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## 1 Introduction

In addition to the currently produced bioethanol and biodiesel, the term “biofuels” covers a wider array of fuels derived from biomass, which potentially includes all possible pathways and end products provided that they can be used as a fuel, e.g. solid biomass, liquid fuels and biogas (Demirbas 2009). The same approach is found in the Renewable Energy Directive (2009/28/EC), where biofuels are defined as “*liquid or gaseous fuel for transport produced from biomass*”. This definition also reflects a specific definitional aspect of biofuels: biofuels implicitly refer to transport fuels, as opposed to fuels used in boilers, heating or power stations. For the purpose of this report, only transport fuels derived from biomass will be considered.

The term biomass probably covers the most diverse range of substances and compounds known to man, as it refers to the mass of all living organisms for a given unit of time and space. This indistinct approach of a multitude of living organisms has been increasingly used to describe the wide array of raw materials potentially convertible into renewable energy. Some of them, like the sugar and starch in corn grains or the vegetable oils from oilseeds, can be readily used for biofuel manufacturing, while others, like lignin, are very difficult to break down into its usable biofuels feedstock. Biomass is defined as the biodegradable fraction of products, waste and residues of biological origin, taking the widest possible approach, which includes all possible materials. Therefore, biomass covers materials from both animal and vegetable origin, i.e. all products usually referred to as “algae” for convenience, including microorganisms not classified as vegetable organisms.

Bioethanol and biodiesel production belong to the tried and tested biofuels production pathways. The conversion of sugar (sugar cane) or starch (corn/wheat grain) into bioethanol, initially intended for alcohol distillation has been used at large scale in the food and industrial sectors for decades. Similarly, the reaction of oils and fats with lower alcohols yielding free glycerol and the fatty acid ester of the alcohol used has been mastered since the XIX<sup>th</sup> century (Mittelbach, 2004). This situation has led to the term “first-generation” biofuels, to which “second generation” biofuels are opposed. Although “first generation biofuels” is generally understood as covering biofuels produced from biomass also used for food and feed, there is no scientific definition for “first generation biofuels” (Knothe, 2009). The artificial nature of the classification in “generations” is particularly clear with algae technologies, as “third generation” is used to describe algae-based fuels without support from a precise concept.

Despite the technological reference implied with the term “generation”, “generations” are not necessarily linked with innovative technologies. In addition, the term “generation” is disconnected from market availability as some advanced biofuels are currently produced economically viable at large scale, while

certain first generation biofuels are not, e.g. sunflower biodiesel. Similarly, “generations” do not reflect the sustainability of the fuels produced, as certain advanced biofuels pathways can emit more greenhouse gas and be linked to greater environmental concerns than the biofuels pathways currently in use, as is the case for hydro-treated vegetable palm oil when compared with rapeseed biodiesel. Therefore, it is only safe to claim that algae can allow the production of advanced biofuels and that algae mainly represents an alternative feedstock to current biofuel production pathways.

There can be environmental benefits common to most algae biofuels. Algae have a low pressure on arable land and the high sunlight conditions in infertile, dry areas may paradoxically make them a perfect location for algae cultivation. Large scale cultivation of (micro) algae in closed systems is a technically challenging issue, however, which still needs to be resolved. Algae may also be cultivated in open systems or even harvested from the wild, which is of course readily feasible.

Refining algae into components that can be easily converted to biofuel feedstock is the main challenge. Several options are available, which each yield a different type of biofuel (e.g. biodiesel, bioethanol, biogas, etc). This paper will describe the production processes for the different types of biofuels and which sources of aquatic biomass are most suitable for which biofuel type. A final table summarizes the respective advantages and drawbacks of the respective pathways.

## 2 Types of algae and aquatic biomass for biofuels

Aquatic biomass relevant for biofuel production mainly concerns algae, which can be divided in macro- and microalgae. Other aquatic plants are also sometimes referred to as possible sources of biomass, but in general only algae are considered to have the potential to yield biofuels in large enough quantities for a realistic alternative to fossil fuel sources.

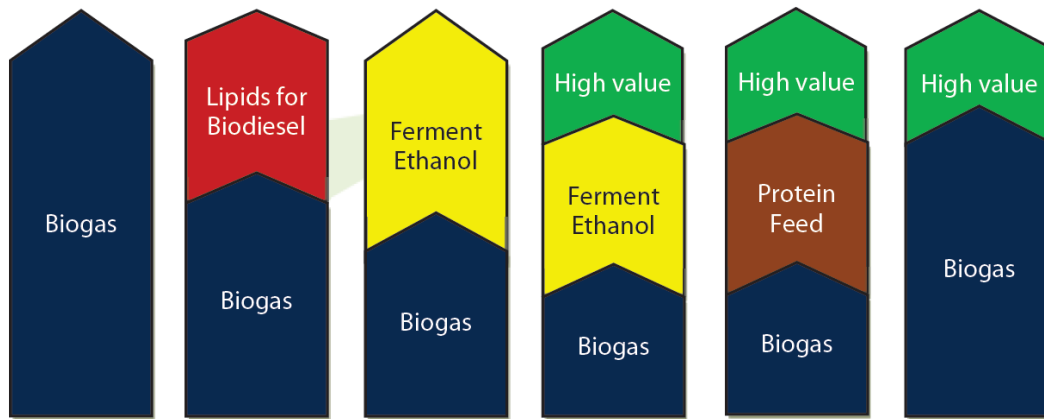
The selection of a suitable organism or strain for biofuel manufacturing depends on many aspects, which are covered in D1.4. In general the type of biofuel that can be produced from the biomass is dictated by the composition of the biomass. For biofuel production the ratio between starch (including sugar), lipids and protein is very important. Table 1 shows the relationship between biomass components and the type of biofuel that can be made with these components.

Type of biofuel \ Type of biomass	Biodiesel	Bioethanol	Biogas	BTL	Hydrogen
Whole biomass			x	x	x
Lipids	x				x
Starch/sugar		x			x
Proteins			x	x	x

\*BTL = Biomass to Liquid. A technique to generate bio oil pyrogenically from biomass

**Table 1. Relationship between biomass components and the relevant type of biofuel that can be produced from it**

This means that for instance a strain or species with high lipid content will preferably be used for biodiesel production, while high starch is better suitable for bioethanol. Combinations are also possible, in which for instance the lipid fraction is used for biodiesel, while the remaining biomass is fermented for biogas production. Some species produce high value compounds (i.e. pigments in microalgae or alginate in macroalgae), which can be isolated and sold separately. Valorization of as many biomass components as possible is crucial to reduce the price of biofuels to the level of fossil fuels (Fig. 1) (Wijffels, 2010).



**Figure 1. Different biofuel production strategies for algal biomass. The optimal usage of the biomass is highly dependent on its composition**

Besides the content of lipids, starch and proteins, other factors are important as well. Water content is very important, especially for pyrogenic techniques. The saline (for salt water algae) or sulphate content is important for fermentation-based techniques because these substances may inhibit growth of microorganisms. Ash content is relevant for the use of the waste material after combustion or fermentation as soil fertilizer. Finally, cellulose and lignine are major components of the biomass that need (bio) chemical conversion steps before they can be used for biofuel (bioethanol) production. Valorization of these components is crucial for biofuels to be competitive as compared to fossil fuels, but also increases the technical difficulty and cost of the overall process. Further details regarding the different biofuel production techniques and their specific demands for biomass composition will be discussed in more detail below.

## 2.1 Macroalgae

Macroalgae for biofuel purposes are mainly harvested from the wild. Cultivation of seaweed does occur, mainly in Asian countries, but this is usually for higher value purposes such as food, or hydrocolloid (i.e. agar-agar and alginate) production. Traditionally harvest is being done by hand, but this is now largely replaced by mechanic harvesting using trawler systems. Several designs and pilot systems have been developed for floating cultivation systems (farms) in open sea, some with surface areas up to 4000 ha, but no full scale systems have been put in operation so far and their economic feasibility remains doubtful. This is certainly true for (partly) closed systems for macroalgae cultivation, which are therefore non-existent.

The world potential of harvesting macroalgae in coastal areas ranges between 10 and 100 million tones per year. Intensive harvesting from the wild is potentially harmful to ocean life when not regulated properly (Bruton et al., 2009).

## 2.2 Microalgae

Microalgae can be harvested from the wild by harvesting natural blooms. This is a highly unpredictable and uncertain source of biomass and can only be exploited when a good opportunity occurs. In general, microalgae for biofuel purposes are cultivated, either in open pond systems or closed photobioreactor systems in order to reach the densities necessary for biofuel production. Open ponds are well established, low tech systems that can yield fairly large quantities of microalgae. These can either be large natural ponds or the so called “race way ponds” in which the algae are actively mixed and gassed in order to increase the productivity. The disadvantages of this system include contamination with other microorganisms or other species of microalgae, low productivity at higher densities. In order to avoid contamination, rather extreme cultivation conditions are chosen (i.e. high pH or high salinity), which limits the choice for which strain of algae can be used to only a handful.

To be able to grow other species of algae and to increase areal productivity a closed reactor system is required. The photobioreactor technology is still at its infancy, especially for larger scale facilities. The biggest systems currently in operation are in the range of 1-10 hectares, for instance the 4-hectare Solix Biofuels plant in Durango (Colorado, USA). In order to be able to compete with other sources of biodiesel, much larger facilities are required with a ground surface of several hundreds hectares.

High growth rate, high photosynthetic efficiency, relatively high content of energy-rich chemicals on one side and experience with large-scale culture and downstream processing technologies concentrate in last decades increasing attention on microalgae as a feedstock for biofuels, presently produced costly from crop plants containing starch (corn, wheat) or sucrose (sugarcane, sugar beet) for production of bioethanol and lipids (rapeseed oil, soybean oil, palm oil) for production of biodiesel. Today projects dealing with algae are focused almost entirely on biodiesel production. Nevertheless, algal strains containing higher amount of lipids are characterized by low growth rate. Slow growth increases the operational costs and demands cultivation in closed bioreactors entailing high infrastructure costs.

It is also possible to produce ethanol from microalgae. One possibility is to produce starch in *Chlorella*. The starch can be converted into ethanol via fermentation in yeasts. Commonly used way of enhanced starch amount in algal cell is cultivation under nitrogen or sulfur limitation; protein synthesis is inhibited and starch, as a energy reserve for metabolic processes is for some time accumulated in the cell. In this way, using the outdoor thin-layer solar photobioreactor, an increase of starch content in sulfur-limited culture up to 50% of algal biomass DW was attained (Branyiková et al., 2010). Ethanol production can also directly

be done in cyanobacteria. Ethanol synthesis pathways have been introduced in cyanobacteria to ferment pyruvate into ethanol in several initiatives such as the Algenol process (Wald, 2009).

The current cost of algal biomass production is not lower than 5 US \$ kg<sup>-1</sup> (Rodolfi et al., 2009). For economical production of bioethanol or biodiesel, it should be at least one order of magnitude lower.

Under favourable climate conditions, yearly yields up to 100 tons dried algal biomass are possible (Borowitzka, 1999), (Rodolfi et al., 2009) resulting thus in further decrease of production cost.

Whilst about 3.000 L bioethanol can be obtained from 1 ha corn, suitable *Chlorella* strains can by protein synthesis control provide more than 50 t starch for production of 25.000-30.000 L bioethanol. The remaining parts of the cell, containing largely proteins, can be used as a feed supplement what further decreases the cost of bioethanol production.

Lipids for production of biodiesel are promising as well. It is expected that per ha of land area 20.000-60.000 liter of lipids can be produced. In comparison via oil palms, 6.000 liter of lipids can be produced (Wijffels and Barbosa, 2010).

### 3 Types of Biofuel and their typical production processes

Biofuels are generally understood as liquid transport fuels produced from biomass. Currently, 75% of transport fuels consumed in the EU are diesel, while petrol represents 25% of the market. Due to the characteristics biodiesel and bioethanol, petrol can be substituted with bioethanol and diesel with biodiesel, thus forming two separate markets for biofuels. However, several types of biofuels can compete on the market for diesel substitution (biodiesel, hydro-treated vegetable oils, Biomass-to-Liquids), while petrol can be substituted by bioethanol, but also by biobutanol. However, research should not exclude other possible uses as transport fuels less economically viable, as hydrogen or pyrolysis, or fuels that are not necessarily intended for transport, e.g. biogas.

#### 3.1 Biodiesel

Biodiesel is a biofuel that is based on the conversion of vegetable or animal fats and oils into a fatty acid methyl ester of the respective fat or oil. In the transport sector, biodiesel may be effectively used both blended with mineral diesel (i.e. petroleum/conventional diesel) and in its pure form. Tests undertaken by motor manufacturers in the European Union on conventional diesel-biodiesel blends of 5-10%, 25-30%

blends and 100% pure have resulted in guarantees for all uses. Minor modifications to conventional engines (seals, piping) are required for use at 100% pure.

The use of biodiesel as a transport fuel does not require any modification in the distribution system, therefore avoiding expensive infrastructure changes, which has greatly favoured its market penetration. In addition, biodiesel contains 83% to 88% of the energy content of petroleum diesel, making it a very energy-efficient fuel. Biodiesel is better combusted in engines due to its higher oxygen content and higher cetane numbers. As an ester, it has a low vapour pressure and increases fuel lubricity and therefore helps to keep engines cleaner than with fossil diesel. From an environmental point of view, it is less polluting than fossil diesel as it is almost free of sulphur, contains fewer aromatic hydrocarbons and emits less particulate matters and also because it can emit up to 86% greenhouse gas less than fossil diesel. Biodiesel is biodegradable within 28 days, greatly reducing the impact on environment linked to a potential “biodiesel spill”. Biodiesel contains almost no carcinogens and is much safer to store than fossil diesel, making it a better fuel for health and safety reasons (EBB, 2008).

It consists of mono alkyl (i.e. methyl) esters of long chain, preferably highly saturated fatty acids, often referred to as Fatty Acid Methyl Esters or (FAME). The formation of FAME involves a rather simple chemical conversion in which triglycerides react in the presence of a catalyst with a short-chain alcohol (usually methanol), resulting in free glycerol and three FAME molecules (Fig 2).

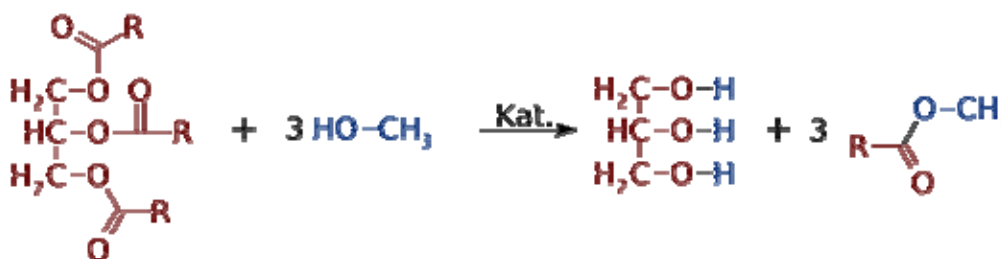


Figure 2. The reaction of a triglyceride with methanol to yield biodiesel

This reaction depends on the type of triglyceride available (saturation and length of alkyl chains), free fatty acid content, the type of alcohol used, the type of catalyst used and the reaction temperature.

Vegetable oil is first extracted from an oilseed, e.g., soybean or rapeseed, and is then delivered to the reactor for transesterification. There are three well-known methods to extract the oil from algae: (1) expeller/press, (2) solvent extraction with hexane, and (3) supercritical fluid extraction, all of them requiring a dry raw material. A simple process is to use a press to extract a large percentage (70–75%) of the oils out of algae. Algal oil can be extracted using chemicals. The most popular chemical for solvent extraction is hexane, which is relatively inexpensive. Supercritical fluid extraction is far more efficient than

traditional solvent separation methods. Supercritical fluids are selective, thus providing the high purity and product concentrations (Paul and Wise, 1971). This can extract almost 100% of the oils all by itself. In the supercritical fluid carbon dioxide (CO<sub>2</sub>) extraction, CO<sub>2</sub> is liquefied under pressure and heated to the point that it has the properties of both a liquid and gas. This liquefied fluid then acts as the solvent in extracting the oil.

Transesterification is the term used to describe the class of organic reactions wherein an ester is transformed into another through the interchange of alkoxy moiety, allowing to convert the vegetable oil into biodiesel. Biodiesel production is based on the transesterification reaction between triglycerides (TG) and methanol, in the presence of a catalyst, producing FAME with glycerol as a by-product. The reaction occurs in stages, with mono- and diglycerides (MG and DG) resulting as intermediate products. The most commonly used catalysts in the industry are basic homogeneous catalysts. In a conventional industrial biodiesel production process, a mixture of methanol and vegetable oil with a molar ratio of 6:1 is applied to carry out the transesterification reaction, resulting in FAME production within 1 hour of reaction time, using NaOH as the catalyst. As the solubility of the oil in methanol is low, two phases are present during such processes. Therefore, stirring (at about 200 rpm) and high temperatures (60°C) are necessary to guarantee acceptable reaction kinetics. In addition, an alkoxy formed from the NaOH and methanol mixture is used as a homogeneous catalyst (Marchetti et al., 2007). After the transesterification reaction is complete, the mixture is allowed to settle under gravity. The heavier glycerol settles in the bottom layer, whereas the upper layer is made up of the FAME. The glycerol is separated, and the FAME is washed with water to remove the catalyst. In addition, a neutralization step is required to handle sodium soap residues from FAME production. The excess methanol present in FAME is recovered by distillation, whereas the glycerol may be refined for further use or directly discarded.

In the two steps process, hexane is efficient in oil extraction and alkali-catalyzed transesterification, which is advantageous in high speed (a matter of seconds or minutes) and mild heating conditions (60°C). However, three energy-intensive operations are involved: hexane vacuum evaporation, process heating, and stirring during transesterification. Among the three operations highlighted, stirring may not be as costly as process heating or methanol recovery in a standard biodiesel production process; however, no stirring or a better manner of stirring should be considered as a potential process cost-saving strategy. The reason why these costly operations are required is that, on the one hand, hexane is methanol-immiscible, and so should be removed from crude oil before adding methanol for transesterification; on the other hand, methanol is oil-immiscible, and so needs stirring to homogenize the reactants during transesterification.

### 3.1.1 Homogeneous acid and base catalysts

In using alternative raw materials with a high acid value, alkaline catalysts react with FFA to produce soap in a secondary reaction during transesterification. This soap remains in the glycerol and in the biodiesel phase, and requires necessary yet expensive glycerol purification methods and processes for biodiesel washing. Therefore, basic catalysts are not suitable for producing esters from alternative raw materials containing FFA higher than 1 mg/g oil (Parawira, 2009). Acid catalysts are suitable for producing esters from alternative raw materials. Using sulfuric acid (as well as other acids as catalysts), FFA can be esterified to FAME without soap formation. Acid catalysts can also be used as a pretreatment step, before the alkaline reaction. In addition, TG can react with methanol to produce biodiesel and glycerol in a transesterification reaction using acid catalysis. But acid catalysis is less favorable than the than the commercially applied alkaline catalysis using NaOH because of the far longer reaction times and the high costs of reaction equipment (Zhang et al., 2003).

In addition, both acid and basic homogeneous catalysts require neutralization and separation from the reaction mixture using large amounts of energy used and wastewater generation (Suehara et al., 2005), (Barnwal and Sharma, 2005).

### 3.1.2 Heterogeneous catalysts

Transesterification using heterogeneous has been carried out in an effort to lower cost and produce biodiesel in a more environmentally friendly manner. Heterogeneous catalysts have two important advantages in comparison with homogeneous ones: easy removal of the catalysts from products and their reuse without expensive treatments. The production of pure glycerin also enhances the process feasibility. Several of these catalysts have been evaluated, but leaching of soluble compounds from the catalysts into liquid products has been reported (Kouzu et al., 2009), (Noiroj et al., 2009). However, the insufficient activity of cheap heterogeneous catalysts and the high cost of enzyme-incorporated active ones have delayed their commercial application.

### 3.1.3 Biocatalysts

To produce biodiesel in a cleaner way, biocatalysts are more preferred to chemical catalysts. The use of biological catalysts such as purified lipases or whole cells (from lipase-producing microorganisms) does not

result in soap formation when alternative raw materials with high acid value are used. In addition, glycerol can easily be recovered, and the purification of FAME is simplified. In fact, immobilized catalysts can be easily separated and removed from the reaction mixture, and are therefore economically and environmentally suitable for biodiesel production (Röttig et al., 2010). Although biocatalyst based FAME formation is critically influenced by some process parameters, like enzyme concentration, molar ratio of oil to alcohol, water concentration, reaction temperature and pH (Salis et al., 2005). Optimization of these parameters is likely to enhance the yield of the biodiesel product.

In the chemically catalyzed transesterification method, extensive downstream processing is required to remove acid or alkali. Water washing is often used for removing these chemical catalysts from the biodiesel product creating much wastewater. Being biodegradable, biocatalysts do not pose such problems as mentioned above, although eutrophication could also be described as an environmental issue. Moreover, biocatalyst based method is less energy intensive than chemical method (Balat and Balat, 2010). Various oils have been tried as substrates to produce biodiesel by free enzymes obtained from different sources. For instance transesterification of rapeseed oil with *Candida rugosa* lipase showed more than 99% conversion (Wu et al., 1997).

In addition many studies have been carried out to avoid the problems related with the separation of catalysts from transesterification. Saka and Kusdiana have proposed catalyst-free transesterification using supercritical methanol. This approach would simplify the separation and purification process and the reaction can be complete in less time than the alkaline method; however, the reaction requires temperatures of 250–400 °C and pressures of 35–60 MPa (Saka and Kusdiana, 2001).

### 3.1.4 New processes

The majority of studies in transesterification of vegetable oils available deal with batch processes. However, batch processes suffer several disadvantages compared to continuous processes: they require larger reactors volumes and hence higher capital investment; they are inherently less efficient due to their start-up and shut-down nature; there are batch to batch variations in the quality of the products; and labor costs are higher. Facing those problems and motivated by the needs to increase its production, the biodiesel industry was obliged to develop large scale continuous processes.

Recently, research on continuous biodiesel production have been focusing on novel reactors and new processing technologies. A membrane reactor was operated, combining transesterification and separation in a single unit (Cao et al., 2008). This reactor was able to not only promote continuous mixing of raw

materials, but also provide a means to retain emulsions. Other authors proposed to overcome the mass-transfer limitations using liquid–liquid reactors, either a packed bed reactor (Ataya et al., 2008) or a film reactor (Narváez et al., 2009). The use of a gas–liquid reactor was also proposed to solve the same problem (Behzadi and Farid, 2009). In the latter case, vegetable oil was atomized and sprayed into a reaction chamber filled with methanol vapor. This process produced micro-sized droplets and thus was able to increase the heat and mass transfer. Other examples of recently proposed continuous process improvements include replacing classical stirring with ultrasounds (Stavarache et al., 2007), employing microwave radiation to provide the needed energy (Lertsathapornsuk et al., 2008) and using supercritical methanol in order to eliminate the need for catalyst (He et al., 2007).

To avoid the problems related with the extraction of lipids from microalgae biomass and its complex nature two alternatives has been proposed, (i) the direct extraction of fatty acids from biomass by means of a direct saponification of biomass and their eventual fractionation, or (ii) the direct production of FAMES through a direct transesterification of wet biomass.

### 3.1.5 Extraction of fatty acids by direct saponification

Direct saponification of microalgae biomass enables fatty acids to be obtained as potassium or sodium salts instead of as crude lipids in a first step. Thus, it is a selective method to recover only the fatty acids that are suitable to be transformed into biodiesel. Hereby removing compounds like unsaponifiable lipids that interfere in the production of biodiesel from microalgae oils (Molina-Grima et al., 1994). Direct saponification can be performed by adding KOH solutions (85%) to solvent mixture used for lipid extraction. The extraction/saponification is performed at room temperature for long time although it can be improved by heating at 60°C. After saponification the unsaponifiable fractions are extracted with hexane. Then, the hydroalcoholic phase containing the fatty acid salts is acidified by HCl addition to pH 1, Followed by recovery of the released fatty acids with hexane.

Direct saponification during extraction from the biomass is faster, less costly and saves operating time compared to lipid extraction followed by saponification. The only downside on this process is that more intensive operating conditions are necessary.

Extraction of fatty acids by direct saponification can be also performed from wet biomass (Ibañez-González et al., 1998). On this case, fatty acid extraction is performed by a three-step method shown in Figure 3: (1) direct saponification of wet biomass, followed by (2) extraction of unsaponifiable constituents and, finally, (3) extraction of purified fatty acids. It should be noted that this study was conducted using ethanol (96%) as extraction system. This gave rise to fewer problems than a hexane:ethanol (96 %) ratio of 1:2.5 v/v. This

caused formation of emulsions, which is the major bottleneck of lipid extraction. The overflow solution leaving the L-L extraction stage was transparent with a weak yellow colour almost free of pigments.

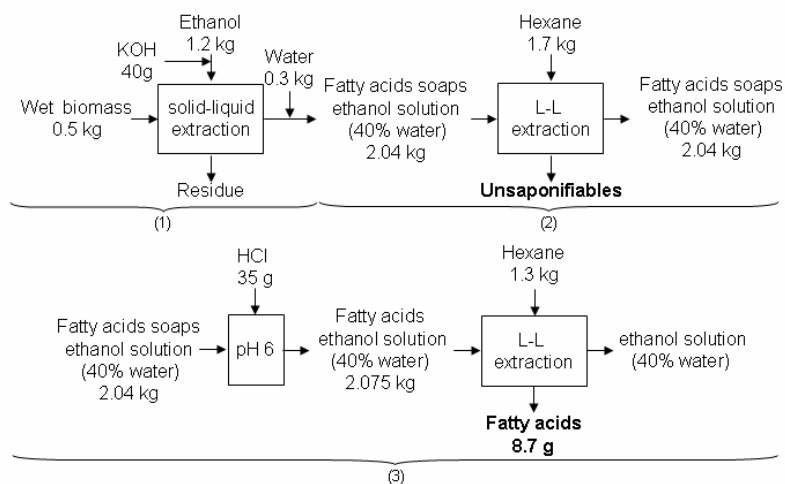


Figure 3. Conventional process for biodiesel production in the extraction-transesterification mode

### 3.1.6 Direct transesterification of wet biomass

This is a low expense process for recovery and fractionation of fatty acids methyl esters from microalgae. The process was developed at the University of Almería, Spain (Belarbi et al., 2000). According to this method the transesterification of fatty acids is performed simultaneously to extraction of methyl esters by reacting wet biomass with methanol in presence of catalysts and hexane. This allows direct production of fatty acid methyl esters (FAMES) from wet biomass, without drying of biomass or lipids extraction. All aspects of the process have been demonstrated at lab scale, using either wet or dry biomass. The flowsheet of the process is showed in Figure 4, also showing a cell disruption step that is optional according to the cell wall of cells.

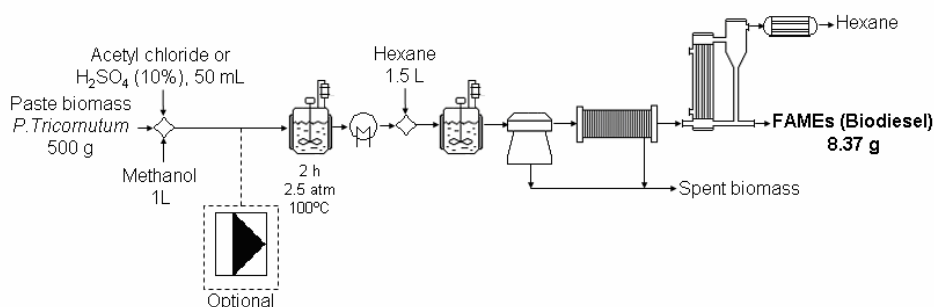


Figure 4. Conventional process for biodiesel production in the extraction-transesterification mode

In a typical run biomass paste was added to a mixture of methanol and acetyl chloride other catalysts like H<sub>2</sub>SO<sub>4</sub> can also be used. The resulting slurry was placed in a stainless steel pressure vessel and kept at boiling temperature for 120 minutes from the time the pressure reached its maximum value of 2.5 bar. After cooling to ambient temperature hexane was added to the slurry reaction mixture and mixed using a gentle level of agitation in the reactor vessel. Next the hexanic FAME solution is separated from the biomass slurry by centrifugation. The crude FAME extract is further polished by filtration and the hexane is finally recovered by evaporation. The FAME recovery yield ranged from 90-92%.

### 3.1.7 Suitability of algae oil to biodiesel production

There are several microalgal strains which are suitable for biodiesel production because they contain a large amount of lipids, Certain microalgae strains have proved to yield up to 87% lipids by weight (Alvarez and Steinbuchel, 2002). Although microalgae strains regarded as relevant for biofuel production include strains that have a lipid range between 10% and 50% by weight, already. Macroalgae hardly contain lipids, they consist more of sugars and carbohydrates, which makes them more suitable for bioethanol or biogas production.

Lipid production by algae can be studied on several levels. Most studies performed focused on bioreactor design and cultivation factors like nutrient depletion and photosynthesis effects. Cultivation factors are important in lipid production as it is known that (starvation) stress during cultivation increases lipid production. For instance during nitrogen depletion microalgae cells stop dividing and the storage products continue to accumulate (Darzins et al., 2010), with the result that dry weight lipid contents can double or triple. However, Rodolfi et al. (2009) showed an almost consistent productivity and almost doubling of the lipid content to 60 percent after switching to nutrient deficient conditions in a outdoor pilot reactor. In addition to nitrogen deprivation, limitations in phosphate and silicate can contribute to an increase in lipid content as well (Roessler, 1990). Photosynthesis inhibition also appears to contribute to higher lipid content, as certain red algae proved to yield more triglycerides with decreasing light intensity (Khotimchenko and Yakovleva, 2005). Metabolic studies, a relatively new research area in microalgae studies, could play a key role in understanding the relation between cultivation factors, lipid production and growth. Metabolic studies are useful in revelation of the regulation networks responsible for microalgal lipid production and growth (Schenk, 2008). Not only metabolic studies but also process engineering could play a role in the improvement of lipid production and growth. By developing (new) cultivation systems and making smart combinations with new or existing downstream processing equipment, improvements in lipid yield can be made as well. Process engineering will play an important role in all the different steps of the biodiesel production from microalgae. A typical process overview for a

biodiesel manufacturing process based on algae is shown in Figure 5. Here, the use of residual biomass streams is incorporated in the process.

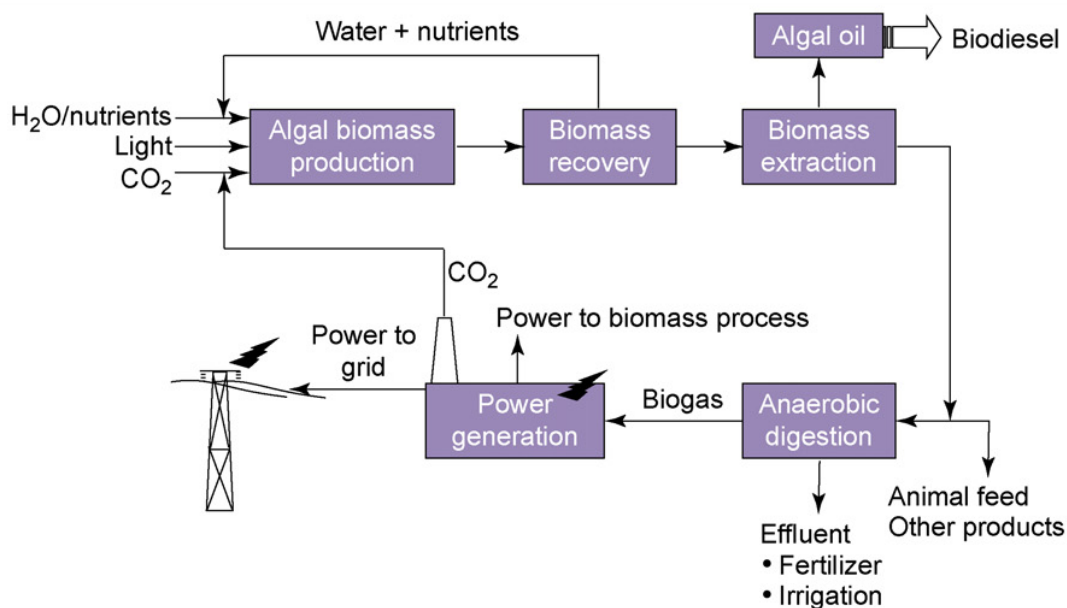


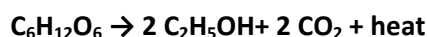
Figure 5. A general schematic of a biodiesel production process based on algal biomass (Chisti 2008)

Depending on the species many different lipids are produced by microalgae. Some lipids have their use as essential oils in cosmetics or production of heavy oils (FAO, 2010). Biodiesel made from lipids of microalgae, usually long-chain fatty acids, between C14 and C18, should theoretically compare favourably with the European standard EN 14214 (Darzins et al., 2010). Indeed, the characteristics of microalgae lipids correspond to the fatty acids found in terrestrial crops, which typically range between C16 and C18 (Knothe, 2010). This would indicate the suitability of the biodiesel pathway to process several types of algae oils. Microalgal biodiesel has also been reported as suitable for use in the aviation industry where low freezing points and high energy densities are key criteria (NREL, 2006). In order to create a realistic assessment of the potential of microalgae biodiesel it is important to test the fuel properties of microalgae biodiesel as they have not been addressed in scientific publications yet (Knothe, 2009). This is important as variations in cultivation conditions can affect the microalgal lipid composition which could have an impact on the fuel quality since the biodiesel feedstock characteristics have an impact on the final fuel properties. Biorefinery will play an important role to make biodiesel production from algae feasible and to mitigate the variation in lipid composition caused during the upstream process. In biorefinery the different biomass components are separated. This will not only improve the biodiesel quality as all the separated components from microalgae each have their own value. By adding up those different values, the overall economics of microalgae biomass will improve. Both improvement of the quality of the microalgae products economics and makes biorefinery an important factor in the success microalgal biodiesel.

Biodiesel production from microalgae still needs research (as can be read in this part). At present, although many small companies are operating, no large-scale processes are available. In order to efficiently produce biodiesel from algae, several aspects have to be approached, including algae strain selection, harvesting and extraction techniques. However, the biodiesel process as such is well established.

## 3.2 Bioethanol

The production of bioethanol basically involves a conversion of as much of the biomass as possible to sugar, followed by a conversion of sugar to bioethanol. The general reaction used in all of these processes involves the use of yeast or other microorganisms to convert sugar into ethanol and CO<sub>2</sub>:



It should be noted that the production of ethanol releases CO<sub>2</sub> in itself, accounting for potentially higher greenhouse gas emissions over the life-cycle than for other biofuels.

Bioethanol can be produced from several different biomass feedstocks such as sugar or starch crops. Sugar cane is the main feed-stock for bioethanol production in Brazil, while corn sugar beet and wheat in United States and Europe (Chiaramonti, 2007). Sugar and starch (which is basically an easily degradable polymer of glucose), have been used for current bioethanol production, because they can be readily fed into a fermenter where their conversion into ethanol by microorganisms takes place. Because the feed-stocks used as a source for sugar and starch are usually sugar cane, sugar beet, grain or corn, this process is in direct competition with human food production as well as it is limited due to unavailability of sufficient cultivable land on earth.

In the advanced bioethanol processes, more complex sugar polymers such as cellulose or hemicelluloses are used. These polymers are widely available in agricultural or forest waste products like straw, sawdust, woodchips, grasses etc. and these low-cost feed-stocks therefore no longer compete with food. However, by using these raw materials the process becomes a bit more challenging because after fractionation of the biomass, the (hemi)cellulose polymer fraction has to be degraded using specific enzymes to yield fermentable sugars that can be readily used by micro organisms for bioethanol production. The feasibility of using these materials as a feedstock is often limited by the low yield and high cost of the hydrolysis (cost of cellulose enzymes) process.

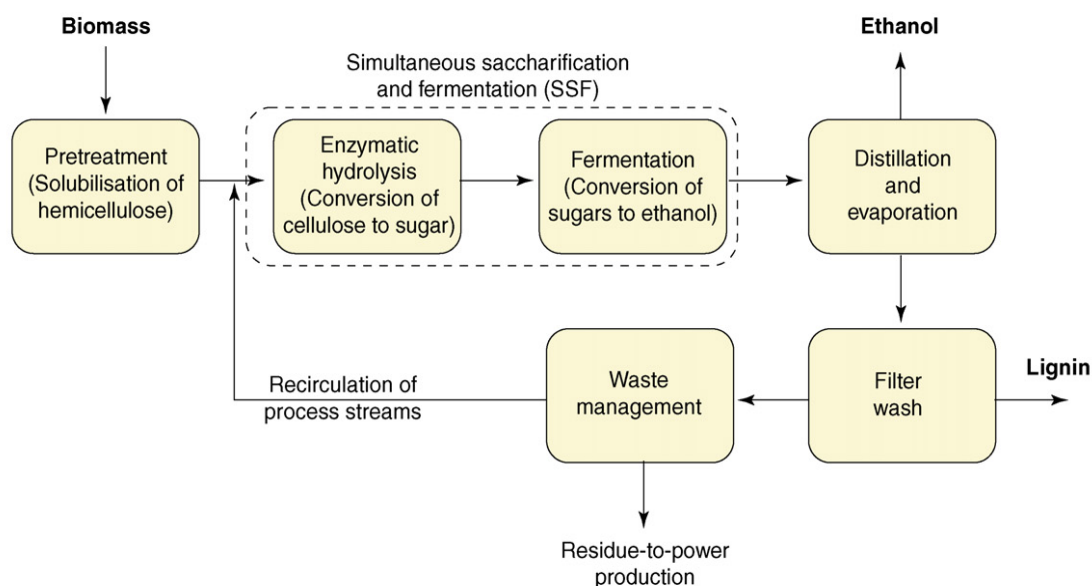


Figure 6. A schematic overview of a typical bioethanol production process (Hahn-Hagerdal et al. 2006)

In comparison to traditional crops and lignocellulosic feed-stocks, algae can provide a high-yield source of biofuels without compromising food supply chains. Besides biodiesel, there are algae accumulating carbohydrates (starch, cellulose), which can be used as a feedstock for the production of bioethanol. The algal starch, cellulose or other carbohydrates can be used for bioethanol production after hydrolysis.

### 3.2.1 Algal biomass for fermentative production of ethanol

Algae can be grouped into two categories (microalgae and macroalgae), based on their morphology and size. Some microalgae (*Chlorella*, *Chlamydomonas*, *Spirulina* etc.) are known to contain a large amount of starch and cellulose, raw materials for bioethanol production (Matsumoto et al., 2003). Many of the properties of starches that determine their suitability for particular end-uses are dependent upon their amylose/amylopectin ratios. These properties include gelatinisation characteristics, solubility, and the formation of resistant starch. It was found that the structure of algal starch (*Chlorella*) was similar to that of cereal starches (ca. 34% of amylase content) and the gelatinisation temperature of algal starch (ca. 65°C), as determined by viscosity measurements, also suggests to a structural similarity between algal and cereal starches (Maršáľková et al., 2010). Macroalgae contain mainly cellulose, which is at their low lignin content easily hydrolysable to fermentable sugars (Adams et al., 2009). These features together with their growth characteristics make these photosynthetic species exploitable in large-scale processes.

As far as bioethanol production is considered, while screening algal strains, high biomass with high starch/cellulose content should also be considered as a desirable characteristic since starch/cellulose can

serve as substrate for ethanol fermentation. Microalgae like *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus*, *Spirulina* are known to contain a large amount (>50% of the dry weight) of starch and glycogen, useful as raw materials for ethanol production (Ueda et al., 1996), (Brányiková et al., 2010). An increase in the production of starch in sulphur-limited culture up to a maximum of 50% starch content of algal biomass (DW) was firstly showed by Brányiková et al. (2010) under field conditions using the outdoor scale up thin-layer solar photobioreactor. Thus, the essential macroelement limitation is a very promising and practical approach. Similarly to any other industrial process, the major challenge in the production of bioethanol from algal biomass is that the process must be cost-effective.

Prior to fermentation the algal polysaccharides (starch, cellulose, hemicelluloses, laminarin) have to be hydrolysed to fermentable sugars, this process is called saccharification (Kelsall and Lyons, 1999). The greatest potential for large-scale bioethanol production renders starch together with cellulose. Their hydrolysis can be carried out by acid or enzymatic hydrolysis. Acid hydrolysis at high temperatures (>100°C) is non-specific and can lead to corrosion of equipment as well as formation of undesirable by-products decreasing the yield and/or toxic to fermenting microorganisms.

High-temperature, liquid-phase enzymatic hydrolysis (alpha-amylase and glucoamylase) is now used for starch hydrolysis (Nitschke, 2010). In order to facilitate the action of amylolytic enzymes upon intracellular starch, the algal biomass can be either mechanically disintegrated (bead mill) or the algal cells can be permeabilised by cellulases (Percival Zhang et al., 2006), a group of enzymes hydrolysing the cellulosic fraction of cell walls. The mechanical disruption of solid cell walls and intracellular membrane structures of algae before the enzymatic hydrolysis resulted in an increase in the yield of starch hydrolysis to 97% (Maršálková et al., 2010). This value is comparable to the enzymatic hydrolysis of corn starch. When applying cellulases prior to starch hydrolysis, not only the enzymatic cell wall degradation can be accomplished, but simultaneously an increased overall yield of glucose is achieved.

In the next step, the fermentable sugars are converted to ethanol by a suitable yeast strain e.g. *Saccharomyces cerevisiae*. In order to achieve the highest possible fermentation yield (theoretically 0.51 g of ethanol/1 g of glucose) an appropriate nutrition of yeast has to be ensured (source of N, P, S etc.). The cost of nutrients can be cut by adding proteases into the step of polysaccharide hydrolysis, which liberates free amino acids (source of nitrogen for yeast) from the protein fraction of the algal biomass. The processes of enzymatic hydrolysis and fermentation can be carried out also simultaneously (Doran-Peterson et al., 2009). Finally the ethanol is purified from the fermentation broth by distillation and the obtained concentrated ethanol (95%) can be blended with fossil fuels or directly used as fuel. The solid residue from the process can be used as animal feed or as a feedstock for biogas production (John et al., 2011).

### 3.2.2 Direct production of bioethanol by algae

As reviewed by Chen et al. (2009) and John et al. (2011) the production of bioethanol via fermentation of starch has its drawbacks: It is necessary to separate intracellular starch by extraction as mentioned above. However, many algae have strong cell walls and significant amount of energy is consumed during cell disintegration. Furthermore, a large amount of organic solvent is required in the starch extraction step. Since the starch separated by extraction is raw, it must be subjected to heat treatment for gelatinisation before being hydrolysed to glucose.

Thus, alternative ways for production of bioethanol directly from certain microalgae are tested: if dark and anaerobic conditions are established, the oxidative reaction of starch becomes incomplete and depending on the type of the microalgae, hydrogen gas, carbon dioxide, ethanol, lactic acid, formic acid, acetic acid and other products are produced in varying proportions. Ueda et al. (1996) used microalgae as starting materials for the production of ethanol. The algal cells contained a large amount of polysaccharides composed of glucose in the cells, which were catabolised rapidly under dark and anaerobic conditions to ethanol. These microalgae fall under classes Chlorophyceae, Prasinophyceae, Cryptophyceae and Cyanophyceae. Typical genera belonging to the class Chlorophyceae include *Chlamydomonas* and *Chlorella*, and typical genera belonging to the class Cyanophyceae include *Spirulina*, *Oscillatoria* and *Microcystis* (Ueda et al., 1996). Hirano et al. (1997) placed algal slurry into a light shielded tube under dark and anaerobic conditions. Conversion from intracellular starch to ethanol under dark and anaerobic conditions was then observed in almost all of the tested strains. But the levels of conversion to ethanol differed significantly from each other. Relatively high conversion rates of 30–40% (vs. a theoretical yield of 0.56 g of ethanol/1 g of starch) were observed in the two strains *Chlamydomonas reinhardtii* (UTEX2247) and Sak-1. The optimal pH for ethanol from *Chlamydomonas* cells were 7–8 at a temperature of 25–30 °C. Hirano et al. (1997) pointed out that ethanol production was directly proportional to the increase of biomass in slurry. This finding was backed up by Ueno et al. (1998). Ueno et al. (1998) also produced ethanol via dark fermentation of cellular starch of *Chlorococum littorale*, in which study, an increase in the incubation temperature affected the mode of cellular starch decomposition and brought about an increase in ethanol productivity up to 30 °C. (For detail review of these alternative ways of ethanol production, see John et al. ,2011).

## 3.3 Biogas

Biogas production results from biological breakdown of organic matter in absence of oxygen, called anaerobic digestion. Currently biogas production is a well-established technology, especially in smaller local

facilities where organic waste streams are fed into an anaerobic digester and the resulting gas (usually methane) is used to heat the facility or generate electricity. Anaerobic digesters are ideal for handling diverse biomass composition or biomass with a high content of difficult to process compounds (such as lignin). Larger scale anaerobic digesters are common in the treatment of municipal waste water. The generation of biogas is usually not the primary objective of these installations. More solid waste streams like vegetable, fruit and yard (VFY) waste or the organic residual of municipal solid waste are sometimes used for biogas production, like in the Dutch Valorga plant (Reith et al., 2003). This type of systems could also be used to make biogas from (residual) algal biomass.

In this process the conversion of the biomass is carried out by a complex mixture of micro organisms, which may each cover a distinct portion of the degradation pathway. The composition of this mixture may vary depending on operating conditions or the type of biomass that is fed into the system.

The conversion of biomass to biogas through anaerobic degradation can be broken down into 4 main conversion steps, as shown in Figure 6. Hydrolysis releases small organic compounds from the more complex biopolymers such as cellulose and protein. This is followed by acidogenesis in which these compounds are converted into volatile fatty acids (VFA) and alcohols. The anaerobic metabolic routes of the organisms can use these compounds to create acetic acid in the acetogenesis phase. Generation of acetic acid is accompanied with the formation of hydrogen as well, which can be captured separately. Finally acetic acid is converted into methane in the methanogenesis phase to yield the final biogas.

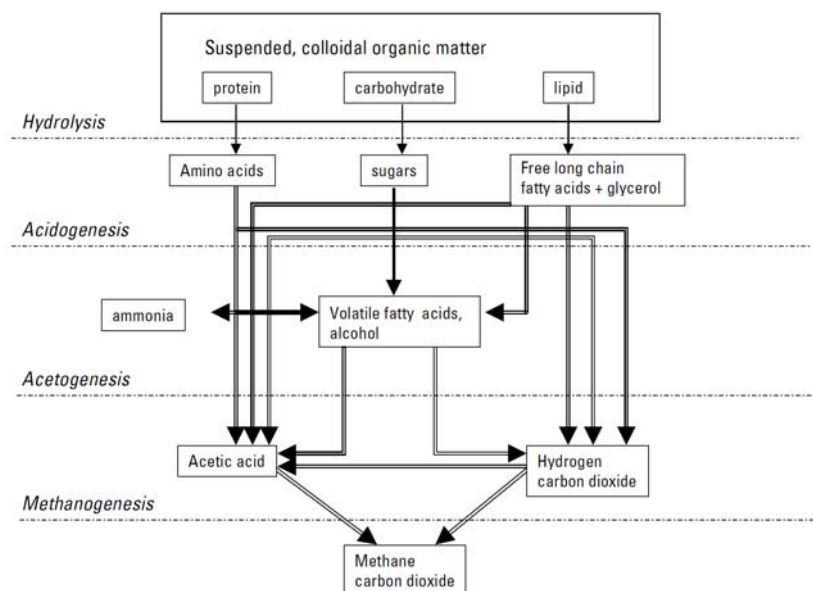


Figure 7. Schematic overview of the anaerobic degradation process

Biomass with low lipid content is especially suitable for biogas production using anaerobic fermentation. In terms of aquatic biomass this means that macroalgae are especially suitable in this case. However, macro algal biomass has some drawbacks over other types of biomass. For marine species, the high saline content

may inhibit growth or productivity of the anaerobic microorganisms in the fermenter. This can be mitigated by mixing algal biomass with other types of biomass to “dilute” the saline concentration<sup>2</sup>. Another problem, typical for green macroalgae, is the formation of H<sub>2</sub>S due to the high sulphate concentration in these species. This can be solved by using iron-based chemicals to bind H<sub>2</sub>S, as has been applied in wastewater treatment systems (Briand and Morand, 1996).

Currently, a 500 kW biomethane plant requires approximately 10.000 to 12.000 tons of biomass feedstock per year with maize currently being the major crop plant feedstock. Microalgae are a major focus of interest for this process as the efficiency of biomass production per hectare is estimated to reach 5 – 30 times that of crop plants. The relatively high lipid, starch and protein contents and the absence of lignin that cannot be fermented easily, make microalgae an ideal candidate for efficient biomethane production by fermentation in biogas plants (Schenk and Thomas-Hall, 2008).

Anaerobic degradation of phytoplanktonic cells is a process which takes place naturally in aquatic environments. This natural process has been the subject of research studies since the 1957, when Golueke et al. published the first study on anaerobic digestion of an algal biomass. In 1960 they proposed associating the production of microalgae in an open pond for the treatment of sewage water and energetic recovery of the algal biomass by anaerobic digestion (Sialve et al., 2009). This process is still currently a focus of intense research.

One of the major factors inducing high operation costs on microalgae production is nutrients feed: nitrogen and phosphorus. Anaerobic digestion can be an answer to this problem as a way to recycle nutrients, since this biotechnological process can mineralise algal waste containing organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be used as a substrate for the microalgae. Thus, if anaerobic digestion is used to process algal waste, it will not only recycle nitrogen and phosphorus but also produce methane. The energetic value of the produced methane can potentially lead to an energetic balance of the microalgae to biofuel process (Sialve et al., 2009).

Anaerobic digestion may be employed to convert the whole microalgal biomass, or also to valorize the residual biomass after extraction of the microalgae oil for biodiesel production. Indeed, the conversion of algal biomass after lipid extraction into methane is a process that can recover more energy than the energy from the cell lipids (Sialve et al., 2009). Christi (2008) reported that the anaerobic production of methane with cell residues is a key issue to balance both energetic and economic aspects. When the cell lipid content does not exceed 40%, anaerobic digestion of the whole biomass appears to be the optimal strategy on an energy balance basis, for the energetic recovery of cell biomass (Sialve et al., 2009).

The three main bottlenecks to digest microalgae are: low digestability, depending on both biochemical composition and the nature of the cell wall; high cellular protein content resulting in ammonia release which can lead to toxicity; and the presence of sodium in marine species that can also affect the digester performance (Rosch et al., 2009).

Indeed, some microalgae species can be very resistant to hydrolysis, which dramatically reduces their anaerobic biodegradability. Pretreatment, co-digestion or control of gross composition are strategies that can significantly and efficiently increase the conversion yield of the algal organic matter into methane (Rosch et al., 2009). The example cited by Sialve et al. (2009) of a full-scale plant for the thermal hydrolysis of sewage sludge reported by Kepp et al. (2000), demonstrates that the improvement of the methane yield can energetically balance the thermal pretreatment.

Finally, a synergy may also apply for the biogas purification through the microalgae production. The filtration of the biogas by algal cultures supplies CO<sub>2</sub> to the culture, thus enhancing the algal growth and productivity. Moreover, methane does not induce any toxicity on the growth of algae. Biogas purification through the algal culture can be very efficient (Sialve, 2009). Indeed, Travieso et al. (1993) evaluated the capacity of a culture of *Arthrospira* sp. to filter a biogas resulting from the digestion of molasses of a sugar refinery. The influent biogas was composed of 55 to 71% of CH<sub>4</sub>. Once the microalgal culture had filtered the biogas, the methane concentration had increased up to 88–97% CH<sub>4</sub> while CO<sub>2</sub> had decreased down to 2.5–11.5% CO<sub>2</sub> (Sialve, 2009).

### 3.4 Hydrogen

Hydrogen combustion leaves only water vapour, without releasing CO<sub>2</sub> as other fuels, which has led to claims that this fuel is highly sustainable (FAO, 2009). However, the assessment of the fuels' respective sustainability nowadays involves life-cycle analysis and thus fuel combustion represents a minor aspect of a fuel's sustainability assessment. Hydrogen is technically the most challenging biofuel to produce, as this byproduct of biogas production from certain types of digesters is difficult to separate from other compounds and to purify.

The biological production of hydrogen by algae was firstly described by Gaffron and Rubin in 1942; research on photosynthetic hydrogen production as a renewable energy source began in the 1970s (Gibbs, et al.,

1973), (Lien and San Pietro, 1975), (Mitsui et al., 1977). Biohydrogen can be produced by three different pathways:

1. Photolysis of water (direct or indirect)
2. Photofermentation
3. Dark fermentation.

Each of those pathways is performed by a different group of organisms. The three pathways can be, however, combined in order to obtain a higher yield of hydrogen.

### 3.4.1 Photolysis of water

The bio-photolysis uses the solar energy captured by photosynthesis to split water and produce hydrogen and oxygen ( $2\text{H}_2\text{O} \rightarrow 2\text{H}_2 + \text{O}_2$ ). The two processes, hydrogen and oxygen evolution can either run simultaneously (direct photolysis) or are temporally and/or spatially separated (indirect biophotolysis). Hydrogen is generated as a result of combining free protons and electrons through the activity of enzymes hydrogenase or nitrogenase. Hydrogenases are classified according to the metal ions at their active sites, the [NiFe] and [FeFe] hydrogenases. These two enzyme classes are evolutionary distinct and evolved independently. The [FeFe] hydrogenases have been described in eukaryotic microalgae; whereas, only the [NiFe] hydrogenases have been reported for cyanobacteria. The better characterized of the two are the [FeFe] hydrogenases. They are extremely oxygen sensitive. The enzyme is not made in the presence of oxygen and its enzymatic activity is quickly inhibited in the presence of traces oxygen. Therefore substantial hydrogen production is only observed in the light under anaerobic conditions. Since oxygen is continuously produced by photosynthesis when the algae is exposed to light, initial attempts describing hydrogen production used purging by argon in order to achieve anaerobiosis. Alternatively, hydrogen can be produced in the dark when no oxygen is produced by photosynthesis and its traces are respired. Hydrogen production under such conditions uses starch reserves and it is in principle indirect photolysis of water (the photosynthesis produces both oxygen gas and starch and is temporally separated from the hydrogen production). Both the direct photolysis under anaerobic conditions and dark production of hydrogen are not practically feasible for technological production of hydrogen since only small amounts of hydrogen for only a short time are produced. Also the anaerobic conditions require costly photoreactors devoid of oxygen.

A major breakthrough has been the use of sulfur deprivation (Melis, 2000). Under sulfur deprivation the photosynthetic activity is decreased allowing for all produced oxygen to be respired and therefore sustaining hydrogen production. This approach allows for a temporal separation of hydrogen and oxygen

evolutions. Firstly, starch as an energy reserve is made under oxygenic and sulphur replete conditions. Next, the cells are exposed to sulphur limitation in anaerobiosis leading to hydrogen evolution. Furthermore, the process can occur in cycles allowing for prolonged hydrogen production. This process is therefore an indirect photolysis of water and it is so far the most promising approach to hydrogen production by eukaryotic algae. Under certain conditions it can occur also in oxygenic atmosphere, which significantly simplifies the production process (no need for specific photobioreactors). Recently, genetic engineering has been extensively used to affect the hydrogen production at different limiting processes. It has been an iterative process, which holds promise for the near future.

The above described are characteristics and drawbacks of eukaryotic hydrogenase. Cyanobacteria can produce hydrogen by either [NiFe] hydrogenase with similar limitation as those described above or by nitrogenase. Nitrogenases are enzymes responsible for fixing of atmospheric nitrogen gas into ammonium. The nitrogenases contain molybdenum and iron in the active site. Hydrogen production catalyzed by nitrogenase occurs as a side reaction at a rate of one-third to one-fourth that of nitrogen-fixation, even in a 100% nitrogen gas atmosphere.

Similarly to hydrogenase nitrogenase is extremely oxygen-labile. Cyanobacteria have developed several mechanisms to protect nitrogenase from oxygen. The most successful one is spatial separation of nitrogen fixation and photosynthesis (oxygen evolution). Filamentous cyanobacteria produce nitrogenase and fix nitrogen gas in heterocyst, while oxygenic photosynthesis is carried out in vegetative cells of the filament. Similarly to the hydrogen production by hydrogenase also the nitrogenase hydrogen production is being improved both by different conditions (nitrogen starvation) and by genetic engineering. However, in contrast to hydrogenase, the nitrogenase requires the energy from ATP in hydrogen production. This makes the process extremely energetically demanding and significantly decreases its efficiency.

### 3.4.2 Photofermentation

Photofermentation is a process carried out by non-sulfur purple photosynthetic bacteria. They capture solar energy by anaerobic photosynthesis and use it to produce hydrogen by nitrogenase. These organisms are unable to split water; instead they use organic compounds, usually organic acids for the hydrogen production yielding both hydrogen and carbon dioxide. This is today the most productive biological pathway for hydrogen production. The hydrogen yield is about 25% of the substrate. It has the potential to use and clean different wastes and it provides complete conversion of the organic acid wastes to hydrogen and carbon dioxide. However, in principle it is less efficient than the process of photolysis of water because

it requires ATP to produce hydrogen; it also requires organic substrate, which makes it more expensive in large scale and less sustainable in the long term.

### 3.4.3 Dark fermentation

Dark fermentative production of hydrogen is very similar to biogas production. During the process a variety of different microbes can be used anaerobically to breakdown carbohydrate-rich substrates to hydrogen and other products, principally organic acids (lactic, acetic, butyric, etc.) and alcohols (ethanol, butanol, etc.). Product distribution differs dependent upon the microorganisms used and the conditions. This process is very simple and can use up a wide variety of wastes (including biomass). However, the yields of hydrogen are low; also there is large amount of side product produced.

It has been proposed to combine the dark fermentation of various waste compounds and/or biomass, which would produce hydrogen and use the side products as substrates for photo-fermentation. This increased the yield of hydrogen up to 60%. It is also possible to use a waste from hydrogen producing algal biomass (by photolysis of water) to feed the dark fermentation. This could further increase the yield.

### 3.4.4 Suitability for “algae hydrogen” production

Three different biological pathways can be used for hydrogen production, photolysis of water, photo and dark fermentations. At this point the most promising and most productive of them is the photofermentation. However, it requires organic substrate for the hydrogen production and it is therefore not sustainable in long term. The photolysis of water by algae is the sustainable variant that holds the biggest promises for the future because it requires only mineral medium and solar energy for the hydrogen production. However, at this point the efficiency of this process is lower than that of photofermentation; the process is limited in several steps and it requires a major research and biotechnological input. The biological production of hydrogen is now only at the research stage, the economically feasible production of hydrogen is so far not achievable and is dependent on the undergoing intensive research and optimization. Beyond biohydrogen production, biohydrogen also requires the adaptation of the fuel distribution infrastructure as well as vehicle adaptation, adding other technical bottlenecks to the need for further research.

### 3.5 Hydro-treated vegetable oils

New kinds of biofuels refinement processes that produce large quantities of hydrocarbon-based diesel fuel have been developed, among which hydrodeoxygenation, with hydro-treated vegetable oils (HVO) as end-products, stand out (Knothe, 2009).

Instead of the traditional esterification, vegetable oils and animal fats are hydrotreated. The process is comparable to usual diesel fuel refinement. The first stage of the production is the pretreatment, where impurities are removed. The actual process has two parts: hydrogenation and isomerisation. The pretreated compound is continuously fed into the hydrotreatment unit. The fatty acids are hydrogenated into paraffinic hydrocarbons.

During the hydrogenation temperature is 330-450 °C and pressure 5 MPa. Catalysts, such as NiMo/Al<sub>2</sub>O<sub>3</sub> or CoMo/ Al<sub>2</sub>O<sub>3</sub> are used (Reinhardt et al., 2006), (Oja et al., 2005). After the hydrotreatment, the mixture of hydrocarbons is isomerised. The molecular structures of the paraffinic hydrocarbons are treated with the help of a catalyst so that the number of carbon atoms remains high and methyl branches are created in the carbon chain. The end results of the reactions are also water, propane and small amounts of carbon oxides. The isomerisation should not go too far, as the end-products cetane number would decrease. During the isomerisation phase, the temperature is about the same as in the hydrotreatment. To stabilize the catalyst, a pressure of 3,5 - 4 MPa is used. Side products of the hydro-treatment process are fuel gas, which is burned for energy, and a small amount of biogasoline (Reinhardt et al., 2006), (Oja et al., 2005), (Rantanen et al., 2005).

The life cycle emissions of HVO is lower than these from FAME production emissions and generally allow slightly better greenhouse gas savings than biodiesel. The renewability of HVOs can be verified by radioactive C<sup>14</sup> analysis. The Engines and Vehicles Group at VTT has made exhaust gas measurements with mixtures of HVOs and ordinary diesel in passenger cars and buses. The measurements were made with fuels consisting of 5, 15, 20 or 85 % of biocomponent. The base fuel used fulfilled basic European requirements. The results showed emission reductions, especially with carbon monoxide and hydrocarbons. With the highest HVO proportion–fuels, the particle and even NO<sub>x</sub> emissions were also diminished.

The characteristics of HVO are as good as or better than with usual good quality diesel fuel, although the density of HVO is lower. With HVO, flammability is good, boiling ranges lower than with normal diesel fuel and the cold operation characteristics are adjustable. Moreover, HVO contains no aromatics or oxygen.

Although the lubricities of the fuels are poor, they can be easily improved with additives (Mäkinen et al., 2005).

HVO can be used with more flexibility and without some restrictions that concern usual FAME –biodiesel. The process also makes a wider range of raw materials possible. Additionally, it enables better product optimization. HVOs have a low cloud point, which can be adjusted from -5 to -28°C by severity of process conditions in order to produce either summer or winter type diesel fuel. The end product is very similar to conventional synthetic diesel fuel and can thus be blended directly with it or be sold as such to be further processed. No upper limits need to be set for HVO blends, as long as the final blend fulfills EN 590 requirements. In practice, the lower density of HVO means that the limits of EN 590 are reached with a 30% HVO blend (Reinhardt et al., 2006), (Mäkinen et al., 2005), (Rantanen et al., 2005).

HVOs can be mainly composed of long-chain alkanes, granting a high cetane number to the fuel and decreasing its suitability to cold climates. However, HVOs may also contain large amounts of shorter-chain and isomerized species, entailing a lower cetane number. HVOs are likely to have good cold flow properties and the possibility to isomerise the straight-chain alkanes from the C15-C17 alkanes produced through hydro-treating make them more suitable for jet fuel use. However, reports on tests against technical standards for diesel fuels (ASTM D975 or EN 590) as well as for jet (ASTM D1655) are not available to support this claim. Similarly, the confidentiality regarding the exact process description and the type of catalysts used makes it difficult to support the claim that hydro-treating vegetable oils is less costly than biodiesel (Knothe, 2009).

In terms of feedstock, HVOs are very well suited to animal by-products or palm oil, requiring less hydrogen for processing because their hydrogen content. However, the limited availability of animal by-products and the sustainability concerns on palm oil jeopardize the future of these fuels. On the opposite, hydro-treating camelina oil has been proved to produce fuels matching the requirements for jet fuels due to their high quantity of unsaturated fatty acid chains, a feature also found in certain algae oils (Hu et al., 2008).

The argument that HVOs can be produced directly from existing oil refineries, which are currently more available for such activities because of the decrease in activities induced by the competition from developing countries and because of the increased use of biofuels, is not entirely convincing. Indeed, the current installed biodiesel production capacity largely exceeds demand (EBB, 2010) and stand-alone HVO production units have been reported extremely costly to produce, which is not the case for biodiesel production facilities (Knothe, 2009). Similarly, the fossil methanol used in the production of FAME is not less sustainable than the fossil hydrogen used for HVO production.

### 3.6 Biomass to liquid (BTL) techniques

Another way to get liquid biofuels from biomass is by heating biomass to the point that fuel components get volatile. After distillation a type of fuel can be obtained that is rich in substances that are interesting for the chemical industry and the remainder can be used as fuel. The high water content of aquatic biomass makes this technique not feasible in most cases, except when the biomass can be dried in a cheap way, for instance by utilizing waste heat from other industrial facilities. In addition, these processes typically run at extremely high temperatures and/or pressures. The energy input required to maintain these conditions has a large negative impact on the overall energy balance of the process. For this reason most industrial applications of BTL for biofuel manufacture have been abandoned.

BTL techniques can be divided in three options:

- Gasification together with the Fisher-Tropsch (FT) process
- Anhydrous pyrolysis
- Hydrous pyrolysis under which thermo (catalytic) depolymerisation (includes other reactions besides pyrolysis)

Non edible parts of biomass feedstock, or even the entire biomass, can be converted to fuel by exposing it to high temperatures, i.e. by means of thermochemical conversion processes. During these processes biomass is decomposed into smaller molecules, which can then be reassembled again to new products like fuels or other chemicals. These technologies have the advantage that a range of potential end products can be produced. Not only a broad variety of fuels can be formed, but also the commonly used raw materials for the chemical industry can be manufactured.

These processes have been developed decades ago, and have in the past been applied to produce a.o. synthetic fuels from coal. These technologies are now being validated to be used in the context of biomass. Up to date, application of these technologies with biomass feedstock, and especially with algae biomass, is not yet commercially feasible. Next to ongoing basic research, demonstration plants are being built in the EU and the US to scale-up from the current stage of development and to improve technology performance. In the case of algae, research is still in an early phase to determine process suitability and develop/adjust conversion technologies to algae feedstock.

From a technical point of view, thermochemical conversion technologies can be subdivided in two groups, namely the ones in which biomass is heated in the presence of oxygen (i.e. gasification), and heating of biomass in absence of oxygen (i.e. pyrolysis). Figure 6 gives an overview of the BtL conversion processes.

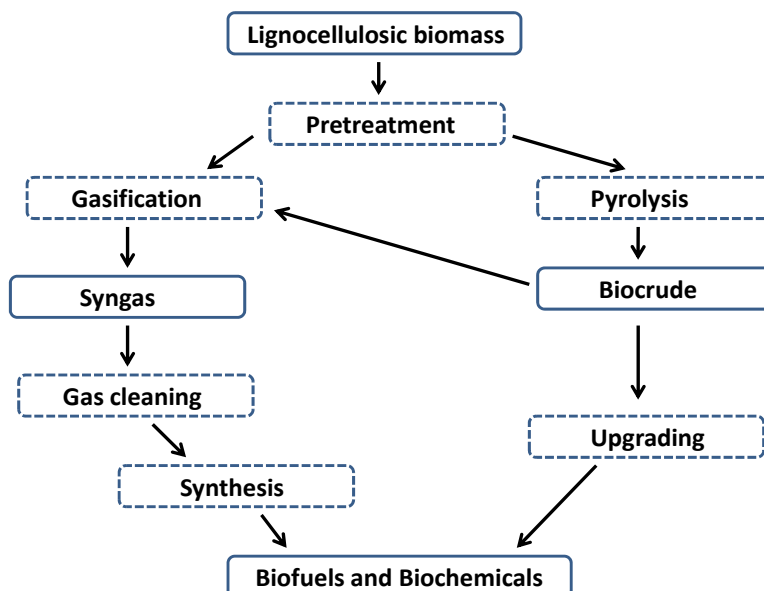
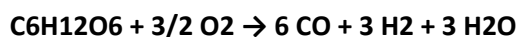


Figure 6 Overview of Biomass to Liquid (BtL) conversion processes

### 3.6.1 Gasification

Gasification implies heating of biomass in the presence of oxygen. The temperature is elevated (> 700°C) to induce various chemical processes. As such, so-called syngas is formed, which is a mixture of carbon monoxide (CO), water (H<sub>2</sub>O) and hydrogen (H<sub>2</sub>). The formation of syngas is typified by the following reaction equation:



Through subsequent catalytic synthesis processes these molecules can be combined again to form a variety of biofuels, depending on the applied synthesis process. Some examples of synthesis processes are the Fisher-Tropsch process and the mixed alcohol synthesis processes.

Gasification allows for the production of a wide array of fuel products. In the case of the Fischer-Tropsch synthesis process, synthetic diesel (also called FT diesel or BtL diesel) is formed. Through the mixed alcohol synthesis processes, mixtures of alcohols are produced, e.g. methanol, ethanol, and butanol. Methanol can be further dehydrated to form dimethyl ether (DME), a substitute for diesel. Products resulting from these processes can also be used as raw materials in chemical industries. Additionally, syngas can be burnt

directly as a fuel. The composition of the resulting syngas has an important influence on the subsequent processing of syngas to biofuels. As such, the development of reliable gasification processes, adapted to the feedstock available is a key research area. In the case of algae, the moisture content of the feedstock imposes a problem to the efficiency of the gasification process.

Research into the effect of the biomass characteristics of algae and the water content of the algae feedstock on the gasification process is increasing, but available data are still scarce. To circumvent the difficulties associated with wet feedstock, increased attention is given to the development of technologies in which the moisture content of algae is not a hindrance to gasification. To this end, the possibilities of hydrothermal conversion processes (as opposed to conventional thermochemical processes) are being explored. These processes apply the same principles of conventional thermochemical processes, but use the water present in the feedstock as a conversion promoting solvent. As such water is the reaction medium in which conversion takes place. To realize feedstock conversion, water has to be brought in a supercritical state. This means that pressure is augmented to 218 atm and temperature is elevated above 374 °C. As such, water reaches its critical point and becomes supercritical. Supercritical water exhibits properties that fall in between those of a gas and those of a liquid. This makes supercritical water an excellent solvent. Using water as a solvent not only eliminates the problem of moist feedstock, it is also cheap, environmentally benign and leaves no residues after conversion. Gasification by means of supercritical water is termed supercritical gasification. Experiments have already indicated that supercritical gasification of algae is a suitable method to produce, e.g. methane.

### 3.6.2 Pyrolysis

In the case of pyrolysis, biomass is exposed to more moderate temperatures between 300°C and 700°C under elevated pressure and in the absence of oxygen. As such, gases are produced from volatile biomass components, which are subsequently condensed to form a bio-crude. This bio-crude oil can then be further refined to biodiesel or used as a feedstock for higher value chemicals. Converting low-energy density biomass to a high-energy density and easily transportable bio-oil allows for further processing at a different location. Process efficiency can be increased by applying flash pyrolysis, in which finely ground biomass is rapidly heated to 500°C for two seconds. Microalgae have the advantage of their small particle size, reducing the need for fractionation of the biomass. There have been conducted some experiments with algae in this context. Although the bio-oil from algae pyrolysis seems to be qualitatively superior, sufficient reduction of the moisture content of the algae feedstock may be a barrier to the use of algae for this application.

Pyrolyzing microalgae to produce liquid fuel was first put forward in Germany in 1986 (Huang et al., 2010). Pyrolysis is the conversion of biomass to biofuel, charcoal and gaseous fraction by heating the biomass in the absence of air to around 500 °C (Miao et al., 2004), or by heating in the presence of a catalyst (Agarwal, 2007). In recent years fast pyrolysis processes for biomass have attracted a great deal of attention for maximizing liquid yields (Miao et al., 2004). The advantage of fast pyrolysis is that it can directly produce a liquid fuel (Bridwater and Peacocke, 2000). Fast pyrolysis tests of microalgae were performed in the fluid bed reactor (Miao and Wu, 2004). They used fast pyrolysis to enhance oil yield from microalgae from *Chlorella prothecoides* after manipulating its metabolic pathway towards heterotrophic growth. The fast pyrolysis of *C. prothecoides* and *Microcystis aeruginosa* grown phototrophically, yielded 18% and 24% of liquid products, respectively (Miao et al., 2004). If flash pyrolysis is used, the conversion of biomass to bio-crude with an efficiency of up to 80% is enabled.

However there are technical challenges as pyrolysis oils are acidic, unstable, viscous and contain solids and chemically dissolved water (Chiaramonti et al., 2007). The process oil will require upgrading hydrogenation and catalytic cracking to lower oxygen content and remove alkalis (Demirbas, 2001).

### 3.6.3 Thermochemical liquefaction

Microalgae usually have a high moisture content. The high water content and inferior heat content makes the microalgal biomass difficult to be used for heat and power generation. As a result the pyrolysis requires a drying process that needs a great deal of energy to vaporize the water (Zou et al., 2009).

A technique related to pyrolysis, but in which the moisture content of wet feedstock does not pose a problem, is liquefaction. Liquefaction occurs when water is in its subcritical state, leading subcritical water to act as a solvent. Subcritical water is water at a temperature between its boiling point (100°C) and its critical point (374°C), but which is kept liquid by applying high pressure (120-200 atm). The high reactivity of water molecules in this state breaks down long-chain biomass polymers into smaller molecules that form a bio-oil that could be used as fuel or feedstock for the chemicals production. This technology can be employed to convert wet biomass material to liquid fuel (Patil et al., 2008). Thermochemical liquefaction is a low temperature (300-500 °C) high process (5-20 MPa) aided by a catalyst in the presence of hydrogen to yield bio-oil (Goyal et al., 2008). Some first experiments indicate that this technology is a potential conversion option for algae biomass.

Dote et al. (1994) reported that *B. braunii* produced liquid oils at 57-64% of dry weight at 300 °C and also declared a positive energy balance for the process (output/input ratio of 6.67:1). In a similar study, an oil

yield of 42% dry weight was obtained from *Dunaliella tertiolecta* and positive energy balance of 2.94:1 (Minowa et al., 1995). Sawayama et al. (1999) investigated the energy balance of a liquid fuel production process from *B. Braunii* using thermochemical liquefaction. The study suggests that microalgae consume low amounts of nutrients and accumulate high caloric materials and nutrient resources which are produced without energy wasting processes.

Aresta et al. (2005) have compared different conversion techniques for production of microalgal biodiesel. The hydrothermal liquefaction technique was more effective for extraction of microalgal biodiesel than using the supercritical carbon dioxide. Zou et al. (2010) investigated the thermochemical catalytic liquefaction of *Dunaliella tertiolecta* and characterized the obtained bio-oils. The main compounds of bio-oils were benzofuranone, fatty acid methyl ester (FAME) and fatty acid hydroxyethyl ester with a long chain from C<sub>14</sub> to C<sub>18</sub>. These bio-oils were presented as potential feedstock for biofuels. In addition, thermochemical liquefaction is more energy efficient than other BTL pathways, accounting for a potentially positive greenhouse gas balance.

### 3.6.4 BTL suitability for algae biofuels production

It is very important to take into account the biomass quality for choosing the most appropriate conversion technology. For biomass grown on waste water or waste nutrients, or if the strain composition can not be tightly controlled, BTL will be the only available option for biofuels production, for sanitary reasons and since by-products will not be marketable anyway. Only BTL will destroy possible pathogens or toxins and retain potential toxic compounds (heavy metals etc) in the combustion residues, that otherwise will dissipate into nature. Also for conversion of macroalgal biomass featuring complex biomaterials of unknown digestibility BTL may be the only reasonable conversion technology available. In addition, thermochemical liquefaction turns the specific drawback of algae, moisture content, as an advantage through reaching supercritical state.

However, algae as a wet feedstock is not well suited for pyrolysis or gasification. Although these two pathways would allow using most of the carbon contained in algae without a complex extraction process, these processes do not leave any co-products to valorise, relying entirely on the biofuel valorisation. Given the relative high price of algae compared with other potential feedstock and the high price of most BTL techniques, BTL would tend to add another variable to the complex equation of algae biofuels rather than helping solving it. Many of the various BTL pilot plants built up in the previous years are not being scaled-up to commercial scale, mainly for their insufficient prospects for economic viability. In addition, these

complex, energy-intensive and therefore rather unsustainable processes give no guarantees of an easy downstream processing or of advanced sustainability characteristics.

## 4 Conclusions

Algae as a category covers such a wide array of microorganisms that most biofuels pathways could potentially be used. Algae mainly constitutes a new feedstock source, but algae can also play the role of a catalyst, as for biogas, biohydrogen and for the direct production of bioethanol from algae. None of the presented pathways currently qualifies as a biofuel production process allowing the economically viable production of renewable fuels. In general, for a biofuel to become truly sustainable, the overall energy balance needs to be positive. This means that the energy required to drive the process to obtain the final biofuel should not exceed the energy that is generated by the biofuel upon combustion. In order to achieve this, many of the techniques discussed in this document need to be optimized or refined in such a way so that the process energy input is minimized. However this document shows that the basic technology to achieve this is available and in some cases even developed on to industrial scale.

Algae have been recognized for their potential to provide an efficient and high quality feedstock without competing with food, feed or arable land surface. The great advantage of using algae as feedstock for current biofuels is the low-cost biofuels processing technology and the established downstream production chains and even the effective integration to fuel markets, as supply chains may not exist for all advanced biofuels (Slade et al. 2009), hindering their market penetration. Future technologies as hydrogen production and BTL techniques would require adaptation on downstream markets, making them less desirable biofuels pathways for algae as compared to other pathways. However, several technical arguments tend to support these pathways: physico-chemical characteristics similar to those of fossil fuels, which implies that the current distribution infrastructure and vehicle design could be used also for these new type of fuels. This aspect could open the doors of the jet fuel market to algae biofuels produced by hydro-treating algae oils, as this production pathways has similar costs to FAME production. On the opposite, the limited success of the BTL pilot plants should inspire caution to public decision-makers and economic operators. Indeed, these technologies have proved to be highly costly. Therefore, these technologies neither represent an option for making algae biofuels commercially viable, nor a solution to reach technical feasibility of algae biofuels. From this group, only hydro-treated vegetable oils would constitute a viable option for algae biofuels, although their production would be limited to the existing oil refineries given the cost of producing stand-alone production units.

Although inexpensive, the production of biogas or biohydrogen in fermenters has low yields, making it an option for small and de-centralised energy production units rather than for industrial production. However, the wide array of possible biogas feedstock typically makes it a possible option for the valorisation of co-

products from other pathways. Biohydrogen is still far from commercial production and although photofermentation seems promising, the need for an organic medium and significant inputs to cultivate these algae makes their sustainability questionable. Thermochemical liquefaction allows bypassing the traditional hurdles of harvesting and extraction, which is technically promising. However, additional research is required for this pathway, which is also energy-intensive and thus questionable in terms of economic and environmental sustainability.

The table included in Annex summarizes the various aspects of each pathway for the production of biofuels from algae. “**Suitability for algae**” reflects the biofuels pathway’s ability to use the natural characteristics of the algae, e.g. carbohydrate accumulation for bioethanol or wet content for thermochemical liquefaction. Cost-effectiveness mainly depends on the input required and on the current technical command of this pathway within the biofuels industry. **Sustainability** mainly refers to greenhouse gas (GHG) life-cycle emissions, reflecting either demanding cultivation conditions for certain algae or the high GHG emissions from certain processes. The results for **cost-effectiveness** and **sustainability** are similar as they depend on the input needed, except for bioethanol: the CO<sub>2</sub> released during the fermentation process makes ethanol production less sustainable than other pathways, while on the opposite algae yielding directly bioethanol remove the need for energy-intensive harvesting and extraction. The early stage of development of most pathways or their functional limitations are reflected through “**scaling-up potential**”, for which only current biofuels can be deemed to have no potential scaling-up limitations. “**Ease of extraction**” mainly reflects the need for extraction specific to the production of biodiesel, bioethanol and HVOs. Extraction adds to the process complexity. Similarly, “**Ease of conversion**” mainly reflects the complexity of BTL pathways. The possibility for **co-products valorisation** are necessarily negative for pathways using the whole algae. The difference between “**final fuel characteristics**” and “**end market suitability**” is the following: a process can yield a fuel adapted to a market, but mixed with so many other co-products that further refining or purification is required. The need for downstream infrastructure and vehicle adaptation is reflected in “**end market suitability**”.

## COMPARISON OF THE RESPECTIVE BIOFUELS PATHWAYS FOR ALGAE BIOFUELS PRODUCTION

	Suitability to algae	Cost-effectiveness	Sustainability	Scaling-up potential	Ease of extraction	Ease of conversion	Co-products valorisation	Final fuel characteristics	End market suitability
Biodiesel	+	+	+	+	-	=	+	+	+
Bioethanol (starch extraction)	+	+	=	+	-	=	+	=	+
Bioethanol (direct production)	=	-	+	-	+	+	+	=	+
Biogas	+	+	+	+	+	+	+	-	-
Biohydrogen (water pyrolysis)	=	+	+	-	+	=	=	-	-
Biohydrogen (photofermentation)	=	-	-	-	+	=	=	-	-
Biohydrogen (dark fermentation)	=	+	+	-	+	=	=	-	-
BTL (pyrolysis)	-	-	-	=	+	-	-	-	+
BTL (thermochemical liquefaction)	=	-	-	=	+	-	-	=	+
Hydrotreated vegetable oils	=	=	+	=	-	=	+	+	+

All indications should be read with biofuels production as a final objective, i.e. a + reported in the column “co-products valorisation” means that this pathway offers co-products valorization options. Blue reflects positive aspects, red accounts for negative aspects and white corresponds to a neutral result.

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