies to convert biomass to fuels, biogas production is the most efficient in terms of net energy gain (91), because anaerobic digestion has the potential to utilise all the degradable components (carbohydrate, protein and lipid) in the feed stock (92) to produce biogas. A similar claim has also been made; it is claimed that the energy/input ratio for AD (anaerobic digestion) technology is very high compared with other biological and thermo-chemical conversion processes, including the conversion of cellulose to ethanol (93). A ratio of 28:1 has been calculated. It is uncertain how the ratio of 28:1 has been calculated. It is true that anaerobic digestion requires very little thermal or electrical energy input. It only requires a small amount of heat to maintain the constant temperature, and the amount of agitation that is actually required is also very small compared with other processes. Although the chemical energy of the starting materials must be ignored to arrive at a ratio of 28:1, it is clear that the chemical energy of the methane in the biogas is not being ignored.

The difficulty with such claims is that attempts to calculate the ratio of output/input energy, or the net gain in energy cannot be generalised for all feed stocks. For each particular starting material a different value of the output/input ratio or the net energy gain must be calculated. When the feedstock material has thick cell walls, and therefore tends to have large quantities of substances which are difficult to digest (for instance cellulose) the net energy gain may be much lower, and other processes using more intense energy features (such as heat) may rival anaerobic digestion in terms of net energy gain. Therefore researchers with starting materials containing large amounts of cellulose will calculate very different values for the output/input energy ratio. On the other hand, if the starting material has no cell wall components, for instance, if it is pure sugar or lipid, values of 28:1 may well be reached.

Another assumption is that in comparison with agricultural biomass crops, algae contain little cellulose and no lignin, and therefore there is the possibility that anaerobic digestion may bring about a more complete hydrolysis. However the algae, in most cases, the macroalgae, contain other carbohydrates instead of cellulose as the main constituent of their cell walls, and these complex carbohydrates may be even more difficult to hydrolyse. This is because the microbial cultures active in anaerobic digestion have developed in response to the preponderance of cellulosic materials in agricultural crops. They are therefore likely to be able to excrete enzymes which are very efficient at hydrolysing the 1,4 β glucan bond, which holds the glucose units together in cellulose. However the microbes have not normally evolved with the capacity to produce enzymes which are efficient in hydrolysing bonds found in the cell walls of macroalgae. It follows that the hydrolysis of seaweeds during anaerobic digestion is more likely to be incomplete than the corresponding case of an agricultural crop, assuming, of course, that both have the same amount of cell wall materials in their structures.

Anaerobic digestion (AD) is a process involving the degradation of organic matter by bacteria in the absence of oxygen (94). Methanogenic organisms, which work synergistically with the bacteria, and are also unicellular and prokaryotic, being also present in anaerobic digesters convert some of the end products of the degradation into carbon dioxide and methane.

The process occurs naturally inside the stomachs of many ruminant mammals, such as cows, and also in marshes, at the bottom of lakes and in the soil. It also occurs in landfill sites. The methane that is produced in these places is also a greenhouse gas. In fact, it is estimated that methane has a global warming potential which is 20 times as high as carbon dioxide (95). In many cases the methane that is produced in landfill sites is derived from waste organic materials, such as unwanted food residues, and therefore it is important to capture this methane before it escapes into the atmosphere and has an influence on climate change. In some industrial applications of anaerobic digestion which have already been built, the same starting materials are used and the same gases are produced. However it is possible to collect the methane from these facilities and by collecting it and using it in ways to produce energy, the methane is converted into carbon dioxide, and the effect on climate change is reduced.

Algal biomass, like all biomass consists of organic matter which is composed of complex polymeric macromolecules, such as proteins, polysaccharides, lipids, and nucleic acids, and also other simpler compounds such as sugars and amino acids. The Anaerobic digestion process converts the organic matter into final products which are methane, carbon dioxide, new

biomass, and inorganic residues. Several groups of microorganisms are involved in the transformations, and the overall process comprises multiple reactions with many intermediate products (96). Generally, the process can be simplified to three groups of biochemical reactions: (1) hydrolysis; (2) acetogenesis; and (3) methanogenesis.

3.1.3. A detailed approach to anaerobic digestion reaction:

Often the anaerobic process is described as having four groups of biochemical reactions as follows: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis and (4) methanogenesis. However since the hydrolysis reactions produce amino acids and fatty acids, it seems that acidogenesis (the generation of acids) occurs as part of the hydrolysis. It seems to be quite difficult for various authors to define what reactions occur in acidogenesis to distinguish them from hydrolysis on the one hand and acetogenesis on the other. In view of this perhaps it is less confusing to avoid the acidogenesis concept as a separate series of reactions altogether.

It is also found that the different series of biochemical reactions are referred to in the literature as consecutive steps which occur chronologically, with the implication that first step must be carried out before the second step, and so on. However, on the contrary, the first activity which occurs when anaerobic conditions are established is the building up of populations of the microbes which will be able to thrive at the temperature, on the different components of nutrients which are made available. This process takes some time to occur, and corresponds to the lag phase, when a single population of micro-organisms is allowed to grow on a given source of nutrients in a batch fermentation process. The requirement to enable this building up activity to take place is energy in the form of nutrients which can be metabolised easily, to generate the basic building blocks for the optimum population of microbes, so that the complete supply of nutrients can be made use of. Therefore it is expected that the first biochemical transformations would be the reactions where soluble sugars are converted to pyruvic acid, and then to acetic acid (the acetate ion), with the release of carbon dioxide and the conversion of NAD^+ to its reduced form NADH.

Conditions inside the cell walls of these microbes are not the same as those outside. Inside the cell, acetic acid is normally found attached to co-enzymes, whereas outside in the aqueous solution the pH is such that acetic acid will dissociate forming the acetate ion. Co-enzymes and nucleotides such as NAD^+ and NADH are not normally able to pass through the cell wall, and therefore remain inside the cells. Because of this, a metabolic pathway to convert the NADH back to NAD^+ must also operate.

The building up of the optimum population of micro-organisms to take advantage of the supply of nutrients is the driving force for all the biochemical reactions, and since this is so, other reactions will begin to take place, once there is a sufficient supply of energy. These include transformation reactions of the amino acids which are present in the nutrients, so that new protein can be built up, and also some reactions where sugars and carbohydrates which are easily hydrated can be metabolised to form new biomass.

It can be expected that these biomass building-up activities, when they rely on the availability of simple sugars, easily hydrolysed carbohydrates and free amino acids, cannot continue indefinitely. Eventually the sources of these particularly easily metabolised nutrients will dry up. However by the time this happens, the biomass which has been built up will contain some micro-organisms which are capable of making enzymes which can be released into the aqueous solution surrounding them, and once there is a sufficient supply of energy, enzymes can be generated and released into the immediate environment. These enzymes are capable of causing the hydrolysis of complex carbohydrates and proteins which are also available as nutrients in the environment. The hydrolysis of complex carbohydrates and proteins produces fresh supplies of the simple sugars and amino acids, so that fresh biomass can be built up.

One aspect of the above account which has not been described is the metabolic pathway which allows NADH to be converted back to NAD^+ . This has to be linked in some way with reactions occurring in methanogenic micro-organisms where either carbon dioxide or acetic acid can be combined with hydrogen provided by a co-enzyme similar to NADH, to release methane with the co-enzyme reverting back to its oxidised state similar to NAD^+ . This reaction helps the microbe community to remove the metabolic product acetic acid by transforming it into a gas which is almost insoluble in water, and therefore escapes completely from the environment where anaerobic digestion is taking place. If such a mechanism did not exist, the product of metabolism, acetic acid, would produce a steadily increasing acidic environment, which would eventually cause the whole microbe community to die.

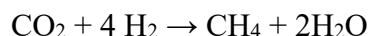
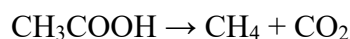
There remains one major difficulty with this description of the reactions involved during anaerobic digestion. If the nucleotide NADH cannot pass through the cell walls of these micro-organisms, how does the NADH in acetogenic bacteria provide the basis for the hydrogen providing co-enzymes in methanogenic organisms? And, once these hydrogen-providing co-enzymes have taken part in the reactions which lead to the release of methane, how does the oxidised form of the co-enzyme, the equivalent of NAD^+ , formed during these reactions, allow

a transfer to take place to produce NAD^+ again in the acetogenic bacteria? The traditional answer given to this question, is that hydrogen itself is formed, and in very dilute aqueous solutions, the hydrogen is able to diffuse between the micro-organisms. However there are difficulties with this explanation. It may be that formic acid is produced and acts as a carrier for the hydrogen. Another possibility is that the hydrogen ion diffuses between the micro-organisms, and electrons are transferred by other mechanisms from one cell to another.

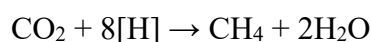
While the above description is incomplete and somewhat speculative, it does provide a perspective about what reactions occur first and what reactions occur at a later time. As mentioned above it is sometimes stated that the groups of biochemical reactions referred to as hydrolysis, acetogenesis and methanogenesis are consecutive steps, with the implication that the first step must be completed before the second step can take place. On the contrary in the above description, circumstances may occur when the acetogenesis reactions occur first, followed by methanogenesis, which must occur soon afterwards to prevent the whole community of microbes from poisoning their environment. It is only afterwards that the bacteria have developed sufficient biomass for the various enzymes to be excreted to allow the much slower hydrolysis to take place.

Hydrolysis occurs where organic polymers, namely proteins, fats and carbohydrates, are broken down into smaller molecules such as amino acids, fatty acids, and simple sugars (97-99). While some of the products of hydrolysis may be used directly by methanogens in the anaerobic digestion process, the majority of the products must firstly be converted in a group of reactions called acetogenesis before they can be made available to methanogenic organisms as nutrients, and these organisms are then able to generate energy from these nutrients, while they convert them to methane. The formation of the acetate ion takes place at this stage, and it occurs when acetogenic organisms metabolise the nutrients in the biomass. The acetogens are fermentative bacteria, and produce an acidic environment in the digestive environment while creating also ammonium salts, CO_2 , and possibly H_2 , as well as trace amounts of other by-products. Methanogenesis constitutes, in one sense, the final stage of anaerobic digestion. During these reactions methanogens create methane from the final products of acetogenesis as well as from some of the intermediate products from hydrolysis (100). Methane is the final product of anaerobic digestion in the sense that once formed, the methane is expelled from the anaerobic environment, because it is an insoluble gas. However this does not imply any chronological order for the different groups of biochemical reactions. The various reactions in methanogenesis can be divided into two pathways, in both of which methane is formed. One

pathway involves acetic acid as the starting material, and in the other carbon dioxide is one of the starting materials. The reactions can be written as follows:



However in view of the uncertainty about whether hydrogen is generated in large quantities at this point, perhaps the second reaction should be written:



Written in this form, the equation allows for the fact that hydrogen can be donated from many different “hydrogen carriers” in the anaerobic environment, and that consequently, perhaps, very little actual hydrogen gas, dissolved as an aqueous solution, exists during anaerobic digestion. The second reaction indicates that CO_2 can be converted into methane, but there is a need for a reducing agent (or a hydrogen carrier) before the reaction can take place. From a different perspective, the second reaction indicates a series of metabolic reactions where the anaerobic culture of micro-organisms is able to remove reducing agents from its environment, and by this method, the whole micro-organism culture can take part in many metabolic reactions without gradually poisoning the environment because of the build-up of reducing agents. In an oxidising environment, for instance in air where oxygen is present, the build-up of reducing agents can be avoided because oxygen can be used to eliminate these reducing agents by forming water:



However this approach is not available to organisms existing in an anaerobic environment, and was not available originally when, it is believed, all environments were anaerobic. Therefore an alternative way of removing reducing agents had to be developed, and was developed by converting CO_2 to methane. Eventually when oxygen became available in the atmosphere, many micro-organisms and subsequently many multi-cellular organisms were able to develop metabolic pathways to remove the reducing agents using oxygen and forming water, and at the same time they were able to benefit from the fact that much greater quantities of energy could also be made available.

The first of the methane producing reaction pathways mentioned above allows methane to be produced from acetic acid. It does not require any reducing agent and methane and CO_2 are produced in equal quantities. In most anaerobic digestion environments this pathway probably

produces most of the methane which is generated. However it would be unusual for it to be the only pathway for the generation of methane.

The second reaction pathway, involving the conversion of CO_2 to methane, occurs frequently when lipids are present in the starting materials, because relatively large quantities of reducing agents (or hydrogen carriers) are produced during the acetogenetic reactions where lipids are converted to acetic acid. In this way the methane content of the biogas rises above 50%.

The second reaction pathway also occurs when the starting material contains only carbohydrates. This is because the metabolic pathways where sugars are converted to acetic acid involve the formation of pyruvic acid, and when this acid is formed reducing agents or hydrogen carriers are also generated. Therefore these reactions could not take place on a continuous basis without some mechanism being present to remove the reducing agent, and this is accomplished in the community of micro-organisms working together synergistically, when the second pathway is followed and some CO_2 is converted to methane.

Once the pyruvic acid has been formed it is converted to acetic acid in acetogenetic bacteria with the release of CO_2 . Therefore in the anaerobic fermentation of pure carbohydrate ingredients, although in methanogenic micro-organisms more methane is generated than CO_2 , some extra CO_2 is also generated by acetogenic bacteria, and therefore the overall result is that equal quantities of methane and CO_2 are produced.

The whole process of groups of biochemical reactions occurring in anaerobic digestion is shown in Figure. 3.

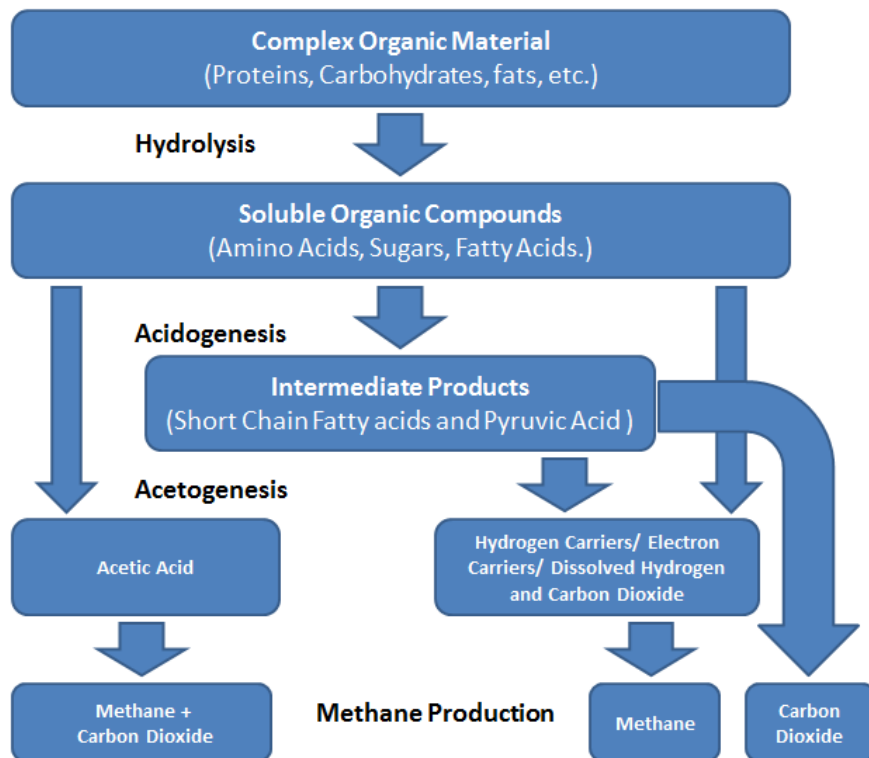


Figure 3: Anaerobic Digestion Process.

A number of procedures have been developed to make it easier to predict the methane concentration in the biogas, and the biogas yield itself. The methods rely on analysing a sample of the starting material. Firstly there is the determination of ash content. This is normally carried out in a furnace at a temperature of at least 500°C, and in air. This causes ingredients in the sample to vaporise and then to burn, forming CO₂. The weight of solid left in the furnace after a sample has been passed through this treatment, divided by the original dry weight of the sample, gives the ash content. Biogas cannot be generated from this ash material, and therefore the amount of ash material in the sample must be determined. The material lost by vaporising or forming gases during this treatment is given the name of “volatile” solids.

The total volume of biogas which can be formed is related to the total volatile solids, being the sum of the carbohydrates, proteins and lipids, which are all volatile solids by this definition. However attempts to predict the gas yield must also take into account the fact that some of the biomass will contain substances which are not easily digested. This occurs when the biomass contains cells with thick cell walls, and in this case the total biomass will have a high concentration of carbohydrate.

It is for this reason that all the methods used for predicting yields can only provide information about theoretical yields, where it is assumed that all of the volatile solids can be digested in

anaerobic digestion. In practice the actual yield will be smaller than this, because some of the solid, although classified as “volatile” remains unchanged during anaerobic digestion.

For those parts of the biomass which can be digested the relative proportions of the two major gases produced by anaerobic digestion can be predicted by different calculation methods. The reason for the complication in these calculations is that the proportion of methane and CO₂ in the biogas can vary depending on the relative amounts of carbohydrates, lipids and protein in the starting material. The atomic ratio of carbon to hydrogen and the atomic ratio of carbon to oxygen are different for each of these types of ingredient, and this leads to different amounts of methane in the biogas, as (Table 4) illustrates:

Table 4: Mole fraction of methane to CO₂ in biogas obtained from Carbohydrate, Lipid, and Protein.

Type of biomass	Typical chemical formula	Carbon to hydrogen ratio	Carbon to oxygen ratio	Mole fraction of methane to CO ₂ in biogas (theoretical)
Carbohydrate	(C ₆ H ₁₀ O ₅) _n	1:1.667	1:0.833	1:1
Lipid	C ₅₇ H ₁₀₄ O ₆	1:1.825	1:0.105	1:0.425
Protein	C _{5.22767} H _{7.93944} O _{1.56355} N _{1.32839} S _{0.05843}	1:1.519	1:0.299	1:0.443

The concept of “oxidation state” has been applied to the covalent compounds of carbon to make it of value in this type of prediction. If an analysis of the elements present in the volatile solids has been carried out, it is possible to use this information to arrive at an average value of the oxidation state of all the carbon in the material. A formula can then be used to predict the amount of methane and the amount of CO₂ (on a theoretical basis) which can be formed during anaerobic digestion.

3.1.4. Inoculum:

The term inoculum can have a number of meanings, and unfortunately this has resulted in some statements occurring in the literature which suggest that some confusion can arise. In the area of industrial microbiology the term usually denotes the source of living bacteria and the other

micro-organisms which are needed for the biochemical reactions. Inoculum is therefore equivalent to seeding sludge as used in the standard VDI 4630. These reactions transform the other materials present at the start of the operation into biogas and residual materials. If the inoculum were not present, the reactions would be limited to chemical and physical changes of the other starting materials. In that case, the physical environment would be more severe in order to achieve any chemical reactions, and no biogas would be produced. The need for an inoculum is fundamental in defining the type of transformations taking place. It is what makes any fermentation possible, and in this case it makes the complex fermentation referred to as anaerobic digestion possible.

The inoculum defines what ingredients in the starting material can be of use in the anaerobic digestion, because these ingredients can be used as nutrients for the micro-organisms in the inoculum to grow and multiply themselves, so that their population can increase. There are two possible outcomes for those parts of the composition of the starting materials which cannot be used as nutrients in this way. Either they do not take part in the anaerobic digestion, and remain unchanged, or the microbes attempt to change them and this may result in the production of chemicals which can harm some of the micro-organisms, and then this can stop the anaerobic digestion proceeding any further. It is preferable, therefore, if the chemical elements in the nutrients are in almost the same proportions as the chemical elements in the micro-organisms of the inoculum which are growing or increasing in numerical population.

There is one exception to this. In order for the nutrients in the starting material to be transformed into more cells of micro-organisms, there is a need for energy to be supplied, to carry out the biochemical transformations. This energy is normally supplied as extra carbohydrate in the nutrients, but it could also be supplied as extra lipid.

Not all of the micro-organisms in the inoculum will grow and increase in population, when they come into contact with nutrients. The nutrients may contain some ingredients which are difficult to digest, or the physical conditions such as the temperature may be such that certain micro-organisms can carry out the biochemical transformations more efficiently than others. There may therefore be changes in the ratios of the populations of the various species of micro-organisms present in the inoculum, depending on these factors. Gradually the microbes which are able to take most advantage of the nutrients will be selected, and therefore these microbes will increase their populations, but there will still be many other micro-organisms present in

relatively small numbers. These are then available when circumstances change or when new nutrients become accessible.

The microbes which carry out the anaerobic digestion form colonies composed of different species which work together so that, although only some of them can hydrolyse some of the ingredients in the starting material, these microbes release, as waste products, other ingredients which can act as nutrients for other microbes. These in turn produce waste products which are suitable as nutrients for still others in the colony. Eventually the final waste products, either, are converted into harmless residual materials, or, they are released from the immediate vicinity as gases.

It is probably the case that the colonies of micro-organisms have been assembled together by chance to carry out anaerobic digestion of the most common starting materials occurring in the environment. Gradually those colonies which are most successful have grown and increased their populations. These colonies are therefore likely to have microbes which have developed the ability to achieve success in efficiently digesting the structural elements which occur frequently in materials in the environment. This includes chemical compounds such as hemicelluloses, and to some extent it includes cellulose itself. Although this material is very difficult to hydrolyse, especially when it occurs in tight, fibrous bundles, some microbes have been able to develop enzyme systems which can be moderately successful in hydrolysing certain sections of the cellulose molecule, especially when the cellulose occurs in isolated fibrils, and free from inhibiting substances.

3.1.5. The relevance of an inoculum to seaweeds:

When, however, the starting material for anaerobic digestion contains some species of macro-algae, such as laminaria species, there may be difficulties in hydrolysing a number of ingredients in the starting material. This is because the polymers which provide support for these types of seaweeds are based on algin and mannitol. They are different chemically from the cellulose and hemicelluloses which are used to provide support for most terrestrial plants.

There is therefore a danger that with starting materials which contain unusual substances, the anaerobic digestion may not be effective, or may take such a long time to produce biogas that the process cannot be used on a commercial scale.

One suggestion has been proposed to overcome this difficulty. It involves using sheep which, over many generations have developed the ability to survive using only various types of seaweeds as their main source of nutrition. Such sheep (particularly the male sheep) exist on

one of the Orkney Islands. It has been suggested that rumen of these sheep must contain some microbes which have developed the ability to hydrolyse the polymers which occur in these seaweeds, and to cope with the inhibiting effects of other chemicals, which are also found in these species of macro-algae. It is assumed that these microbes must be part of the colonies of micro-organisms, which carry out anaerobic digestion in the same way as other colonies of micro-organisms carry out anaerobic digestion on cellulose-based materials in, for instance, the rumen of cows. It is thought that such anaerobic cultures, which would be suitable for starting materials containing seaweeds, could be obtained by collecting suitable droppings from the male sheep on the island of North Ronaldsay. However there have been conflicting reports on this topic, and it is evident that various other factors complicate the picture (101).

As has already been suggested, the solid waste products of ruminant animals can be a convenient source of the inoculum needed for laboratory trials and commercial operations based on anaerobic digestion. It is also possible to obtain useful anaerobic cultures from other examples in nature where anaerobic digestion is occurring, such as stagnant pools. Obviously once a culture has been established in a commercial operation, samples of useful anaerobic cultures can be obtained for new trials. In commercial operations, the micro-organisms are continuously increasing in number, and can therefore normally be made available in the waste product from the anaerobic facility, being surplus to requirements.

The account given above about sheep on one of the Orkney Islands, is an example where an anaerobic digestion microbial culture might be especially effective when the starting material is seaweed. It may be possible to find in nature other examples of specialised anaerobic cultures. However it is more unlikely that cultures will be found which can tolerate high ammonia concentrations, or heavy metal concentrations, because such conditions do not occur often in nature.

3.1.6. Confusion about the concept of an inoculum:

A number of statements about this topic can be found in the literature, which seem to indicate confusion and misunderstanding. In their report Migliore et al (102) state that they studied the anaerobic digestion of seaweeds with and without the addition of an inoculum. In the case where it was reported that no inoculum was added, use was made of a marine sediment taken from the same environment as the algae, presumably to be used instead of a traditional inoculum, and sea water was used as the solvent during the subsequent anaerobic digestion. If these authors had been able to initiate anaerobic digestion without an inoculum, they would

have succeeded in the spontaneous creation of living organisms. The authors report that these circumstances produced a slower acidogenesis, compared with more normal anaerobic digestion systems. When the anaerobic digestion was carried out at 55°C, the main gas was hydrogen and the concentration of methane was negligible.

These results are compared in the report with the results of anaerobic digestion in which a standard inoculum was added to identical samples of the seaweeds. In this case it is assumed that the inoculum was obtained from a conventional source, such as from other anaerobic digestion systems, where, perhaps lingo-cellulose would be present among the starting materials.

In the case where it was reported that no inoculum was added, it has to be assumed that the microbes, which carried out the anaerobic digestion, were obtained because they already existed on the surfaces of the seaweeds, or in the marine sediments, or, perhaps the seaweeds themselves were suffering from parasitic organisms or diseases. Perhaps the seaweed had been dead for some time and had been stored in such a way to produce anaerobic conditions. It is also possible that autolytic enzymes in the seaweed itself were responsible for the hydrolysis of cell wall materials, but this series of reactions would probably not produce a gas such as hydrogen, without there being active microbial organisms also present.

It is concluded that the study by these authors indicates differences in the performance of anaerobic digestion systems because the inocula were obtained from different sources. In the first case a conventional inoculum was added as intended by the authors. In the second case an inoculum was assembled in spite of the fact that this was not the intention of the authors. In both cases anaerobic digestion resulted with the production of biogas.

In another paper Tedesco et al (103) reported an effect which seemed to occur when too much inoculum was added to the starting material. The result seemed to be that a high concentration of ammonia was obtained and this caused the premature termination of the anaerobic digestion. The authors therefore made the recommendation that the ratio of the sludge volume to algae volume should be 2:3 to obtain optimum conditions for anaerobic digestion.

The inoculum is normally understood to refer to the population of active micro-organisms which multiply their populations by using the other ingredients in the starting materials as nutrients, and in doing so they cause the series of biochemical reactions of anaerobic digestion. In that sense, one cannot ever have too large an amount of inoculum, bearing in mind that the purpose in human terms, of carrying out anaerobic digestion is to convert the biomass into

biogas. A large amount of the inoculum can only result in higher reaction rate for the biochemical reactions.

It is often true that the inoculum contains some microbes which cannot make use of some of the nutrients in the starting material efficiently, and there may be a need for the ratios of the populations of the various microbes to change, so that colonies of the microbes which can make use of the starting materials more efficiently can grow more quickly while those unable to use some of the starting materials will grow more slowly. However, in that case, it is even more important to use as large an amount of the inoculum as possible, so that the most efficient colonies of microbes, even though present initially in very small numbers can grow, and in this way, in general terms, the whole of the new population of microbes will make use of the available nutrients more efficiently.

The most probable explanation for the experimental observations reported by these authors is that the sludge which was used to provide the microbial population required for anaerobic digestion also contained many other substances, which could be used as nutrients for the microbes to use to increase their numbers. However, there was an imbalance with respect to the ingredients in this nutrient component of the sludge. It was stated above the different chemical elements in the nutrients must match the chemical elements in the microbial colonies which are able to grow by making use of the available nutrients most efficiently, with a small number of exceptions. In order to assimilate these nutrients there is a need to expend chemical energy, and therefore the nutrients must also be the source of this chemical energy. Generally this energy is supplied in the form of carbohydrates, and therefore there needs to be extra carbohydrate material available in the nutrients.

In the special case of the circumstances reported by Tedesco et al, it seems likely that the imbalance is due to a high concentration of protein in the nutrient component of the sludge, and a low concentration of carbohydrates which could be used to provide the energy. The authors indicated that the sludge was mixed with the algae to allow anaerobic digestion to take place. It seems probable therefore that the algae feedstock supplied the carbohydrate. This is suggested even though other reports state that the Carbon to Nitrogen ratio is low in algal biomass, and this leads to high ammonia concentrations when algae biomass is used without other sources of biomass (see below). When the ratio of sludge to algae was not optimal, with too much sludge and too little algae, a high concentration of ammonia was produced which resulted in the premature termination of the anaerobic digestion. The protein, since it contains

nitrogen, is converted during the initial stages of the anaerobic digestion to ammonium salts and other residual substances. If there had been an abundance of energy rich substances in the nutrients available to the microbial population, there would have been exponential growth in the populations of micro-organisms. In this case the nitrogen in the ammonium salts would have been converted to the specific forms of protein which can be found in these microbial populations. However because of the lack of energy rich substances, the growth in the population of micro-organisms was stunted, and so the ammonium salts began to accumulate. The problem therefore was not that there was too much inoculum, but that the other component in the sludge only provided an unbalanced set of nutrients for the anaerobic digestion.

3.1.7. Controlling the physical parameters for AD:

Methanogenic archaea, which are present in the colonies of microbes which are active in anaerobic digestion, are classified according to the temperature ranges in which they exhibit maximum growth and substrate utilization rates. The optimal temperature for the growth of the organisms designated as psychrophilic is between 10°C and 15°C, for mesophilic organisms it is between 35°C and 40°C, and for thermophilic, it is between 58°C and 68°C. These methanogens are very sensitive to temperature variation, so that a sudden temperature change can make it necessary for one species of methanogen to be replaced by another in the colony. This causes delays as the populations change, with some organisms dying and others growing. The delay results in a decrease in the methane generation rate, and can cause an accumulation of volatile fatty acids, especially under thermophilic conditions – a disadvantage which is particularly acute when operating in the higher temperature range. On the other hand the rate of methane generation by psychrophilic micro-organisms is significantly slower than rates of generation by mesophiles and thermophiles, and therefore the psychrophilic regime is rarely used for large-scale methane production (96). Similar observations were made by Vanegas (104). The mesophilic temperature range has therefore been recommended for the anaerobic digestion of seaweeds in different studies (104, 102).

The choice of the temperature range can also have an effect on other micro-organisms in the colonies. Some authors have indicated that the temperature impacts directly on the production by bacteria of enzymes which hydrolyse insoluble polymers in the biomass, to make them available as nutrients. (105, 106) Changes in the temperature can also cause an increase or

decrease in the concentration of inhibitors, and affect the ratio of growth rate of methanogenic micro-organisms to that of bacteria.

The micro-organisms which can carry out the series of biochemical reactions in anaerobic digestion are able to do so if the pH of the aqueous solution surrounding them is maintained within specific ranges. For optimal performance, these ranges are slightly different for each of the biochemical conversions. For instance enzymes released from bacteria to hydrolyse insoluble polymers of carbohydrates achieve maximum activity in the pH range between 5.5 and 6.5, whereas the optimum pH range around methanogens has been stated to be between 7.8 and 8.2 (107). The activity of methanogens is very sensitive to pH variation. It is reported that the maximum biogas yield in anaerobic digestion can be achieved in the pH range between 6.5 and 7.5 (96, 108- 110). A decrease in the pH value can be prevented by adding lime. In continuous production systems, if the solution after the treatment of residues of anaerobic digestion is alkaline, it can also be used to prevent pH values falling by recycling it into the anaerobic digestion equipment.

3.1.8. Controlling the balance of nutrients for AD:

Both macro- and micro-nutrients are required for anaerobic digestion, which is achieved by the stable growth of anaerobic micro-organisms. In the case of macro-nutrients the control of the C to N ratio is an important aspect of optimising the digester performance. It is often achieved by mixing two different sources of biomass and introducing both of them together into the anaerobic equipment, a practice referred to as co-digestion. Protein is an important nutrient for digestion, but it can lead to inhibitory effects if high concentrations of ammonia are allowed to form. This happens when too much protein has been supplied in the starting materials with insufficient amounts of available carbon-rich substances. Relatively high concentrations of ammonia increase the pH levels in the digester and lead to toxic effects on the population of methanogens. One author (111) seems to suggest that seaweeds are rich in substances with large quantities of available carbon, but relatively poor in Nitrogen, so that benefits can be achieved in the co-digestion of seaweeds with other substances which are richer in Nitrogen. However others seem to report that the C to N ratio of algal biomass (around 10:1) is unusually low in comparison with other types of biomass, so that the benefits of co-digestion are realised when the algal biomass is supplemented by the addition of substances which are richer in carbon and less rich in Nitrogen (112, 113). In order to avoid this imbalance, carbon rich materials were added by these researchers in order to improve digester performance. This was

demonstrated as follows. Adding waste paper to algal sludge at a rate of 50% based on volatile solid matter, increased the methane production rate by more than 100%. Results suggested an optimum C to N ratio ranging between 20:1 and 25:1 (113). It has been recommended that the ratios of Carbon to Nitrogen and to Phosphate should be in the range between 75:5:1 and 125:5:1 by some authors (96). Zhong et al. (114) observed that the addition of corn straw to the digestion of Taihu blue algae, to achieve a similar C to N ratio of 20:1, caused an increase in the methane yield of 62%, with a yield of 325 mL per g. of VS being recorded. Similarly, blends of *Saccharina latissima* and straw produced a maximum methane yield when the C to N ratio was about 30:1 (115).

The same approach has been followed when carrying out anaerobic digestion of residues formed as by-products in the production of biodiesel from micro-algae. In this process the lipid which forms in the micro-algae (*Chlorella* sp.) is converted to biodiesel by the chemical reactions of trans-esterification, and sometimes the whole of the micro-algae biomass is subjected to the reaction conditions which allow the chemical reactions to take place, without prior separation of the lipid from the rest of the biomass. After the biomass has been exposed to these conditions, it is separated from the biodiesel, and then introduced to anaerobic equipment to allow a second energy product, biogas, to be also produced. The biomass material, by this stage, on its own, has a low Carbon to Nitrogen ratio (about 12:1), and this results in a poor yield of biogas. However another product of the trans-esterification, glycerol can also be separated from the biodiesel. When some of this is added to the micro-algal biomass, after it has been exposed to the trans-esterification reaction conditions, it increases the Carbon to Nitrogen ratio, and this allows a higher yield of methane (more than 50%) to be achieved (116). However the addition of too much glycerol can have negative consequences. If the Carbon to Nitrogen ratio is too high, there will be a lack of Nitrogen. This can limit the formation of protein, which limits the growth of the anaerobic micro-organisms. While there may be an abundance of available energy in the digestion, if the micro-organisms cannot grow, they cannot transform this energy into biogas quickly enough, and so the result is a poor yield of biogas.

In the case of micro-nutrients, a balanced composition of ingredients in the digesters is also absolutely necessary (117- 119). The light metal ions of Na, K, Mg, Ca and Al are important nutrients for the growth of micro-organisms, although, repeating similarities with the effects of Nitrogen, excessive concentrations of these ions can lead to severe inhibition (120).

3.1.9. The advantages and disadvantages of using of Macro-algae as a source of biogas compared with deriving this fuel from other biomass sources

Advantages and disadvantages were summarized from different research studies (66, 89, 111, 121- 125) and listed in (Table 5).

Table 5. The advantages and disadvantages of choosing macro-algae as the starting material for the production of biogas, as opposed to choosing other types of biomass.

Advantages	Disadvantages
No lignin, low lignocellulose content	High protein content leads to production of high ammonium concentration and may lead to toxicity
removal of P and N from the sea	low digestibility of algal cell wall
mitigate marine eutrophication	The possible need for the co-digestion with other substrates.
comparable or even higher productivity than terrestrial plants	
the ability of algae to treat wastewater and fix CO ₂ from waste gas streams	
minimal requirement of nutrients	
Higher Photosynthetic efficiency	
Doesn't compete with agriculture	
Less feedstock cost	
Can be used in combination with biodiesel production of lipid wastes.	
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	

3.2. Deriving Biodiesel from algae

3.2.1. Introduction to Biodiesel:

Biodiesel is an alternative fuel formulated exclusively for diesel engines; it is renewable, but it is not a totally clean-burning diesel replacement. It can be produced from vegetable oil, animal oil and fats and waste cooking oil. In his pioneering work on diesel engines, Rudolph Diesel produced methyl esters from fatty acids obtained from crops, for some of his earliest trials in 1900 (65), but since then attention has been focussed mainly on diesel oil produced from the mineral deposits of crude oil. Biodiesel is now of interest as an alternative because it is renewable and its use has better environmental consequences (126) when compared with conventional diesel oil.

It is the combustion and vaporisation characteristics of a fuel, which make it suitable for use in diesel engines, and these characteristics are present in some esters formed from alcohols with carboxylic acids. To be useful for biodiesel applications, the alcohols need to have short chains,

and are primarily methanol or ethanol. The carboxylic acids are fatty acids with chain lengths between C₁₄ and C₂₂.

Biodiesel would be much in demand if it can replace diesel derived from fossil fuels, which may have limited availability in the future. However an even more important reason for using it as a replacement for conventional diesel is the possibility of a reduction in net carbon dioxide emissions. This is achieved because the major part of biodiesel, the carboxylic part, is derived from plants or algae which absorb carbon dioxide from the atmosphere as they grow. Therefore, when the biodiesel is consumed, and carbon dioxide is released into the atmosphere, the net effect is that less carbon dioxide is released than would be the case for conventional diesel. The other component from which diesel is made is the alcohol, the methanol or ethanol. This is usually a product of the petrochemical industry, particularly when methanol is used. Since this is not derived from organisms which absorb carbon dioxide from the atmosphere, there is a small net increase in carbon dioxide emissions when biodiesel is consumed, and because methanol is a smaller component than the carboxylic acids, it is usual to refer to a net carbon dioxide emissions reduction of 78% (127- 129). It is also possible to manufacture ethanol from biological materials (bio-ethanol), and when this is used in the manufacture of biodiesel, there is a net reduction of carbon dioxide emissions of 100%.

In its pure form, while biodiesel remains a liquid at room temperature, it is not easy to set it alight. However, in its commercial form, it usually contains some unreacted methanol, which has toxic effects. It is biodegradable, unlike conventional diesel (130, 50).

There may be some advantages in the fact that it has a high viscosity in comparison with petrodiesel (131). It can therefore be used to some extent as a lubricant (126). On the other hand, such high viscosity fuels are difficult to combust and leave deposits on the fuel injector systems of diesel engines. Therefore, biodiesel is usually blended with conventional diesel. For example B20 contains 20% biodiesel and 80% diesel (132). Algal biodiesel has a calorific value between 39 and 41 MJ/kg. This is close to that of petro diesel, which has a value of 46 MJ/kg [43]. A comparison of the elemental and chemical content of biodiesel and conventional diesel is presented in Table 6 (131, 133).

Table 6: A Comparison of the elemental and chemical content of biodiesel and conventional diesel.

Components	Biodiesel content (%)	Diesel content (%)
Carbon	79.6	86.4

Components	Biodiesel content (%)	Diesel content (%)
Hydrogen	10.5	13.6
Oxygen	8.6	–
Nitrogen	1.3 (This is due to impurities.)	–
C/H	7.6	6.5
n-aliphatics	15.2 (referring only to the	67.4 (normal and branched aliphatics)
Olefinics	84.7 (hydrocarbon part of the part of	3.4
Aromatics	- the carboxylic acid)	20.1
Naphthenics	–	9.1

3.2.2. The reasons for considering micro-algae as a source for biodiesel:

Microalgae produce fatty acids and lipids by metabolism and they form storage structures where the lipids can be stored so that they can be used as sources of energy. They also use the properties of lipids as components of membranes in the organisation of the cells (134, 135).

It is true that uncontrolled growth of micro-algae in natural water environments has negative consequences, which have some similarities with the uncontrolled growth of macro-algae. In this case, the micro-algae can quickly cover the surfaces of lakes, and when they die, their remains can cause there to be a lack of dissolved oxygen in the water, which can result in the loss of aquatic life.

Bearing this in mind, engineering solutions need to be developed to minimise uncontrolled growth. On the other hand, there is a major problem at present in third world countries where oilseed crops are grown for the manufacture of biodiesel as a means of making money by unscrupulous land owners, with tragic consequences for local poor populations. It would be better from an environmental point of view to use only algal sources of the lipids required in its manufacture. Also the United Kingdom consumes nearly 25 billion L of diesel annually; to produce this amount of biodiesel from oilseed crops, more than half of the land in the UK would be required. Therefore, currently microalgae are being considered promising sources of biodiesel because of their high reproduction rate and lipid content, which may be at least as high as 50% to 70% (136). Therefore attention towards this alternative source is increasing.

After collecting the algal biomass, it is often dried in preparation for extracting lipids from it using solvents. The literature indicates that solvent extraction is more efficient if it is preceded by some form of mechanical or thermal treatment, to cause the lipid storage structures to become exposed to the solvent. Hexane is the most common choice for the solvent extraction

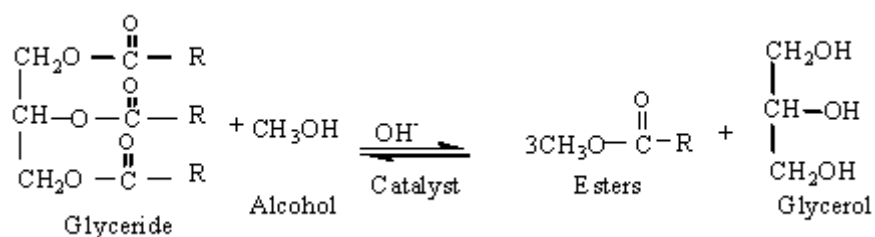
of lipids from algae on a large scale. The extraction process consists of bringing the material into contact with hexane, and this causes the oil to dissolve in the hexane forming a solution referred to as a miscella. Subsequent evaporation and condensation allows the hexane absorbed by the material, and in the solution to be recovered. The hexane thus recovered is reused for the extraction of further batches of the biomass. The low boiling point of hexane (67°C / 152°F) and the high solubility of oils and fats in it are the properties exploited in the solvent extraction process (137, 138). Other solvents and mixtures of solvents can be used, but they tend to also extract various pigments and chlorophyll, which are present in the biomass, and this causes impurities to be formed in the biodiesel.

Biodiesel can be produced from oil and fat by any one of the following methods: 1. Base catalysed trans-esterification of the oil with alcohol, 2. direct acid catalysed trans-esterification of the oil with methanol, and 3. the conversion of the oil to fatty acids, followed by the conversion of the fatty acids to alkyl esters with acid catalysis. The majority of biodiesel produced at present is carried out using the base-catalysed process because it is the most economic for several reasons (139):

- Low temperature (150 F) and pressure (20 psi) processing.
- High conversion (98%) with minimal side reactions and reaction time.
- Direct conversion to the methyl ester with no intermediate steps.
- Exotic materials of construction are not necessary.

3.2.3. Trans-esterification process:

Trans-esterification is the reaction of a triglyceride (fat or oil) with an alcohol to form esters and glycerol. During the esterification process, the triglyceride reacts with the alcohol in the presence of a catalyst, typically sodium hydroxide or potassium hydroxide. Excess alcohol is normally used to ensure total conversion of the fat or oil to its esters. The alcohol reacts with the fat or oil to form the mono-alkyl ester, which is the active ingredient of biodiesel and glycerol is also formed. A successful trans-esterification reaction is signified by the separation of the oil phase containing the esters and a glycerol layer at the end of the reaction time. The equation below shows the chemistry for the formation of the methyl ester, which is the most common component of biodiesel (140).



The glycerol is significantly denser than biodiesel and the two liquids can be separated by gravity, with the glycerol layer being simply drawn off from the bottom of the settling vessel. In some cases, a centrifuge is used to separate the two materials more efficiently. Once the glycerol and biodiesel phases have been separated, the excess alcohol in each phase can be partially removed by a flash evaporation process or by fractional distillation which causes the removal to be more complete. Figure 5 illustrates the process of trans-esterification (20, 141), with some ancillary process steps. Before being used as a commercial fuel, it is necessary to analyse the biodiesel to ensure it meets the required specifications. The most important properties of biodiesel to ensure trouble-free operation in diesel engines are: the absence of glycerol, catalyst, unreacted alcohol and free fatty acids (140).

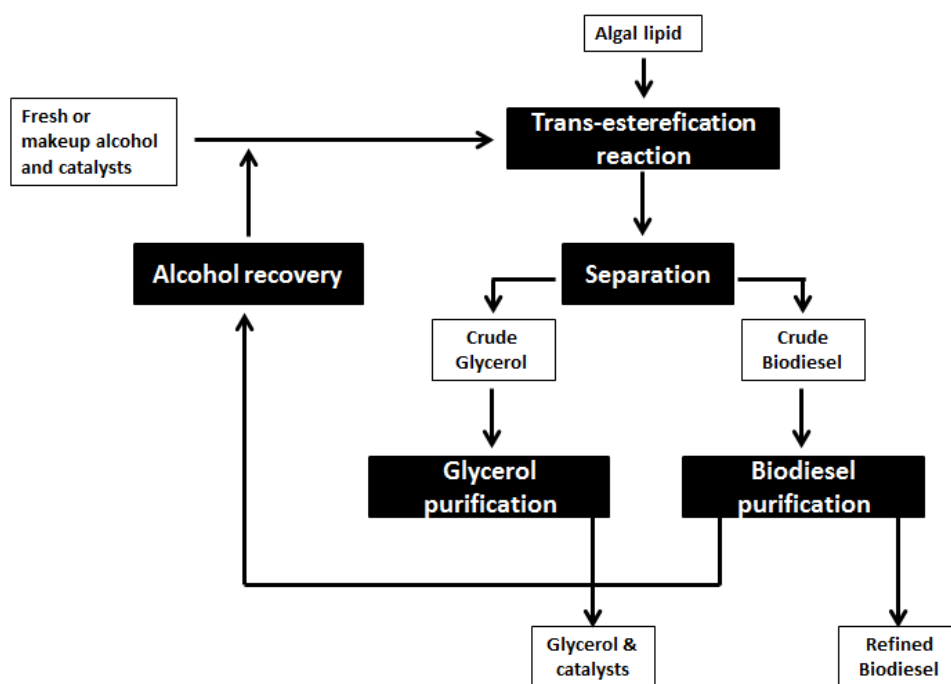


Figure 5: Process of Trans-esterification

Energy efficiency, inexpensive lipid extraction and a low cost trans-esterification process are needed for algal biodiesel to compete with petroleum fuels in a market driven by low costs. Several studies have suggested that the biomass obtained after lipid extraction has been carried out, can be used to generate biogas in a subsequent anaerobic digestion facility. The biogas would then be a separate product, generated in addition from the original algal material without decreasing the yields of biodiesel which can be obtained. If this biogas can also be sold to generate an income, the total costs of production can be reduced, allowing the biodiesel sales price to be reduced also, and allowing biodiesel to compete with petroleum fuels more effectively (88, 142- 145).

3.2.4. The advantages and disadvantages of using micro-algae as a source of diesel-type fuel, compared with deriving this fuel from other biomass sources.

Advantages and disadvantages were summarized from different research studies (79- 81, 126, 133, 146- 148) and listed in (Table 7).

Table 7. The advantages and disadvantages resulting from choosing micro-algae as the starting material for the production of a diesel-type fuel, as opposed to choosing another biomass as the starting material

Advantages	Disadvantages
High oil content in the biomass. For instance there is a high per-acre yield (7–31 times greater than the next best crop—palm oil)	Low biomass concentration at the end of the micro-algal culture
No additional land use	Higher harvesting cost after micro-algal growth is completed
High photosynthetic efficiency	Drying and extraction of oil from biomass, after the micro-algal growth completed, is difficult
High growth rate	
Opportunity to carry out the biofixation of waste CO ₂ when growing the algae.	
Doesn't compete with agricultural land where food products are made.	
Fresh water may not be a requirement	
Easier to provide optimal nutrient concentrations in comparison with growing plants in soil, because the aqueous environment can be mixed well.	

4. Summary:

This paper describes different types of algae, and presents different technologies in making biofuel from them in order to help researchers all over the world to do further investigations in this vital area. The importance of producing biogas and biodiesel from algae was revealed. Popular types of seaweeds and microalgae were described, and their chemical composition, properties, and cultivation techniques were outlined. Anaerobic digestion of seaweeds was investigated in depth, while factors affecting the process and algal biogas were covered. Biodiesel production from microalgae species using the trans-esterification process was explained. Advantages and disadvantages of choosing seaweeds and microalgae as sources of biogas and biodiesel respectively, compared with choosing other biomass feedstock were summarized.

References:

- 1: The world fact book website, <https://www.cia.gov/library/publications/the-world-factbook/geos/xx.html>.
- 2: Ecotricity Website, <https://www.ecotricity.co.uk/our-green-energy/energy-independence/the-end-of-fossil-fuels>.
- 3: Renewable energy can power the world, says landmark IPCC study. [Fiona Harvey](#). Intergovernmental Panel on Climate Change (IPCC). The guardian. <http://www.theguardian.com/environment/2011/may/09/ipcc-renewable-energy-power-world>
- 4: Renewable Energy Futures: Targets, Scenarios, and Pathways. Annual Review of Environment and Resources. Vol. 32: 205-239 (November 2007). Eric Martinot, Carmen Dienst, Liu Weiliang, and Chai Qimin.
- 5: Review of Environmental, Economic and Policy Aspects of Biofuels. The World Bank Development Research Group Sustainable Rural and Urban Development Team September 2007. Deepak Rajagopal and David Zilberman.

- 6: IPCC, 2011: IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation. Prepared by Working Group III of the Intergovernmental Panel on Climate Change [O. Edenhofer, R. Pichs-Madruga, Y. Sokona, K. Seyboth, P. Matschoss, S. Kadner, T. Zwickel, P. Eickemeier, G. Hansen, S. Schlömer, C. von Stechow (eds)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1075 pp.
- 7: Biofuels Make a Comeback Despite Tough Economy. World Watch Institute. <http://www.worldwatch.org/biofuels-make-comeback-despite-tough-economy>.
- 8: Biofuel Website. <http://www.biofuel.org.uk/>.
- 9: Relation between biofuels versus fossil fuels. Luis Cortez and Rogerio Cezar de Cerqueira Leite. Petroleum Engineering_ Downstream. Encyclopaedia of Life Support Systems (EOLSS).
- 10: The Advantages of Biofuels over Fossil Fuels. November 13, 2013. <http://www.carsdirect.com/green-cars/the-advantages-of-biofuels-over-fossil-fuels>.
- 11: Roos, Dave. "Biofuels vs. Fossil Fuels" 20 August 2012. HowStuffWorks.com. <<http://auto.howstuffworks.com/fuel-efficiency/biofuels/biofuel-fossil-fuel.htm>> 03 December 2014.
- 12: Conserve Energy Future. <http://www.conserve-energy-future.com/advantages-and-disadvantages-of-biofuels.php>.
- 13: Biofuels - A Way To Reduce Your Carbon Footprint. Carbon Neutral Earth. <http://www.carbonneutralearth.com/biofuels.php>.
- 14: First Generation Biofuels. Biofuel Website. <http://biofuel.org.uk/first-generation-biofuel.html>.
- 15: Energy from waste and wood. <http://energyfromwasteandwood.weebly.com/generations-of-biofuels.html>.
- 16: UN Report; Sustainable Bioenergy: A Framework for Decision Makers; April 2007
- 17: Climate change Task Force Website: <http://climate.uu-uno.org/topics/view/46923/>.
- 18: Singh, A., Nigam, P.S. et Murphy, J.D., (2011), "Renewable fuels from algae: An answer to debatable land based fuels". Bioresource Technology, Vol.102, pp. 10-16.
- 19: Wei Han, William Clarke, Steven Pratt. Composting of waste algae: A review. Waste Management 34 (2014) 1148–1155.

- 20: Yusuf Chisti. Biodiesel from microalgae. *Biotechnology Advances* 25 (2007) 294–306.
- 21: Ayhan Demirbas and M. Fatih Demirbas. *Algae Energy. Algae as a New Source of Biodiesel*. Springer-Verlag London Limited 2010.
- 22: 28: Lardon 2009. Life cycle assessment of biodiesel production from macroalgae.
- 23: 22: Anders S Carlsson, Jan B van Beilen, Ralf Möller and David Clayton. MICRO- AND MACRO-ALGAE: UTILITY FOR INDUSTRIAL APPLICATIONS. EPOBIO: Realising the Economic Potential of Sustainable Resources - Bioproducts from Non-food Crops © September 2007, CNAP, University of York.
- 24: 23: McHugh DJ (2003) A guide to the seaweed industry. Rome, FAO. FAO Fisheries Technical Paper No. 441.
- 25: 84: Philippe Morand† and Michel Merceron. Macroalgal Population and Sustainability. *Journal of Coastal Research* 21 5 (1009–1020) West Palm Beach, Florida (2005).
- 26: 88: Jaap W. van Hal, W.J.J. Huijgen, and A.M. Lopez-Contreras. Opportunities and challenges for seaweed in the biobased economy. *Trends in Biotechnology*, May 2014, Vol. 32, No. 5.
- 27: 165: The Marine Life, Information Network Website. <http://www.marlin.ac.uk/>
- 28: 166: Fish, J.D. and Fish, S. (1989) A student's guide to the seashore. Unwin Hyman Ltd, London.
- 29: 167: Budd, G.C. & Pizzola, P. (2002) *Enteromorpha intestinalis*. Gut weed. Marine Life Information Network: Biology and Sensitivity Key Information Sub-programme [on-line]. Plymouth: Marine Biological Association of the United Kingdom. (November, 2003)
- 30: 168: The seaweed Site: information on marine algae. <http://www.seaweed.ie/>
- 31: 169: Irish Seaweeds Website: <http://www.irishseaweeds.com/>
- 32: 170: Devil's Apron". Webster's Revised Unabridged Dictionary. C. & G. Merriam Co. 1913.
- 33: 171: Guiry, M. D. ; Guiry, G. M., 2014. **Algaebase. World-wide electronic publication, National University of Ireland, Galway.**
- 34: 172: Abbott, I.A. and Hollenberg, G.J. 1976. *Marine Algae of California*. Stanford University Press. ISBN: 9780804721523.

- 35: 173: Brodie, J.A. and Irvine, L.M. 2003. Seaweeds of the British Isles. Volume 1 Part 3b. The Natural History Museum, London.
- 36: 174: Algae Base Website: <http://www.algaebase.org/>
- 37: 175: Wildscreen Arkive Website: <http://www.arkive.org/>
- 38: 176: Seaweed Industry Association Website: <https://seaweedindustry.com/>
- 39: 26: Luning K, Pang SJ (2003) Mass cultivation of seaweeds: current aspects and approaches. *Journal of Applied Phycology* 15: 115-119.
- 40: 89: Gian Powell B. Marquez, Wilfred John E. Santiañez, Gavino C. Trono Jr, Marco Nemesio E. Montaña, Hiroshi Araki, Hisae Takeuchi, Tatsuya Hasegawa. Seaweed biomass of the Philippines: Sustainable feedstock for biogas production. *Renewable and Sustainable Energy Reviews* 38 (2014) 1056–1068.
- 41: 85: V. K. DHARGALKAR AND NEELAM PEREIRA. SEAWEED : PROMISING PLANT OF THE MILLENNIUM. *SCIENCE AND CULTURE*, VOL. 71, NOS. 3-4 (2005).
- 42: 122: A.F. Bezerra, E. Marinho-Soriano. Cultivation of the red seaweed *Gracilaria birdiae* (Gracilariales, Rhodophyta) in tropical waters of northeast Brazil. *biomass and bioenergy* 34 (2010) 1813- 1817.
- 43: 123: J. Naylor. Production, trade and utilization of seaweeds and seaweed products. *FAO Fish Tech Pap*, 159 (1976).
- 44: 91: Roberts, T. et Upham, T., (2012), “Prospects for the use of macro-algae for fuel in Ireland and the UK: An overview of marine management issues”. *Marine Policy*, Vol.36, pp. 1047–1053.
- 45: 125: Lüning, K., Pang, S.J., Mass Cultivation of Seaweed: Current Aspects and Approaches, *J. Appl. Phycol.*, 2003, vol. 15, pp. 115–119.
- 46: 94: Chynoweth, D.P., (2002), “REVIEW OF BIOMETHANE FROM MARINE BIOMASS”.
- 47: Carpenter RC (1986) Partitioning herbivory and its effects on coral reef algal communities. *Ecol Monogr* 56:345—36
- 48: 92: Sanderson, J.C. et al., (2012), “Culture, yield and bioremediation potential of *Palmaria palmata* (Linnaeus) Weber & Mohr and *Saccharina latissima* (Linnaeus) C.E. Lane,

C. Mayes, Druehl & G.W. Saunders adjacent to fish farm cages in northwest Scotland”. *Aquaculture*, Vol. 354-355, pp. 128–135.

49: 93: Neori, A. et al., (2004), “Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture”. *Aquaculture*, Vol. 231, pp. 361–391.

50: 48: A.L. Ahmad, N.H.M. Yasin, C.J.C. Derek, J.K. Lim. Microalgae as a sustainable energy source for biodiesel production: A review. *Renewable and Sustainable Energy Reviews* 15 (2011) 584–593.

51: 96: N. Rashid, M.S.U. Rehman, S. Memon, Z. Ur Rahman, K. Lee, J.-I. Han. Current status, barriers and developments in biohydrogen production by microalgae. *Renew Sustain Energy Rev*, 22 (2013), pp. 571–579.

52: 97: A.L. Gonçalves, J.C. Pires, M. Simões. Green fuel production: processes applied to microalgae. *Environ Chem Lett*, 11 (2013), pp. 315–324.

53: 98: V.H. Smith, B.S. Sturm, F.J. Denoyelles, S.A. Billings. The ecology of algal biodiesel production. *Trends Ecol Evol*, 25 (2010), pp. 301–309.

54: 99: A.E. Atabani, A.S. Silitonga, I.A. Badruddin, T.M.I. Mahlia, H.H. Masjuki, S. Mekhilef. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renew Sustain Energy Rev*, 16 (2012), pp. 2070–2093.

55: 100: Ana I. Barros, Ana L. Gonçalves, Manuel Simões, José C.M. Pires. Harvesting techniques applied to microalgae: A review. *Renewable and Sustainable Energy Reviews* 41 (2015) 1489–1500.

56: 40: Guschina IA, Harwood JL. Lipids and lipid metabolisms in eukaryotic algae. *Prog Lipid Res* 2006;45:160-186.

57: 41: Hu Q, Sommerfeld M, Jarvis E, Ghirardi ML, Posewitz MC, Seibert M et al. Microalgal triacylglycerols as feedstock for biofuel production: perspectives and advances. *The Plant J* 2008;54:621 639.

58: 60: Dragone, G., Fernandes, B., Vicente, A.A. and Teixeira, J.A. (2010), Third generation biofuels from microalgae in Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Mendez-Vilas A (ed.), Formatex, 1355-1366.

- 59: 61: Koh, L.P., Ghazoul, J. (2008), Biofuels, biodiversity, and people: understanding the conflicts and finding opportunities, *Biological Conservation*, 141:2450-2460.
- 60: 62: Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussnug, J., Posten, C., Kruse, O., and Hankamer, B. (2008), Second generation biofuels: high efficiency microalgae for biodiesel production, *BioEnergy Research*, 1:20-43
- 61: 63: Li, Y., Horsman, M., Wu, N., Lan, C.Q. and Dubois-Calero, N. (2008), Biofuels from microalgae, *Biotechnology Progress*, 24:815-820.
- 62: 101: M.R. Bilad, Hassan A. Arafat, Ivo F.J. Vankelecom. Membrane technology in microalgae cultivation and harvesting: A review. *Biotechnology Advances* 32 (2014) 1283–1300.
- 63: 102: Brennan, P. Owende. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products *Renew Sustain Energy Rev*, 14 (2) (2010), pp. 557–577.
- 64: 103: M. Heasman, J. Diemar, W. O'connor, T. Sushames, L. Foulkes. Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. *Aquaculture Research*, 2000, 31, 637- 659.
- 65: 54: A. Ramadhas, C. Muraleedharan, S. Jayaraj. Performance and emission evaluation of a diesel engine fueled with methyl esters of rubber seed oil. *Renewable Energy* 30 (2005) 1789–1800.
- 66: 71: Ward RL, Jeffries TW, Timourian H. Methane from algae Grown in Desert regions of California using available Ground Waters. Workshop on energy Resources Conservation, Sacramento, California, USA. July 1977. Report No. UCRL-79716
- 67: 105: T.J. Lundquist, I.C. Woertz, N.W.T. Quinn, J.R. Benemann. A realistic technology and engineering assessment of algae biofuel production. Energy Biosciences Institute, Berkeley, California (2010).
- 68: 106: C.M. Beal, R.E. Hebner, E.W. Michael, S.R. Rodney, A.F. Seibert, C.W. King. Comprehensive evaluation of algal biofuel production: experimental and target results. *Energies*, 5 (2012), pp. 1943–1981.
- 69: 107: A. Darzins, P.T. Pienkos, L. Edye. Current status and potential for algal biofuels production. *IEA Bioenergy Task*, 39 (2010), pp. 1–131.

- 70: 108: Guanyi Chen, Liu Zhao, Yun Qi. Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: A critical review. *Applied Energy* 137 (2015) 282–291.
- 71: 119: L. Jiang, S. Luo, X. Fan, Z. Yang, R. Guo. Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO₂. *Appl Energy*, 88 (2011), pp. 3336–3341.
- 72: 120: M.K. Lam, K.T. Lee, A.R. Mohamed. Current status and challenges on microalgae-based carbon capture. *Int J Greenh Gas Con*, 10 (2012), pp. 456–469.
- 73: 121: N. Hempel, I. Petrick, F. Behrendt. Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. *J Appl Phycol*, 24 (2012), pp. 1407–1418.
- 74: 109: W. Bank. *World Development Indicators 2012* World Bank Publications (2012).
- 75: 110: D. Obaja, S. MacÉ, J. Mata-Alvarez. Biological nutrient removal by a sequencing batch reactor (SBR) using an internal organic carbon source in digested piggery wastewater. *Bioresour Technol*, 96 (2005), 7–14.
- 76: 111: N.N. Bich, M.I. Yaziz, N.A.K. Bakti. Combination of *Chlorella vulgaris* and *Eichhornia crassipes* for wastewater nitrogen removal. *Water Res*, 33 (1999), 2357–2362.
- 77: 112: S.S. Ahluwalia, D. Goyal. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour Technol*, 98 (2007), 2243–2257.
- 78: 51: Ayhan Demirbas. Use of algae as biofuel sources. *Energy Conversion and Management* 51 (2010) 2738–2749.
- 79: 55: Ting Cai, Stephen Y. Park, Yebo Li. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews* 19 (2013) 360–369.
- 80: 56: H.M. Amaro, A.C. Macedo, F.X. Malcata. Microalgae: an alternative as sustainable source of biofuels? *Energy* 44 (2012) 158–166.
- 81: 57: T. Wang, H. Yabar, Y. Higano. Perspective assessment of algae-based biofuel production using recycled nutrient sources: the case of Japan. *Bioresource Technology* 128 (2013) 688–696.

- 82: 115: M.K. Lam, K.T. Lee, A.R. Mohamed. Current status and challenges on microalgae-based carbon capture. *Int J Greenh Gas Con*, 10 (2012), pp. 456–469.
- 83: 118: Bing Hu & Min Min & Wenguang Zhou & Yecong Li & Michael Mohr & Yanling Cheng & Hanwu Lei & Yuhuan Liu & Xiangyang Lin & Paul Chen & Roger Ruan. Influence of Exogenous CO₂ on Biomass and Lipid Accumulation of Microalgae *Auxenochlorella protothecoides* Cultivated in Concentrated Municipal Wastewater. *Appl Biochem Biotechnol* (2012) 166:1661–1673.
- 84: 116: Fadhil M. Salih. Microalgae Tolerance to High Concentrations of Carbon Dioxide: A Review. *Journal of Environmental Protection*, 2011, 2, 648-654.
- 85: 117: P Chelf, L.M Brown, C.E Wyman. Aquatic biomass resources and carbon dioxide trapping. [Biomass and Bioenergy](#). Volume 4, Issue 3, 1993, Pages 175–183.
- 86: 24: James, M.A., (2010) A review of initiatives and related R&D being undertaken in the UK and internationally regarding the use of macroalgae as a basis for biofuel production and other non-food uses relevant to Scotland. Report commissioned by the Marine Scotland, 79pp. 24: <http://www.scotland.gov.uk/Resource/Doc/295194/0115064.pdf>
- 87: 67: Anon. (2010). Oilgae Guide to Fuels from Macroalgae. A Report by Oiigae. 74pp – source <http://www.oilgae.com/>
- 88: 34: Sialve, B., Bernet, N., Bernard, O. *Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnology Advances* 27 (2009) 409–416.
- 89: 72: E.A. Ehimen, J.-B. Holm-Nielsen, M. Poulsen, J.E. Boelsmand. Influence of different pre-treatment routes on the anaerobic digestion of a filamentous algae. *Renewable Energy* 50 (2013) 476e480.
- 90: "Methane (CHEBI:16183)". Chemical Entities of Biological Interest. UK: European Bioinformatics Institute. October 17, 2009. Retrieved October 10, 2011.
- 91: 126: D. Deublein, A. Steinhauser. Biogas from waste and renewable sources: an introduction. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (2008).
- 92: 127: R. Chandra, H. Takeuchi, T. Hasegawa. Methane production from lignocellulosic agricultural crop wastes: a review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews* 16 (2012) 1462–1476

- 93: 68: D. Deublein, A. Steinhauser. Biogas from waste and renewable resources: an introduction. Wiley-Vch Verlag GmbH & Co. KGaA, Boschstr. 12 69469 Weinheim (2011).
- 94: 31: Ye Chen, Jay J. Cheng *, Kurt S. Creamer. Inhibition of anaerobic digestion process: A review. Bioresource Technology 99 (2008) 4044–4064.
- 95: 69: D. Rutz, R. Janssen. Biofuel technology handbook @WIP Renewable Energies Version 1, February 2007.
- 96: 142: Pavlo Bohutskyi and Edward Bouwer. Chapter 36 Biogas Production from Algae and Cyanobacteria Through Anaerobic Digestion. Advanced Biofuels and Bioproducts 2013, pp 873-975.
- 97: 81: Michael Biarnes. Biomass to Biogas- Anaerobic Digestion. 2014 Instruments International, LLC. <http://www.e-inst.com/biomass-to-biogas/>
- 98: 82: A. K. Kurchania. Chapter 2: Biomass Energy. Biomass Conversion. The interface of Biotechnology, Chemistry and Materials science. <http://www.springer.com/engineering/energy+technology/book/978-3-642-28417-5>
- 99: 83: Silvia Tedesco. Mechanical Pre-treatment Assessment of Marine Biomass. School of Mechanical and Manufacturing Engineering, Dublin City University (2013).
- 100: 87: Swedish Biogas Association (2004) Biogas – renewable energy from organic waste, brochure.
- 101: Williams, A.G., Withers, S. and Sutherland, A.D.* (2013) The potential of bacteria isolated from ruminal contents of seaweed-eating North Ronaldsay Sheep to hydrolyse seaweed components and produce methane by anaerobic digestion *in vitro*. Microbial Biotechnol. Vol 6 (1) p45-52.
- 102: 132: Migliore, G., et al. (2012), “Anaerobic digestion of macroalgal biomass and sediments sourced from the Orbetello lagoon, Italy”. Biomass and Bioenergy, Vol. 42, pp. 69-77.
- 103: 133: Tedesco, S., Benyounis, K. Y. et Olabi, A. G., (2013), “Mechanical pretreatment effects on macroalgae-derived biogas production in co-digestion with sludge in Ireland”. Energy, Vol. 61, pp. 27-33.

- 104: 131: Vanegas, C. et Bartlett, J., (2013), "Anaerobic Digestion of *Laminaria digitata*: The Effect of Temperature on Biogas Production and Composition". *Waste Biomass Valor*, Vol. 4, pp. 509–515.
- 105: 128: Romagnoli, F., Blumberga, D. and Gigli, E., (2010), "Biogas from Marine Macroalgae: a New Environmental Technology—Life Cycle Inventory for a Further LCA", *Scientific Journal of Riga Technical University. Environmental and Climate Technologies*, Vol.4 (1), pp. 97-108.
- 106: 129: Angelidaki, I. et Sanders, W., (2004). "Assessment of the anaerobic biodegradability of macropollutants". *Reviews in Environmental Science and Bio/Technology*, Vol. 3, pp. 117-129.
- 107: 143: Khanal SK (2008) *Anaerobic biotechnology for bioenergy production: principles and applications*. Wiley- Blackwell, Ames.
- 108: 146: Richa Kothari, A.K. Pandey, S. Kumar, V.V. Tyagi, S.K. Tyagi. Different aspects of dry anaerobic digestion for bio-energy: An overview. *Renewable and Sustainable Energy Reviews* 39 (2014) 174–195.
- 109: 147: Liu C, Yuan X, Zeng G, Li W, Li J. Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresour Technol* 2008; 99:882–8.
- 110: 148: Xiao-Shuang Shi, Xian-Zheng Yuan, Yu-Ping Wang, Shu-Juan Zeng, Yan-Ling Qiu, Rong-Bo Guo, Li-Sheng Wang. Modeling of the methane production and pH value during the anaerobic co-digestion of dairy manure and spent mushroom substrate. *Chemical Engineering Journal* 244 (2014) 258–263.
- 111: 75: Cecchi F, Pavan P, MataAlvarez J: **Anaerobic co-digestion of sewage sludge: application to the macroalgae from the Venice lagoon**. *Resources, Conservation and Recycling* Volume 17, Issue 1, July 1996, Pages 57–66.
- 112: 134: R. Chandra, H. Takeuchi and T. Hasegawa. (2011) Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable and Sustainable Energy Reviews* 16(3), pp. 1462-1476.
- 113: 135: H. Yen and D. E. Brune. (2007) Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour. Technol.* 98(1), pp. 130-134.

- 114: 136: Weizhang Zhong, Zhongzhi Zhang, Yijing Luo, Wei Qiao, Meng Xiao, Min Zhang. Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. *Bioresource Technology* 114 (2012) 281–286.
- 115: 137: V. Vivekanand, V. G. Eijsink and S. J. Horn. (2012) Biogas production from the brown seaweed *saccharina latissima*: Thermal pretreatment and codigestion with wheat straw. *J. Appl. Phycol.* 24(5), pp. 1295-1301.
- 116: 138: E. Ehimen, et al. (2011) Anaerobic digestion of microalgae residues resulting from the biodiesel production process. *Appl. Energy* 88(10), pp. 3454-3463.
- 117: 139: Biogas from Waste and Renewable Resources. An introduction. - Dieter Deublein and Angelika Steinhauser (2008).
- 118: 140: Carbon/nitrogen-ratio effect on fermentative hydrogen production by mixed microflora - C.Y. Lin, C.H. Lay (2003)
- 119: 141: Effects of temperature, salinity, and carbon: nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater - Q. Fontenot, C. Bonvillain, M. Kilgen, R. Boopathy (2006)
- 120: 150: Soto, M., Méndez, R. and Lema, J., (1993), "Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental set up", *Water Res.*, Vol.27 (8), pp. 1361-1376.
- 121: 76: Muhammad Rizwan Tabbsum, Umer Rashid, Muhammad Ibrahim, Saba Shahid Gill, Muhammad Aamer Mehmood. Marine Macro Algae ULVA: A Potential Feed-stock For Bio-ethanol and Biogas Production. *Asian J Agri Biol*, 2013, 1(3): 155- 163.
- 122: 77: Romagnoli F., Blumberga D., Gigli E. Biogas from Marine Macroalgae: a New Environmental Technology – Life Cycle Inventory for a Further LCA // *Scientific Journal of RTU*. 13. series., *Vides un klimata tehnoloģijas*. - 4. vol. (2010), pp 97-108.
- 123: 73: **Adam D Hughes, Maeve S Kelly, Kenneth D Black, and Michele S Stanley.** Biogas from Macroalgae: is it time to revisit the idea?. *Biotechnology for Biofuels* 2012.
- 124: 79: Shiplu Sarker, Anette Bruhn, Alastair James Ward, Henrik Bjarne Moller. BIO-FUEL FROM ANAEROBIC CO-DIGESTION OF THE MACRO-ALGAE ULVA LACTUCA AND LAMINARIA DIGITATA. *Renewable Energy and Energy Efficiency*, 2012 Biogas and biofuel production technologies.

- 125:** 80: Sanjeev Kumar Prajapati, Prachi Kaushik, Anushree Malik, Virendra Kumar Vijay. Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: Possibilities and challenges. *Biotechnology Advances* 31 (2013) 1408–1425.
- 126:** 43: Ayhan Demirbas. Progress and recent trends in biodiesel fuels. *Energy Conversion and Management* 50 (2009) 14–34.
- 127:** 44: A.E. Atabani, A.S. Silitonga, I.A. Badruddin, T.M.I. Mahlia, H.H. Masjuki, S. Mekhilef. A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renewable and Sustainable Energy Reviews* 16 (2012) 2070–2093.
- 128:** 45: I.M. Atadashi, M.K. Aroua, A.A. Aziz. High quality biodiesel and its diesel engine application: a review. *Renewable and Sustainable Energy Reviews* 14 (2010) 1999–2008.
- 129:** 46: J.V. Gerpen. Biodiesel processing and production. *Fuel Processing Technology* 86 (2005) 1097 – 1107.
- 130:** 47: A.S. Silitonga, A.E. Atabani, T.M.I. Mahlia, H.H. Masjuki, I.A. Badruddin, S. Mekhilef A review on prospect of *Jatropha curcas* for biodiesel in Indonesia. *Renewable and Sustainable Energy Reviews* 15 (2011) 3733–3756.
- 131:** 49: Sevil Yücel, Pınar Terzioğlu, Didem Özçimen. Lipase applications in biodiesel production, biodiesel – feedstocks, In: Fang P.Z. (Ed.), *Production and applications*. 2012. ISBN: 978-953-51-0910-5, InTech.
- 132:** 53: Ali Bahadar, M. Bilal Khan. Progress in energy from microalgae: A review. *Renewable and Sustainable Energy Reviews* 27 (2013) 128–148.
- 133:** 42: M.Y. Noraini, Hwai Chyuan Ong, Mohamed Jan Badrul, W.T. Chong. A review on potential enzymatic reaction for biofuel production from algae. *Renewable and Sustainable Energy Reviews* 39 (2014) 24–34
- 134:** 36: Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of Energy's aquatic species program—biodiesel from algae. NREL/TP-580- 4190. U.S. Department of Energy's Office of Fuels Development.
- 135:** 37: Dijkstra AJ (2006) Revisiting the formation of trans isomers during partial hydrogenation of triacylglycerol oils. *European Journal of Lipid Science and Technology* 108: 249-264.

- 136: 35: Sharif Hossain, A.B.M., A. Salleh, A.N. Boyce, P. chowdhury and M. Naquiuddin, 2008. Biodiesel Fuel Production from Algae as Renewable Energy. Am. J. Biochem. Biotechnol., 4: 250-254.
- 137: 155: NIRAJ S. TOPARE, SUNITA J. RAUT, V. C. RENGE, SATISH V. KHEDKAR, Y. P. CHAVAN and S. L. BHAGAT. EXTRACTION OF OIL FROM ALGAE BY SOLVENT EXTRACTION AND OIL EXPELLER METHOD. Int. J. Chem. Sci.: 9(4), 2011, 1746-1750.
- 138: 157: Solvent Extraction. SRS International Corporation.
<http://www.srsbiodiesel.com/technologies/solvent-extraction/>
- 139: 153: Biodiesel Production. National Biodiesel Board. <http://www.biodiesel.org/docs/ffs-production/production-fact-sheet.pdf?sfvrsn=4>
- 140: A. Demirbas, Biofuels, Chapter 4: Biorenewable Liquid Fuels. © Springer 2009.
- 141: 39: Altun S. Performance and emission characteristics of a diesel engine fuelled with biodiesel obtained from a hybrid feedstock. Energy Educ Sci Technol Part A 2011;27:79–86.
- 142: 29: Patrick E. Wiley, J. Elliott Campbell and Brandi McKuin. Production of biodiesel and biogas from algae: a review of process train. Water Environment Research, Volume 83, Number 4.
- 143: 64: Stephen Park, Yebo Li. Evaluation of methane production and macronutrient degradation in the anaerobic co-digestion of algae biomass residue and lipid waste. Bioresource Technology 111 (2012) 42–48.
- 144: 65: Anoop Singh, Poonam Singh Nigam, Jerry D. Murphy. Renewable fuels from algae: An answer to debatable land based fuels. Bioresource Technology 102 (2011) 10–16.
- 145: 66: B.G. Subhadra, M. Edwards. Coproduct market analysis and water footprint of simulated commercial algal biorefineries. Applied Energy 88 (2011) 3515–3523.
- 146: 50: Yanqun Li, Mark Horsman, Nan Wu, and Christopher Q. Lan, Nathalie Dubois-Calero. Biofuels from Microalgae. Biotechnol. Prog. 2008, 24, 815- 820.
- 147: 58: P. Nautiyal, K.A. Subramanian, M.G. Dastidar. Production and characterization of biodiesel from algae. Fuel Processing Technology 120 (2014) 79–88.

148: 59: A. Kirrolia, N.R. Bishnoi, R. Singh. Microalgae as a boon for sustainable energy production and its future research & development aspects. *Renewable and Sustainable Energy Reviews* 20 (2013) 642–656.