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Algae Biorefinery – Material and energy use of algae

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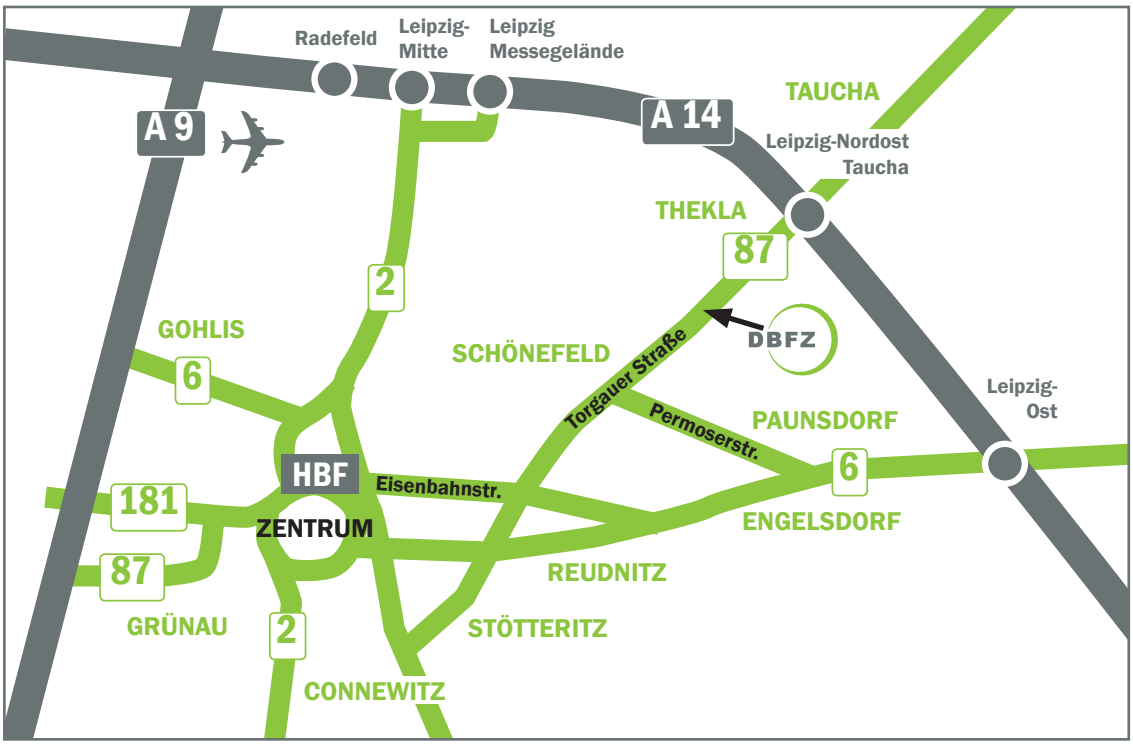

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List of abbreviations

AA	Arachidonic acid
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CED	Cumulative Energy Demand
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty Acid Methyl Ester
FCC	Fluid Catalytic Cracking
FPA-PBR	Flat Plate Airlift Photobioreactor
FA	Fatty Acid(s)
GLA	γ-linolenic acid
HHV	Higher Heating Value
HMF	Hydroxymethylfurfural
HRT	Hydraulic Retention Time
HTC	Hydrothermal Carbonisation
HTL	Hydrothermal Liquefaction
HVP	High Value Product
IRR	Internal Rate of Return
wt.%	weight percent
MSW	Municipal Solid Waste
PBR	Photobioreactor
p_c	Critical pressure
PUFA	Polyunsaturated Fatty Acid(s)
RON	Research Octane Number
sc-CO_2	Supercritical CO_2
SFE	Supercritical Fluid Extraction
TAG	Triacylglycerol(s)
T_c	Critical temperature

dm	Dry matter
Vol.%	Percent by volume
VS	Volatile Solids
ρ_c	Critical density

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1 Introduction

Algae offer as much as 30 times greater biomass productivity than terrestrial plants, and are able to fix carbon and convert it into a number of interesting products.

The numerous challenges in algae production and use extend across the entire process chain. They include the selection of suitable algal phyla, cultivation (which takes place either in open ponds or in closed systems), extraction of the biomass from the suspension, through to optimal use of the obtained biomass. The basic suitability of aquatic biomass for material use and energy supply has been demonstrated in a large number of studies. Numerous research projects are concerned with identifying the optimal processes to enable its widespread implementation.

An overview of the current status of the application of micro-algae as renewable resources is given in (Rosello Sastre and Posten 2010). The food and animal feed industries, including aquaculture, are currently the main markets. The fine chemicals sector (pigments, PUFAs and polysaccharides) is the most profitable. A variety of factors influencing the economic viability of producing motor fuels from micro-algae are described in (Stephens et al. 2010). The key statement is that co-production of 0.1 % of the biomass as a high-value product (600 USD/kg) or an oil price of > 100 USD/bbl with a high level of biomass productivity and low investment costs would give rise to an expected internal rate of return (IRR) of >15%.

A summary relating to the production of energy source materials from micro-algae is contained in (Demirbas and Demirbas 2010).

This report details the progression of the algae suspension downstream of the photobioreactor (PBR), its dewatering and drying where appropriate, through the cell decomposition (lysis) to the processes of recovering energy sources and raw materials.

Biomass consists of a large number of materials with corresponding physical and chemical properties. Depending on its origins, it may be converted into energy by a variety of different means. A wide range of different conversion technologies are available for the purpose. They include physical, thermo-chemical, biochemical and biological treatments to create energy-rich products from the source biomass.

The thermo-chemical conversion treatments studied here comprise (Figure 1.1):

- Hydrothermal liquefaction (section 5)
- Hydrothermal carbonisation (section 6)
- Pyrolysis (section 7.3)
- Hydrogenation (section 7.5)
- Gasification (section 7.6)

The report is rounded off by research on the application of micro-algae as a substrate for alkaline digestion plants, for biodiesel production and as animal foodstuff.

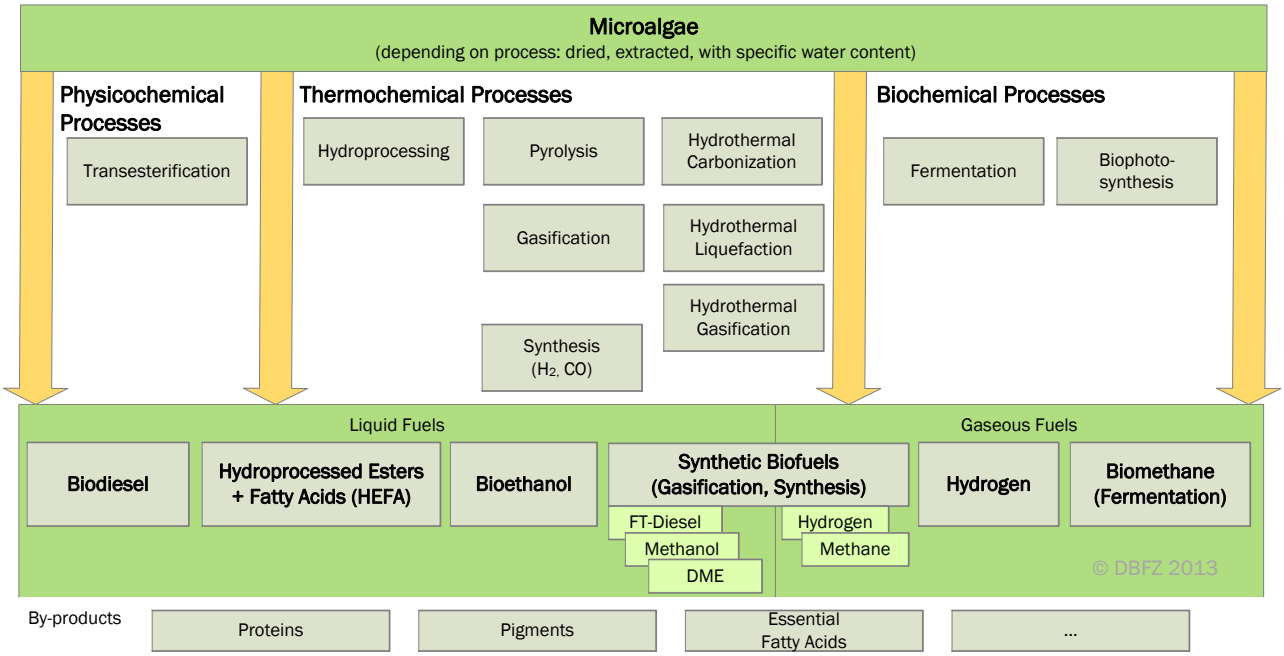


Figure 1.1 Overview of thermo-chemical conversion treatments (DBFZ 2011)

The research partners Hochschule Lausitz (Senftenberg), Deutsches Biomasseforschungszentrum (Leipzig) and Technische Universität Bergakademie Freiberg are studying and assessing the potential material and energy use pathways for micro-algae. In order to obtain specific results, the algae *Chlorella vulgaris, Scenedesmus obliquus* and *Selenastrum rinoi* are being studied in terms of their potential.

2 Algae production and species used

Algae are among the oldest organisms on Earth. It was the existence of algae, in fact, which first led to the enrichment of oxygen in the Earth's atmosphere and enabled higher life forms to be created. Fossil records from the Pre-Cambrian period document the presence on algae stretching back 2.5 billion years (Ecke 2003). Today, algae play a key role as CO_2 consumers, as oxygen and biomass producers and as the bases of marine food webs.

The term 'algae' encompasses organisms exhibiting common physiological properties. Since the system is not based on familial relationships, algae form a paraphyletic group. Algae are aquatic organisms similar to plants which are capable of autotrophic life. Algae require water at least temporarily, though they are able to withstand lengthy dry phases. They even occur in deserts and semi-desert environments. The algal group is very heterogeneous. It comprises organisms with or without genuine nuclei – that is to say, both procaryotes (so-called blue-green algae or cyanobacteria) and eukaryotes. Eukaryotes include the green, red, brown and diatom algae groups, as well as gold and yellow-green algae and others. For the reasons set forth, no exact systematic classification of algae based on the classic nomenclature of kingdom, phylum, class, order, family, genus has yet been accomplished. However, a future taxonomy will be based on genetic sequences, and thus on lineage.

At present over 100,000 species of algae are known. According to general estimates, however, some 400,000 algal species exist worldwide. Algae populate virtually all known habitats. Even extreme habitats such as ice or hot springs pose no obstacle to them.

The basic form of algal growth is planktonic. In that form they are free-moving. They may also be sessile however. This means the algae grow on solid surfaces in the form of slime. Algae can be classified by cell size (e.g. macro-algae or micro-algae), though these taxa are likewise not botanically defined.

In order to grow, micro-algae require light as an energy source, CO_2 as a source of carbon, optimal species-specific temperatures, and nutrient salts in dissolved form. Sources of nitrogen and phosphorus are essential. And sulphur must also be available. Algal growth additionally needs a variety of different trace elements. In the course of cultivation, the effect of the aforementioned factors in interaction with the specific enzymes of the respective species creates micro-algal biomass. The following average chemical formula $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti 2007) is frequently used.

Micro-algae can in principle be cultivated in open or closed systems (Borowitzka 1997; Xu et al. 2009; Ugwu et al. 2008). Open systems include natural ponds, shallow river mouths, lakes or oceans. Other open systems are artificial bodies of water or so-called open ponds. Open ponds are ponds of approximately 20 cm depth in which the algal culture is kept in continuous motion by paddle wheels. Open systems are easy to handle and can be cheaply produced. However, these advantages are countered by some serious disadvantages: The small amount of sunlight penetration limits growth. Other disadvantages are evaporation losses, the large areas which they take up, and contamination risks. In order to prevent bacterial or zooplankton infection, such constructions remain restricted to the cultivation of extremophile alga species.

Closed systems include photobioreactors (PBRs). Cultivation takes place in pipes, tubes, plates or tanks. PBRs offer a number of advantages which justify their high procurement costs (Pulz 2009): Process control adapted to specific conditions enables a reproducible production process to be implemented. Thanks to the ability to sterilise the medium and the enclosed construction of such systems, the risk of contamination is low. Additional CO_2 input results in increased concentrations of the carbon source in the reactor and thus faster algal growth. Furthermore, the pH value of the suspension can be kept constant by appropriate sensor and control technology. There is no metabolically related increase in pH value which would impede growth. Additionally, it is possible to cultivate micro-algae in regions not suitable for agricultural use. This eliminates the potential conflict with food production needs. The closed circuits in PBRs also reduce water consumption thanks to lower evaporation losses. As a sustainable system, the water can even be used for recultivation simply by adding back in the spent nutrient salts.

Biomass productivity by area differs very widely across the various cultivation systems (Pulz 2009): Open systems achieve a productivity rate of 10-20 g/m²d, closed systems 35-40 g/m²d and thin-film systems 80-100 g/m²d.

The *Chlorella vulgaris* and *Scenedesmus obliquus* species are already being cultivated at the pilot plant operated by GMB GmbH. At the start of the project also the species Selenastrum rinoi was considered for cultivation. Consequently, the specific properties of those species must be considered for the individual technological steps. All three species are green algae which grow in freshwater.

Chlorella vulgaris is a unicellular spherical to oval freshwater alga. Its diameter is 4 µm to 13 µm. It proliferates by way of autospores. *Chlorella vulgaris* is one of the fastest-growing micro-algae, which is why it has been identified as a suitable species for many production plants. One reason for its high productivity rate might be its ability to feed mixotrophically. That is to say, this micro-alga is able to procure nutrition from organic carbon sources. Its lack of flagella means this alga species is stationary. No evidence could be found of other methods of movement, such as by changes in density.

Selenastrum rinoi is an approximately 10 µm long and approximately 3 µm wide crescent-shaped green alga. This alga is likewise assumed not to be capable of changing position by its own means. As this is a green alga, it can be assumed – like *Chlorella vulgaris* – to have a highly stable cell wall.

The spherical green alga *Scenedesmus obliquus*, approximately 13 µm in size, is the only species used in the studies which is known to form coenobia (colonies with a specific number of cells). These mostly consist of four, less often eight, or even 16 cells. In contrast to other species of the genus Scenedesmus, *S. obliquus* does not have flagella, so is not capable of changing position by its own means.

The following Table 2.1 summarises the data collated at the Hochschule Lausitz for the three alga species with illumination of 100 µE/m²s at approximately 25 °C and 2 vol% CO_2 in the 2 L bubble column. The detailed data sheets are presented in appendix Algae data sheets. More results are contained in (Hempel et al. 2012).

Table 2.1 Summary of data for the selected micro-algae

	Chlorella vulgaris	*Selenastrum rinoi*	*Scenedesmus obliquus*
Biomass productivity in g/Ld	0.145 – 0.148	0.235 - 0.279	0.119 – 0.129
Lipid content in % dm	20.7 ± 0.9	22.4 ± 2.6	22.7 ± 0.9
Lipid productivity in mg/Ld	12.9 ± 0.6	18.7 ± 2.2	9.4 ± 0.4
Protein content in % dm	38.1 ± 1.2	42.1 ± 1.7	33,6 ± 0.75

3 Dewatering and drying[1]

3.1 Introduction

This section sets out the results of the literature research on dewatering and drying of the produced micro-algae. Dewatering involves separating the extracellular water from the algal suspension. This enables dry matter contents (dm) of approximately 30 % to be achieved. A subsequent drying stage removes all water from the biomass (approximately 95 % by mass dm). In addition to the conventional methods, such as centrifugation, filtration, contact and spray drying, the aim is to identify treatments entailing low energy input. An overview of the manufacturers of plants to implement the processes cited is contained in section 3.5. Owing to the differing amounts of water required for various downstream processes, we investigate which methods, and combinations of methods, promise to deliver advantages in this respect. In order to provide alga-specific results, we also consider in detail the species of alga to be used. The energy consumption and costs of the various treatment methods are indicated, where available.

The density of the living cells of *Chlorella vulgaris* is given by Henderson et al. (Henderson et al. 2008) as 1070 kg/m³. The sedimentation velocity resulting from these parameters can be considered non-existent. *C. vulgaris* has a highly stable cell wall, enabling it to withstand even high pressures without harm (up to 10 MPa Salecker 2009). This makes cell decomposition considerably more difficult.

In the case of *Selenastrum rinoi*, owing to its smaller cell volume and slim shape, no sedimentation under the influence of gravity is expected (appendix Algae data sheets).

The green alga *Scenedesmus obliquus* is the only species used in the studies which is known to form coenobia (colonies with a specific number of cells). These mostly consist of four, less often eight, or even 16 cells. Consequently, its sedimentation behaviour is not dependent on the single cell, but on the properties of the cellular complexes. So, overall, sedimentation is possible. According to the available information, *S. obliquus* also sediments (Hochschule Lausitz (FH) 2011) within 24 hours, which further underpins this assumption. The work by (Salim et al. 2011) reported how this alga autonomously flocculates. In this process, some *Chlorella sp.* content was also included in the flocs. In contrast to other species of the genus Scenedesmus, *S. obliquus* does not have flagella, so is not capable of changing position by its own means.

3.2 Dewatering

The processing of the micro-algae demands a higher biomass concentration than is commonly encountered in the production plants (Chisti 2007). For example, the algal suspension in the culture system of the species *Scenedesmus obliquus* cultivated in the flat-plate airlift photobioreactors (FPA PBR) made by Subitec used at GMB GmbH had a dry matter content of 3-5 g/l. In applications of algae for energy use especially, it is essential to produce at costs comparable to those for the production of established regenerative energy sources. The dewatering accounts for a large part of the total production cost (Carlson et al. 2007; Molina Grima et al. 2003; Bruton und u.a. 2009). This

[1] This section was authored by the Deutsches Biomasseforschungszentrum (DBFZ)

demonstrates the necessity to identify an optimal method of process control for the purpose. The following sets out the various methodological and technological approaches to dewatering. A comparison of the various dewatering methods is set out in section 3.4.1.

3.2.1 Gravity sedimentation

Alga harvesting by means of gravity sedimentation is the technically simplest method of obtaining biomass from the suspension. The algal medium is transferred to a sedimentation tank, where the algae sink to the bottom while the supernatant is scooped off and can be re-used as a growth medium. Depending on the alga species and the retention time, dry matter contents between 1.5 % and 5.0 % can be attained in the sump of the tank (van Harmelen und Oonk 2006), (Lundquist et al. 2010).

Advantages are offered primarily by the ease of handling of such a plant and the widespread use of the technique. Gravity sedimentation does not entail high investment cost. The energy input is restricted mainly to the operation of pumps for the various flows. In order to increase the particle size and accelerate sedimentation, in the case of unicellular algae especially it is necessary to employ coagulants to ensure that the process is completed within a reasonable period of time. A further disadvantage is the large amount of space taken up by the sedimentation tank. The tanks take up roughly a tenth of the total area of the plant (Burlew 1976) and have approximately 50 % of the volume of the photobioreactors (PBRs) (Weissman und Goebel 1987).

3.2.2 Filtration

Filtration entails the use of a variety of different methods depending on the properties of the algae and the desired downstream processing, from vacuum/pressure filtration through surface filtration to depth filtration. Filters are characterised by their low space take-up compared to sedimentation. In filtration by gravity, energy is needed only to transport the media; for vacuum/pressure filtration additional pumping power must be planned. Filtration is a widely used technique, as a result of which solutions have already been developed for virtually all conceivable applications. The main disadvantage of this method is the clogging of the filter pores and the resultant reduction in filter throughput. For this reason, only algae which form colonies (such as *Spirulina sp.*), or already flocculated algae, can be effectively extracted from the suspension. It is possible to use filtration aids (such as lime) to separate even small particles, but the consumption of such ancillary materials (Dodd 1979) and the influence of the filtration aid on the downstream processes (Molina Grima et al. 2003) makes any such use unattractive for the mass production of algae.

In the harvesting experiments conducted by Sim et al. (Sim et al. 1988), the pump energy input for filtration of the micro-algae and to back-flush the filter was between 0.3 and 0.5 kWh per cubic meter of algal suspension. Here mixed algae cultures were used for waste water treatment which also contained smaller species such as *Chlorella sp.* and *Oocytis sp.* (Sim et al. 1988). A drum filter was investigated.

3.2.1 Flotation

Small bubbles created by electrolysis or pressure relief are introduced into the suspension, adhere to the surface of the alga cells and transport the algae to the surface of the water where they can be skimmed off (Figure 3.). Flotation as a method of harvesting algae has to date been used only to a

limited extent. Its main advantages are the small amount of space taken up and the comparatively rapid separation of particles from the suspension. The yield is 75 % (Schmack et al. 2008) to 98 % (Sim et al. 1988). However, the harvesting of micro-algae only produced satisfactory results with the aid of coagulants (G. Shelef et al. 1984).

In a study of the alga harvesting method, 1.6 kWh of power was consumed to obtain an algal suspension with an average 4 % total solid content (dm) from a culture medium with just under 0.1 % algal biomass per kilogram of dry algae (Sim et al. 1988). This data correlates to the power consumption specified by the manufacturer STULZ-PLANAQUA GmbH of at least 1.3 kWh per cubic meter medium, depending on plant size.

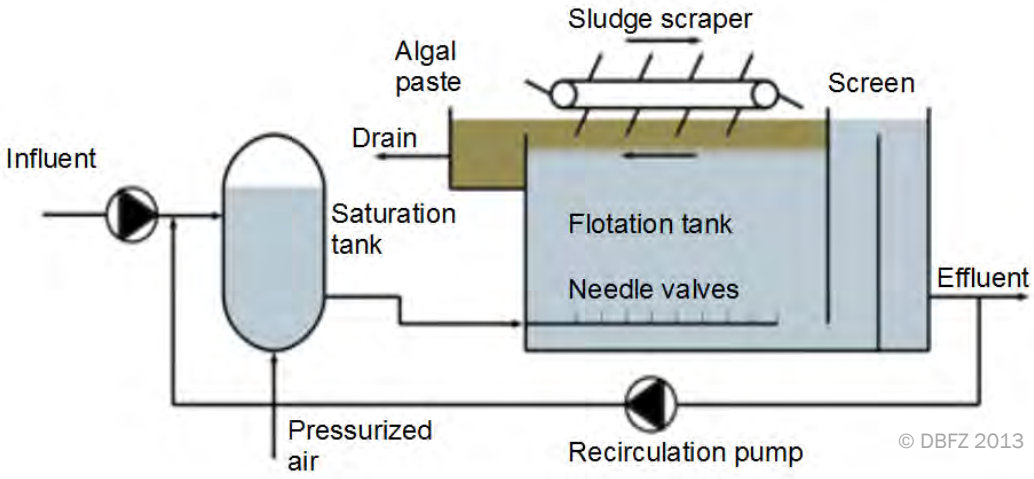


Figure 3.1 Method of operation of a flotation plant (DBFZ 2013)

An alternative is offered by so-called microflotation. This technique can cut energy demand considerably. A power consumption rate of 0.1 kWh/m³ is specified, corresponding to a reduction in energy input of more than 90 %. This reduction results from the lower pressure in the pressure saturator and the special design of the pressure-relief valves. Whereas the conventional pressure-relief flotation technique operates with saturation pressures of 5 to 8 bar, for microflotation 2 to 4 bar is sufficient. The pressure-relief valves prevent the formation of larger bubbles which would destroy the combinations of particles and air bubbles by their faster rate of rise (Stark et al. 2008). The descriptions lead to the conclusion that the use of flocculants can also be significantly reduced, or even becomes entirely superfluous (Damann 1998). World Water Works Inc. has likewise developed a solution for alga harvesting by means of pressure-relief flotation. Here, too, a much reduced energy demand compared to conventional flotation systems of 20 to 50 watt-hours per kilogram of harvested algae is specified (Schnecker 2011).

3.2.1 Centrifugation

The use of centrifuges or decanters for alga harvesting is widespread (Carlson et al. 2007), (Sim et al. 1988), (Bruton et al. 2009). The main advantages of centrifuges are their small space take-up and the fact that they are in widespread use. Disadvantages are high energy demand and maintenance effort (Molina Grima et al. 2003) as well as a relatively high residue of biomass in the outflow (Sim et al. 1988). The aforementioned disadvantages make centrifuges only viable and affordable for the

harvesting and production of high-value products (Molina Grima et al. 2003), (Schmack et al. 2008). The biomass concentration downstream of the centrifuge is approximately 15 % to 30 % (Sim et al. 1988). Based on an initial pre-concentration and centrifugation as the secondary harvesting process, the energy demand can be significantly reduced (Sazdanoff 2006). As a concrete value a cost reduction to approximately 1/50 is cited (Benemann und Oswald).

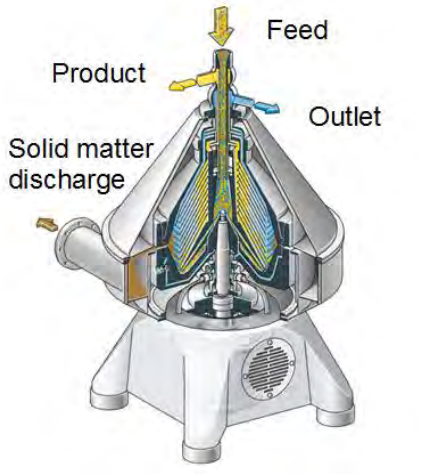


Figure 3.2 Plate centrifuge (by courtesy of GEA Westfalia Separator Group)

The manufacturer Alfa Laval specifies the power consumption of its Clara 500 centrifuge as 43 kW at a throughput rate of 50 m^3/h. Referred to one kilogram of algae in a suspension with 0.1 % solid content, this results in a specific energy demand of approximately 0.9 kWh/kg (algae). In response to an inquiry, the manufacturers GEA Westfalia and Pieralisi also confirmed this order of magnitude (0.4 to 0.6 kWh/kg and 1.5 kWh/kg respectively).

According to data from FLOTTWEG (Steiger 2012), by combining flotation and centrifugation as much as 76 % of the electrical energy input can be saved. After flotation with 0.13 kWh/m^3 a dm content of 2.5-4 % by mass is attained. The FLOTTWEG SEDICANTER® concentrates to approximately 25 % by mass with an energy input of 2.5 kWh/m^3. The energy saving results from the lower water volume needing to be accelerated up to centrifugation speed in the Sedicanter compared to plate centrifuges.

3.2.1 Dewatering aids

Coagulants

In the case of particles of the order of magnitude of unicellular micro-algae, many of the aforementioned treatment methods can only be implemented with the aid of flocculants. These must be selected according to the application and the alga species. The use of flocculants is linked to other circumstances too. For example, the culture medium cannot be re-used after flocculation of the algae, as residues of the flocculant may significantly impede operation of the production plant. The flocculant may likewise have a considerable influence in processing of the algal biomass, as it remains bound to the biomass. Flocculation can be achieved by adding polymers, salts or biological flocculants, as well as increasing the pH value.

The polymers may be of biological origin, such as chitosan, which is produced by deacetylation of the exoskeletons of crustaceans, or may be synthetic, such as the coagulant Ultimer from NALCO®. The polymers cited have been successfully used for alga harvesting (Schmack et al. 2008), (Ahmad et al. 2011). What all polymers have in common is that they are not suitable for harvesting of marine micro-algae, because the high salt content of the medium severely reduces the efficacy of the agents (Bilanovic and Shelef 1988).

Another potential group of flocculants are trivalent iron or aluminium compounds.¬ These form insoluble hydroxides which are deposited on the alga cells. These agents are little used in alga harvesting, as they can significantly influence the downstream processing steps (for example flocculated algae can as a result no longer be used as foods or animal foodstuffs).

It is also possible to flocculate the algae by means of bioflocculation. Some bacteria, such as Paenibacillus sp., produce biopolymers which have been successfully employed as coagulants (Oh et al. 2001), (Kim et al. 2011). Another method is the combined culture of flocculants, i.e. less productive alga genera with highly productive alga species, as described by Salim et al. (Salim et al. 2011). In this, the flocculating alga genus embeds the other species into the flocs, but to do so must occur in large quantities in the medium. By this method a maximum of 60 % of the biomass was removed from the suspension, even when the flocculating species was present in a higher concentration than the species to be flocculated (Salim et al. 2011). The advantage is that no differing cultivation conditions for the two species need to be created, as is the case for the biopolymers from bacteria as described.

Coagulation of the algae can also be initiated by raising the pH value to approximately 12 (autoflocculation). In practice, however, this meant adding more than 0.03 mol/l NaOH (Schmack et al. 2008). This corresponds to a mass concentration of 0.12 %. Therefore, in order to achieve coagulation by raising the pH value comparatively large quantities of sodium hydroxide or other lyes have to be consumed (Table 3.1).

Table 3.1 Coagulants compared

Coagulant	Dosage in mg/l, source	Price in EUR/kg[2]	Spec. cost referred to algae in EUR/kg
Alum	342 (Kim et al. 2011)	0.10…0.13	≈ 0.05
Iron (III) chloride	162 (Kim et al. 2011)	0.40…0.45	≈ 0.08
Ultimer (polymer)	10 (Schmack et al. 2008)		
Prosedim (polymer)	10 (Schmack et al. 2008)		
Chitosan	10 (Ahmad et al. 2011)	15…20	≈ 0.18
CTAB (surface-active agent)	40 (Kim et al. 2011)	6.40	≈ 0.25
NaOH	1,200 (Schmack et al. 2008)	2.80	≈ 3.36
Cationic polyacryl amide		1.50	

Ultrasound

Ultrasound can be employed to support flocculation and cell decomposition in order to improve the efficiency of the processes. An experiment showed that the solid particles aglomerate at the nodal points of standing (ultra)sound waves and form clumps (Food and Agriculture Organization of the United Nations (FAO) 2009). Liang Heng et al. report that the coagulation of micro-algae with flocculant can be improved by brief ultrasound application (Heng et al. 2009).

3.3 Drying

The intracellular water remaining in the cells after dewatering of the algal suspension can only be removed by thermal processes (Food and Agriculture Organization of the United Nations (FAO) 2009). Although many further processing steps with a water content of over 75 % are feasible, it may be necessary to dry the biomass almost completely. The water content must be substantially reduced especially when readying the algae for storage, as otherwise they will decay rapidly. And for transportation too – especially over long distances – reducing the mass delivers considerable savings on transport costs, and so may also be economical. The various drying methods are comparatively assessed in Table 3.3.

3.3.1 Drying by solar energy

The technically simplest and most economical method of drying the biomass dewatered by the aforementioned mechanical processes is by using solar energy. This makes it an ideal method for simple applications in developing countries. The technique is, however, heavily dependent on climatic conditions, and entails the risk that the algal paste may decay during the process. Drying is effected either by direct sunlight or by means of a circulating air flow heated by solar energy. Drying the algae under a cover made of glass or transparent plastic film enables higher temperatures to be reached, and

[2] Price calculated using www.alibaba.com

so speeds up drying. However, these relatively low temperatures are in no circumstances sufficient to sterilise the product, or indeed to permit cell decomposition (Becker 1994). Moreover, the method takes up a lot of space and time.

3.3.1 Flash dryers

Flash drying methods enable very rapid drying. The moist biomass is sprayed into a rising stream of hot gas at the bottom. The finely distributed biomass is carried upwards by the gas stream, whereby the water is evaporated and incorporated into the gas. At the same time the gas is cooled by the evaporation. The gas phase and solid matter are separated in a downstream cyclone. Residues of the solid matter in the gas phase can then be separated off by a filter unit.

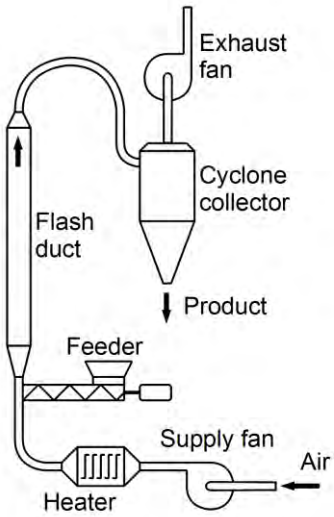


Figure 3.3 Method of operation of a flash dryer (DBFZ 2011)

Technologies Private Ltd. specifies a heat demand of 1.2 MWh per ton of water being evaporated. A further 180 kWh/t of electric power is needed to operate the dryer. Based on a 30 % solid content in the algal paste, this result in a specific energy demand of 3.2 kWh/kg (product).

3.3.1 Spray dryers

Spray drying is a method frequently used in the production of algae for food purposes, because a large number of constituents are retained. Just as in the case of flash dryers, in this continuous process the paste is dried in a few seconds.

The algae are loaded into the spray dryer against a flow of hot gas. The dried product can be removed from the bottom of the dryer. Residual particles in the air stream are separated off by a cyclone.

According to data from the manufacturer (TREMA Verfahrenstechnik GmbH), to dry 200 kg of algae with a moisture content of 80 % approximately 300 kg of steam at a temperature of 150 °C is required. Some 30 kWh of electric power is additionally consumed in operation. The residual moisture in the product is 4 %. Complete cell decomposition cannot be guaranteed due to the short retention time and the relatively low temperatures (Becker 1994). Based on this data, the energy demand can be estimated at 6.4 kWh/kg (product) (without taking into account the boiler efficiency and any heat recovery).

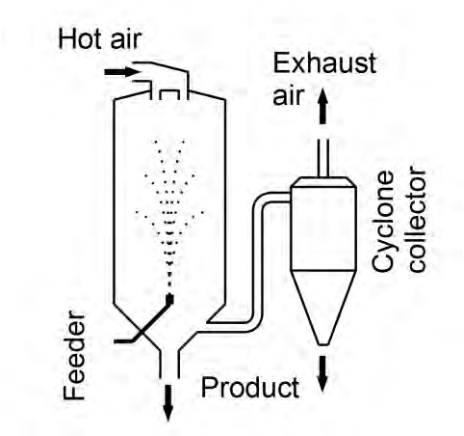


Figure 3.4 Method of operation of a spray dryer (DBFZ 2011)

3.3.1 Drum dryers

Drum dryers consist of a heated drum in which the material being dried is conveyed from one end to the other by gravity and built-in baffles. For industrial processes a wide range of such dryers have been developed which are heated either directly by a hot stream of air or gas, or indirectly by an external source. The material is heated up in just a few seconds, but remains for much longer in the drum dryer, enabling simultaneous sterilisation and cell decomposition.

An example of the necessary energy input is provided by a dryer from mineralit® GmbH which to obtain one ton of water-free solid matter from a paste with 25 % solid content requires 5.1 MWh of thermal energy and 110 kWh of electric power (mineralit GmbH 2011). In this case the residual moisture would be approximately 20 %. Referred to the product, this results in an energy demand of 5.2 kWh/kg. In this case the low efficiency results primarily from the low drying temperature of less than 100 °C.

3.3.1 Conveyor dryers

Conveyor dryers are used to dry bulk goods, fibrous products, pastes and moulds. The material is dried without placing any mechanical strain on it. In the conveyor dryer the material is placed in a product feeder module on a usually horizontal-running perforated conveyor belt, on which it passes through one or more drying chambers and, where appropriate, is turned over by relaying the belt (Christen 2010). In the drying chambers a flow passes through the material from the top or bottom, thereby evaporating the water contained in it. The dried material is collected in the discharge module. The speed of the belt and the temperature of the individual drying modules are adjustable, enabling the plant to be adapted to

different materials and mass flow rates (Jacobs 2009). It is possible to use different heat transfer media, such as air, gas, oil and water, as well as a variety of different heat sources. Conveyor dryers are mostly operated with waste heat, as they are able to utilise the low-temperature heat energy efficiently (Jacobs). If higher temperatures are required, heat can be generated specially for the purpose. The main area of application for conveyor dryers is in the drying of sewage sludge and digestion residues.

Additional parameters for application of this process to micro-algal paste can be derived from data relating to the drying of sewage sludge, as the substances are similar at least in terms of their physical properties. Usually residual moisture levels of 20 % in single-stage processes and 10 % with two-stage dryers are attained (Laxhuber 2009; Kügler et al. 2004). Owing to the occurrence of dust, the process is not suitable for complete dewatering. The amount of thermal energy required to evaporate one kilogram of water is between 1.0 kWh (Laxhuber 2009) and 1.4 kWh (NEUERO Farm- & Fördertechnik GmbH 2011). A small amount of electric power is additionally needed to operate the dryer. The demand is approximately 0.025 kWh per kilogram of evaporated water (Laxhuber 2009).

Figure 3.5 Conveyor dryer: A) Overview; B) Product feeder module with extruder; C) Schematic diagram; (by courtesy of Hans Binder Maschinenbau GmbH 2013)

According to our research, conventional conveyor dryers have not yet been employed to dry micro-algae. There are a number of reasons for this. Firstly the comparatively small capacities of the existing production plants. Conveyor dryers for applications in chemical process technology and environmental technology are built with capacities of around 300 kW and upwards (Laxhuber 2009; Arlt 2003) (exceptions are niche applications such as for textile printing, rapid prototyping, and others). This corresponds to a throughput of approximately 300 kg of algal paste per hour, with a dry matter content of 30 %. For drying subject to lesser performance requirements more simple equipment is used. Micro-algal paste with a water content of approximately 30 % differs in its properties from the materials normally processed by a conveyor dryer. Such a dryer is primarily suitable for granular or pelletised materials, because the belt is perforated and a stream of air or gas has to pass through the material being dried. By way of pre-treatment the algae must be granulated, which requires either thermal or chemical treatment, or the algae are extruded onto the conveyor belt (Green and Perry 2008). A further disadvantage of this method is the unavoidable residual moisture. Owing to the low level of chemical and thermal loading on the micro-algae, no cell decomposition is expected in this drying process. The main advantages are the efficient utilisation of the heat input and the possibility of using waste heat at temperatures of 80 to 90 °C.

3.3.1 Freeze dryers

Freeze drying is employed primarily in the food industry. The frozen water is sublimated out of the product by means of vacuum. Residual water can be removed from the algae by heating under atmospheric pressure. The method is employed primarily to conserve sensitive materials. Freeze drying has to date been employed to dry algae only on a laboratory scale. In continuous-running industrial plants, 1.0 kWh of electric power and 2.1 kg of steam is required to evaporate one kilogram of water (batch 1.1 kWh and 2.2 kg) (Green und Perry 2008).

3.4 Assessment

3.4.1 Dewatering

Table 3.2 indicates that for the economically viable production of algae for energy use the principal methods at present are a combination of flocculation, sedimentation and centrifugation and, following further investigation, also microflotation and centrifugation. For material use, as is today already being implemented, the influence of the flocculants on product quality must primarily be considered.

Table 3.2 Assessment of the dewatering methods

Method	Suitable alga species	Energy demand	Achievable solid concentration	Comments
Sedimentation	*Scenedesmus*	Low (only to transport media)	1.5…5 %	Very time and space consuming
Filtration	*Chlorella, Scenedesmus, Selenastrum*	Low (may rise due to recorded pressure increase)	≈ 3 %	Consumption of filters and filtration aids
Flotation	Not compatible with the species used without flocculant			
Microflotation	*Chlorella, Scenedesmus, Selenastrum*	Low	4…8 %	No independent assessment regarding alga harvesting available
Centrifugation	*Chlorella, Scenedesmus, Selenastrum*	Very high	15…30 %	Very fast
Combinations				
Flocculation & Sedimentation	*Chlorella, Scenedesmus, Selenastrum*	Low (see above)	1.5...5 %	No re-use of culture medium; time and space consuming
Flocculation & Filtration	*Chlorella, Scenedesmus, Selenastrum*	Low		No re-use of culture medium, filters and filtration aids
Flocculation & Flotation	*Chlorella, Scenedesmus, Selenastrum*	High	≈ 4 %	No re-use of culture medium
Flocculation, Sedimentation & Centrifugation	*Chlorella, Scenedesmus, Selenastrum*	Relatively low	15…30 %	No re-use of culture medium; time and space consuming
Microflotation & Centrifugation	*Chlorella, Scenedesmus, Selenastrum*	Relatively low	15…30 %	No independent assessment regarding alga harvesting available

3.4.2 Drying

Table 3.3 provides an overview of the various methods potentially usable for drying algae. In order to make the data comparable, the manufacturers and literature sources cited are referred to the amount of water to be evaporated. The water content of the added algal paste is assumed to be 70 %. A residual moisture of 5 % is assumed, as complete drying is not attainable by all methods.

Table 3.3 Assessment of drying methods

Method	Elec. power per kg of dry algae in kWh	Therm. energy per kg (dm) of algae in kWh	Source
Flash dryer	0.41	2.74	Transparent Technologies Private Ltd. (manufacturer)
Spray dryer	0.43	3.23	TREMA Verfahrenstechnik GmbH (manufacturer)
Drum dryer	0.09	4.32	mineralit® GmbH (manufacturer)
Freeze dryer (continuous)	2.28	3.28	(Green und Perry 2008)

The values indicated should be regarded as guides, as they were merely derived from the data relating to the drying of other products, and do not take into account the specific properties of the algal paste and potential means of process optimisation, such as heat recovery, were not included in the calculation.

3.5 Manufacturers

Table 3.4 Manufacturers of drying/dewatering plants

Manufacturer	Products	Description
ISYKO Filtersysteme Am Stauweiher 11a 51688 Wipperfürth www.isyko.de	Band pass filters	Filters have already been used for alga harvesting; no special designs
Lenzing Technik GmbH Werkstraße 2 4860 Lenzing, AUSTRIA www.lenzing.com	Microflotation plants, among others	
HUBER SE Industriepark Erasbach A1 D-92334 Berching www.huber.de	Flotation plants, among others (offers complete sewage and waste treatment solutions)	
STULZ-PLANAQUA GmbH Hemelinger Hafendamm 18 28309 Bremen www.stulz-planaqua.de	Water, sewage and environmental technology, including flotation plants	
GEA Westfalia Separator Group GmbH Werner- Habig- Straße 1 59302 Oelde www.westfalia-separator.com	Decanter centrifuges, separators, …	On enquiry, lowest energy demand of separators (0.4 … 0.6 kWh/m³)
Evodos Algae Technologies B.V. Takkebijsters 17A 4817 BL, Breda, Netherlands www.evodos.eu	Special alga centrifuges	Centrifuges developed specially for single-stage alga harvesting, two or three phases
Flottweg AG Industriestraße 6 - 8 84137 Vilsbiburg www.flottweg.com	Wide range of separation systems (decanters, centrifuges, …)	"enalgy" process specially for alga harvesting (combination of flotation and decanting)
ThyssenKrupp Polysius AG Graf-Galen-Straße 17 59269 Beckum www.polysius.com	Flash dryer	
Lübbers Anlagen-und Umwelttechnik GmbH Am Fliegerhorst 19 99947 Bad Langensalza www.luebbers.org	Spray dryer	
World Water Works, Inc. 4061 NW 3rd St. Oklahoma City OK 73107 USA www.worldwaterworks.com	Manufacturer of equipment for process and waste water treatment	DAF process modified specially for alga harvesting with very low energy input
NOVAgreen - PM GmbH Oldenburger Str. 330 49377 Vechta-Langförden www.novagreen-microalgae.com	Development, planning and manufacture of alga production plants and harvesting systems	

4 Cell decomposition and extraction[3]

4.1 Introduction

Algae produce a broad range of chemically valuable products (Spolaore et al. 2006).

The algal key compounds to the production of biofuels are lipids (Figure 4.1). They may comprise between 1 % and 50 % of the total solid content, depending on the alga species and the cultivation conditions (Demirbas et al. 2011). Of particular interest are the triglycerols (TAGs) and free fatty acids (FAs).

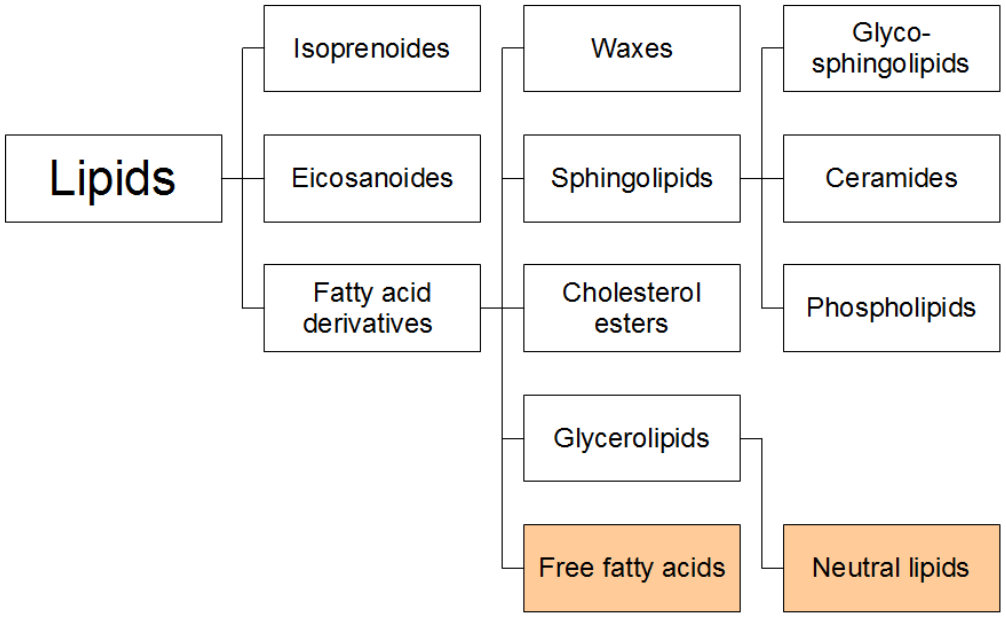


Figure 4.1 Overview of lipid classes according to (Ebermann and Elmadfa 2008)

A special case is the micro-alga *Botryococcus braunii*, which is distinguished by a very high content of long-chained (C30-C37), widely branched, unsaturated hydrocarbons – the so-called isoprenoids (HILLEN et al. 1982).

If the intracellular products are not released to the surrounding environment by the alga itself, one possibility is to genetically manipulate the cells so that they separate off produced valuable materials themselves; alternatively, the products can be extracted by means of physical, chemical and/or enzymatic methods (Chisti and Moo-Young 1986). The object of all decomposition and extraction techniques is to release as much oil as possible from the alga, taking into account the economic viability of the process. Environmental factors such as temperature, intensity and duration of illumination, the pH value of the nutrient medium, nutrients, CO_2 content etc. have a decisive influence on the chemical composition of the algae (Table 4.1).

[3] This section was authored by the Hochschule Lausitz (cell decomposition subsection) and the TU Bergakademie Freiberg (extraction subsection).

Table 4.1 Coarse chemical composition of selected alga species (% of dry matter) (Becker 1994)

	Protein	Carbohydrates	Lipids
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Scenedesmus obliquus	50-56	10-17	12-14
Spirulina maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9

Free fatty acids (FAs) and triglycerols (TAGs) can be separated from the algal biomass by a wide variety of methods (Amin 2009).

- Extraction by organic solvents
- Combinations of cold pressing and solvent extraction
- Extraction by supercritical solvents or
- Enzymatic extraction.

4.2 Cell decomposition

To be able to recover the cell compounds, they must be released into the surrounding medium (usually water). To do so, it is necessary to overcome the cell wall.

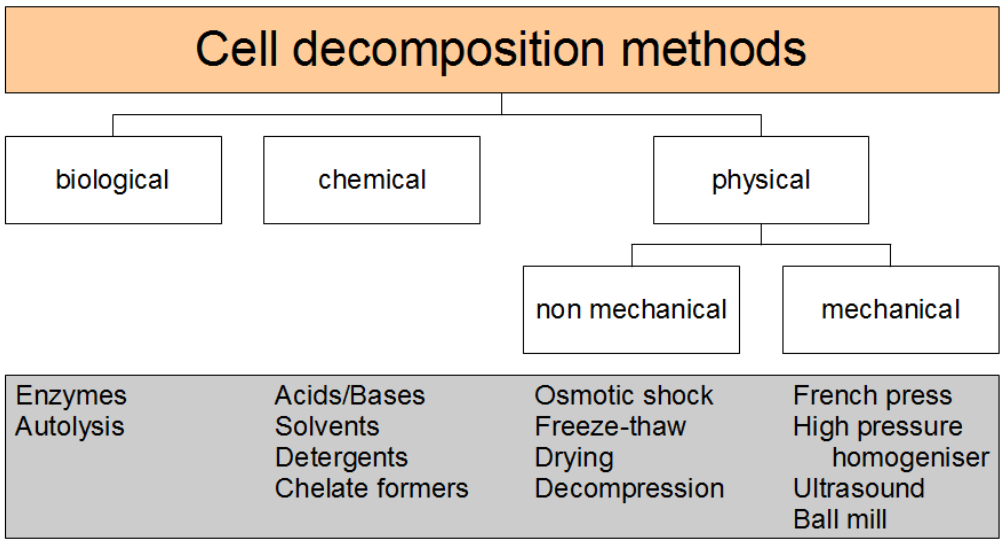


Figure 4.2 Selection of cell decomposition methods (adapted according to (Chisti and Moo-Young 1986), (Kampen; Middelberg 1995)

For effective cell decomposition, the various methods are subject to the following requirements (Kampen 2005):

- No product damage or denaturing
- High degree of decomposition
- No product contamination
- Cell decomposition apparatus capable of being sterilised
- Cell fractions easily separable
- Low energy demand
- Low time commitment
- Low investment cost

The literature describes a wide range of different methods for extracting valuable cell constituents from algae (Table 4.2.).

Table 4.2 Selected decomposition and extraction methods for algae

Method	Alga species	Main constituents of the oil	Literature source
Solvent extraction/ Saponification	*Porphyridium cruentum*	EPA, AA	(Guil-Guerrero et al. 2001)}
sc-CO_2	*Isochrysis galbana Parke*	EPA, DHA	(Perretti et al.)
Autoclaving Ball mill Microwave Ultrasound 10 % NaCl	*Chlorella sp.* *Nostoc sp.* *Tolypothrix sp.*	Oleic acid, linolenic acid	(Prabakaran and Ravindran 2011)
Ultrasound	*Anabaena cylindrica*	No data	(Simon 1974)
Autoclaving Ball mill Microwaves Ultrasound 10 % NaCl	*Botryococcus braunii* *Chlorella vulgaris* *Scenedesmus sp.*	Oleic acid	(Lee et al. 2010)
Pulverising Ultrasound Ball mill Enzymatic Microwaves	*Chlorella vulgaris*	Palmitic acid, oleic acid,	(Zheng et al. 2011)
Ball mill French Press Ultrasound Wet milling	*Scenedesmus dimorphus* *Chlorella protothecoides*	No data	(Shen et al. 2009)

The chemical treatment of alga cell with lyes is an effective method of decomposing the cell wall. It is not suitable for isolating sensitive products such as proteins, however, because denaturation occurs.

With the aid of lyes, free fatty acids can be recovered from micro-algae (Molina Grima et al. 2003). In this way, free fatty acids have been extracted from Porphyridium cruentum (Giménez Giménez et al. 1998) and Phaeodactylum tricornatum (Cartens et al. 1996) by direct saponification of the moist biomass with a KOH-ethanol mixture.

The use of antibiotics such as penicillin acts at the peptide formation level. This prevents the construction of an intact cell wall by disturbing the transpeptidation of the peptidoglycan strands (Berenguer et al. 1982). Industrial-scale application is unsuitable due to the high cost of antibiotics (Kampen 2005).

Chemical methods are not very selective, and when using lyes, acids or detergents there is a risk of permanent contamination of the desired product and of the product being destroyed (such as

denaturing of proteins). Owing to these problems, industrial application for the recovery of HVPs is rather unlikely.

Mechanical methods tear apart the cell walls of micro-organisms by creating stresses and strains. They are used mostly when the desired product is intracellular (inclusion bodies) and cannot diffuse out into the surrounding medium by damaging the cell wall (Kampen 2005).

Pressing and homogenisation are based on the exertion of pressure on the cell wall, while pulverisation is based on the use of grinding media (usually small balls). These methods offer the advantage that little contamination by external sources, such as solvents, occurs (Mercer and Armenta 2011).

High-pressure homogenisers essentially consist of a high-pressure reciprocating pump and a downstream pressure-relief valve. The pump generates a non-pressure-sensitive, virtually pulsation-free volume current which is pressed through a valve. In the valve, the liquid passes through a narrow gap, causing the flow rate to increase dramatically. After passing through the gap, the liquid is rapidly depressurised. In the valve the liquid is exposed to a wide variety of different forces, which decompose the cells (Greenwell et al. 2010).

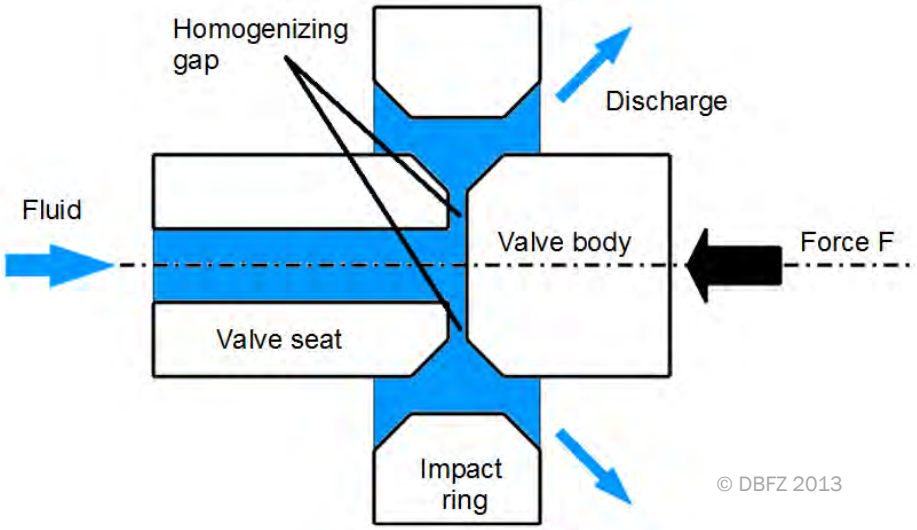


Figure 4.3 Schematic of a high-pressure homogeniser valve (DBFZ 2013)

Some research groups have investigated the influence of process parameters of high-pressure homogenisers on the decomposition of biomass (Doucha and Lívanský 2008; Kelly and Muske 2004).

Homogenisers are used in aquaculture to increase the absorption of carotenoids from *Haematococcus* for fish. The cell decomposition increases the bio-availability of the pigment. The lower the degree of decomposition, the lower will also be the bio-availability, resulting in less enrichment inside the fish (Molina Grima et al. 2004).

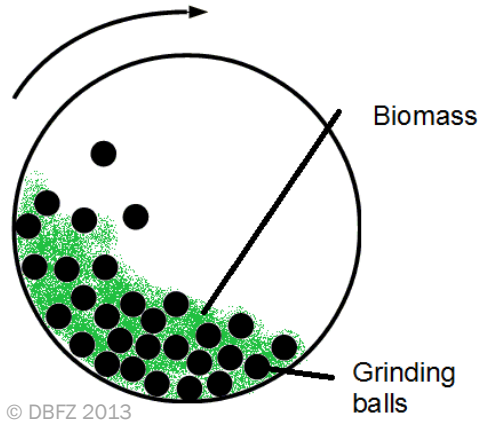


Figure 4.4 Principle of the ball mill

Cell decomposition by ball mill is one of the most effective techniques. Figure 4.4 illustrates the principle of the ball mill.

This assembly comprises a tubular vessel made of metal or thick glass containing the cellular suspension as well as small metal or glass balls. By rotating around their axis, the balls roll in the opposite direction to the direction of rotation of the vessel. At higher velocities, some of the balls move along the vessel wall before dropping back down onto the other balls and the cell mass. The cell decomposition is effected by the grinding motion due to the variously rolling and dropping balls. Mao et al. (Huihua Mao and Moo-Young 1990) deployed a new-style high-speed ball mill in order to decompose bakers' yeast as a model substance. Cells of *Chlorella vulgaris*, *Scenedesmus obliquus* and *Spirulina sp.* were decomposed using the ball mill (Hedenskog and Ebbinghaus 1972; Hedenskog and Enebo 1969). The energy input needed to decompose the cells depends heavily on the cell concentration and the thickness of the cell wall. Cell decomposition is most effective when the concentration is high and when the cell debris can be easily separated (Greenwell et al. 2010).

Ultrasound can likewise be used for cell decomposition (Furuki, et al., 2003; Luque-Garciá, et al., 2003). To do so, a sonotrode which generates the ultrasound waves is placed in the biomass suspension.

Usually 25 kHz is used for cell decomposition. The frequency used also depends on the types of cell. The sound waves alternately generate high-pressure cycles (compression) and low-pressure cycles (rarefaction). During the low-pressure cycles the ultrasound waves form small vacuum bubbles or cavities in the liquid (Hielscher Ultrasonics GmbH). When a certain volume is reached the bubbles cannot absorb any more energy and burst during a high-pressure cycle. As a result, mechanical energy is released in the form of shock waves which destroys the surrounding cells (Chisti and Moo-Young 1986). There are different sized sonotrodes depending on the volume. The sound may be either continuous or pulsed. This enables the constituents to be released into the solvent (Harun et al. 2010). An example is provided by Wiltshire et al. (Wiltshire et al. 2000), who were able to extract over 90 % of the fatty acids and pigments in the micro-alga *Scenedesmus obliquus* by means of ultrasound. Pernet and Tremblay (Pernet and Tremblay 2003) investigated the lipid extraction yield as a function of storage time and treatment method for the diatom *Chaetoceros gracilis*. Ultrasound was used for the complete extraction. It was found in this that ultrasound increases the extraction rate. In the case of the marine micro-alga *Crypthecodinium cohnii*, the ultrasound method improved the extraction rate from 4.8 % to

25.9 % (Mata et al. 2010). Although extraction of FAs and TAGs by means of ultrasonic is already being implemented on a laboratory scale, there is still a lack of adequate information regarding the feasibility or cost of its application on a commercial scale (Harun et al. 2010).

The pulsed electric field (PEF) (de Vito 2006) and supersonic fluid (SSF) (Tempest 1950) methods work in a similar way (Figure 4.5).

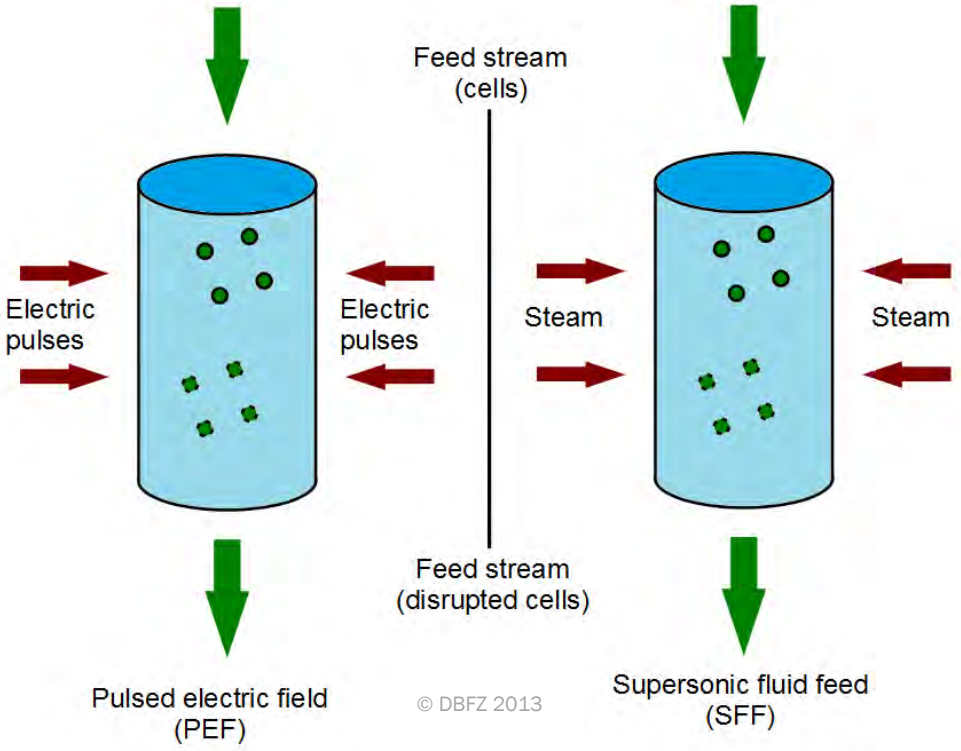


Figure 4.5 PEF and SSF methods

The OriginOil corporation has developed a process which combines ultrasound with electromagnetic pulses in order to decompose alga cells. CO_2 is then introduced into the algal suspension in order to lower the pH value. In the process the cell fractions sink to the bottom and the oil settles on the aqueous phase (Heger 2009).

Solvent extraction of algal biomass is used to extract metabolites such as astaxanthin, beta-carotene and essential fatty acids (Molina Grima et al. 2003). With the aid of organic solvents such as hexane, ethanol, acetone and diethyl ether fatty acids such as EPA, DHA or arachidonic acid can be extracted from *Isochrysis galbana* (Molina Grima et al. 1994; Robles Medina et al. 1995), *Porphyridium cruentum* (Giménez Giménez et al. 1998) and *Phaeodactylum tricornatum* (Cartens et al. 1996). For an efficient extraction process it is vital that the solvent should be able to penetrate fully into the cells and that the target molecule is soluble in the solvent (e.g. non-polar solvents for non-polar lipids) (Mercer and Armenta 2011). One possible way to make the cell constituents accessible to the solvent is to first mechanically decompose the biomass (Cooney, et al., 2009). Shen et al. (Shen et al. 2009) investigated the influence of various decomposition methods (wet milling, ball mill, French Press and ultrasound) with subsequent lipid extraction with hexane from *Scenedesmus dimorphus* and *Chlorella protothecoides*. They found that in the case of *S. dimorphus* wet milling with subsequent hexane extraction was the most effective in terms of lipid extraction (25.3 %). By contrast, soxhlet extraction

yielded only 6.3 % lipids. It was likewise shown that for *Chlorella protothecoides* a combination of ball mill with subsequent hexane extraction is the most suitable. Similar studies were conducted by Prabakaran and Ravindran (Prabakaran and Ravindran 2011). They used *Chlorella sp.*, *Nostoc sp.* and *Tolypothirx sp.* and decomposed them respectively by autoclaving, ball mill, microwave, ultrasound and 10 % NaCl (osmotic shock). The fatty acids were extracted with a chloroform-methanol mixture. The results showed that ultrasound decomposition was the most suitable and the highest amount of fatty acids was extracted from *Chlorella sp.*

In addition to the extraction methods described here, there are a number of others, though none of those have to date been implemented on a large scale. They include enzymatic degradation (Sander and Murthy 2009), osmotic shock (Hosono, 1991) and the use of microwaves (Pasquet et al. 2011).

Industrial-scale methods are mainly mechanical ones such as high-pressure homogenisers and ball mills (Chisti and Moo-Young 1986). For organisms with a highly resistant cell wall, a combination of different decomposition methods is advisable to make the process more economical, because a purely mechanical decomposition would be too cost-intensive (Becker 1983).

Considering the complete process from the alga to the product, high energy input is entailed in particular by harvesting, dewatering and extraction. There are currently not yet any technologies available which are targeted at algae.

At the 2011 International Algae Congress in Berlin, Brentner set forth a life cycle analysis comparing various extraction and conversion technologies (Table 4.3) (Brentner 2011).

Table 4.3. Comparison of various extraction and conversion methods to generate 10,000 MJ of biodiesel (Brentner 2011)

Parameter	**Press + co-solvent + esterification**	**sc-CO_2 extraction + esterification**	**Ultrasound + direct esterification**	**Supercritical methanol**
Extraction efficiency	91 %	95 %	-	-
Conversion efficiency	98 %	98 %	98 %	98 %
Power consumption Extraction (kWh)	59	1830	3190	-
Conversion (kWh)	10	10	-	141
Heat input				
Drying (MJ)	16360	-	14885	-
Extraction (MJ)	1000	-	-	-
Conversion (MJ)	225	225	400	7388
CED[4] (MJ)	**30280**	**8180**	**47200**	**6080**

This demonstrates that most of the energy consumption is caused by the drying. Similar results are also obtained by Dufour et al. (Dufour et al.).

To create an economically viable process it is essential to deploy and develop technologies capable of using moist biomass.

The assessment of a decomposition method is based primarily on the degree of decomposition. There are direct and indirect methods of measuring the success of cell decomposition.

Direct methods are based on counting of intact cells. The simplest way is to colour cells and subsequently carry out a microscopic analysis. For example, methylene blue (Bonora and Mares 1982) or grey colouring can be used to differentiate intact cells from damaged or dead ones (Middelberg 1995). This method is very involved and time-consuming however.

Indirect methods involve the measurement of released cell constituents. Examples of this include the release of proteins or of nitrogen, or measurement of the activity of a specific enzyme. The Bradford (Bradford 1976), Lowry (Lowry et al.) and BCA assays (SMITH et al. 1985) are the most commonly used methods of quantifying protein release. A comparison of the three methods is provided by Berges et al. (Berges et al. 1993).

The release of nitrogen can best be determined by the Kjeldahl method.

Which method is applied depends on the objective. Indirect methods are often quicker and easier to implement. Where a specific product is to be obtained, an assay developed specially for the product in question can be used. Direct methods are suitable for modelling a decomposition process. Indirect methods fail in terms of accuracy at higher product concentrations (Middelberg 1995).

[4] CED = Cumulative Energy Demand

4.3 Solvent extraction

The principle of extracting lipids from micro-algae is based on the basic chemistry concept of "like dissolving like". According to Halim et al. (Halim, et al., 2012) the extraction process can be divided into five steps:

1. Penetration of organic solvent through the organic solvent mixture
2. Interaction of organic solvent with the lipids
3. Formation of organic solvent-lipid-complex
4. Diffusion of organic solvent-lipids complex across the cell membrane
5. Diffusion of organic solvent-lipids complex across the static organic solvent film into the bulk organic solvent

The use of a specific solvent depends on various criteria:

- The targeted molecule needs to be soluble in the used solvent/solvent mixture
- Low boiling point
- Non-toxic
- Easy recovery of the solvent

Hexane is cited as a highly efficient solvent. It is characterised by high extraction capability and low cost (Harun et al. 2010). In addition, Fajardo et al. (Fajardo et al. 2007) investigated a two-stage process by which the lipid extraction could be improved. In the first stage ethanol was used as the solvent, and in the second stage hexane, in order to clean the extracted lipids and to attain oil yields of over 80 %.

Chloroform/methanol (1:2 v/v) is the most frequently used solvent system for lipid extraction from any living tissues. Folch et al. developed this method for isolating brain tissue. The residual water in algal cells acts as ternary component. That enables the complete extraction of both neutral and polar lipids. The use of a chloroform/methanol mixture leads to a fast and quantitative extraction of lipids but due to its toxicity chloroform implies a great environmental and health risk. Alternatively hexane/isopropanol (3:2 v/v) (HIP) has been suggested as a low-toxic substitute (Guckert, et al., 1988; Lee, et al., 1998; Nagle, et al., 1990). For micro-algae lipid extraction HIP showed more affinity towards neutral lipids compared to chloroform/methanol. Guckert and collegues attributed this towards HIP's inability to extract membrane-bound polar lipids (Guckert, et al., 1988).

Alcohols have a strong affinity towards membrane-bound lipid complexes as they are able to form hydrogen bonds. Their polar nature, however, limits the extractability of neutral lipids. For this reason, alcohols are usually combined with non-polar solvents such as hexane or chloroform (Halim, et al., 2011).

Studies on TAG extraction efficiency of single solvents and solvent mixtures from *Selenastrum rinoi* were carried out at the University of Applied Science at Senftenberg. The standard extraction method with chloroform/methanol was used as reference.

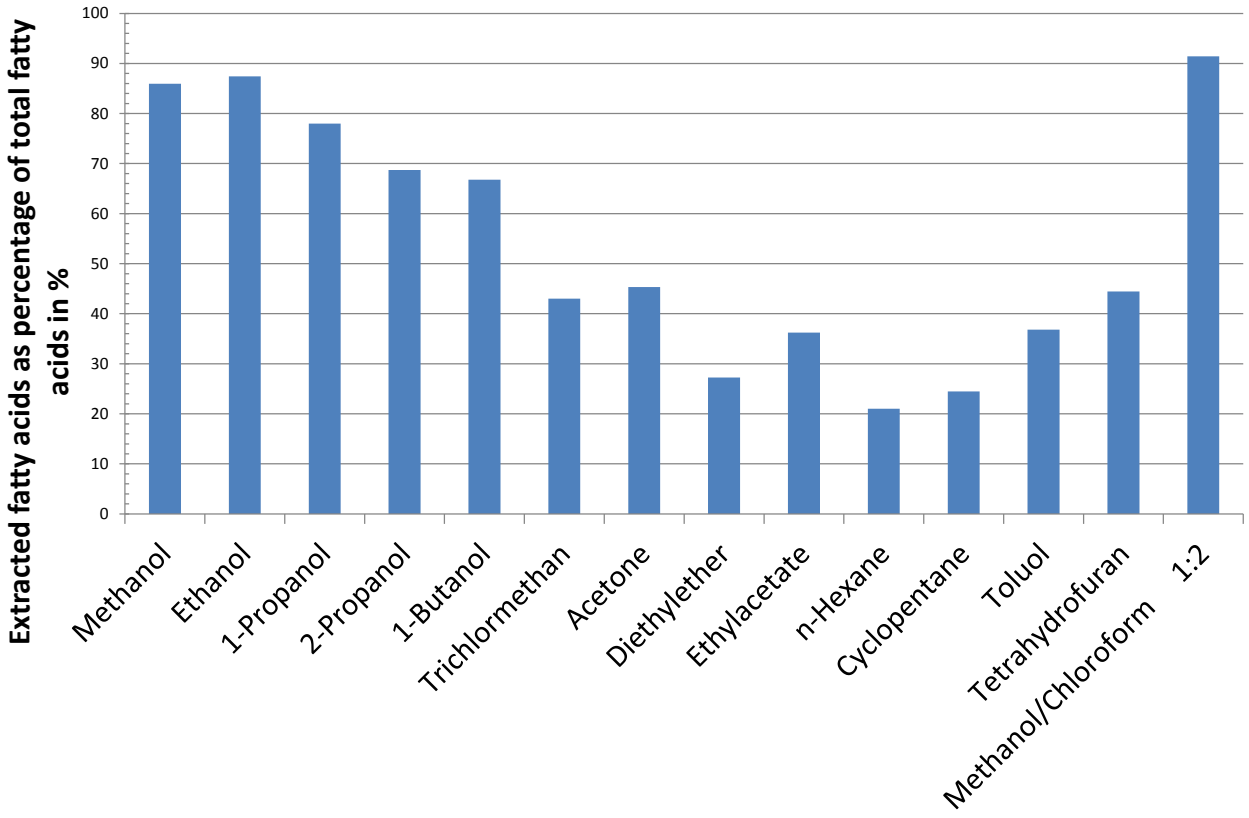


Figure 4.6 Solvent screening (alga*: Selenastrum rinoi*) (HS Lausitz 2013)

Compared to the chloroform/methanol extraction method especially short-chained alcohols show great potential for extracting TAGs from the used alga. Increasing of chain length resulted in a drop of the TAG content in the entire extract.

For solvent mixtures an increasing ratio of non-polar solvent leads to a higher TAG content in the extract when compared to the single solvents.

Studies, carried out by Nagle and Lemke, came to similar results. They evaluated lipid extracting efficiency of three solvents (1-butanol, hexane/isopropanol, ethanol) from *Chaetoceras muelleri* (Nagle, et al., 1990). The ternary water/methanol/chloroform mixture served as control. The control was found to be the most effective extraction mixture. Compared to this all used solvents were fairly effective at extracting a pure lipid product with 1-butanol being the best of all three.

Most of the laboratory-scale solvent extractions reported in literature were performed as batch-processes, although they are limited by lipid mass transfer equilibrium. Continuous extraction processes however require a large amount of solvent (Halim, et al., 2012).

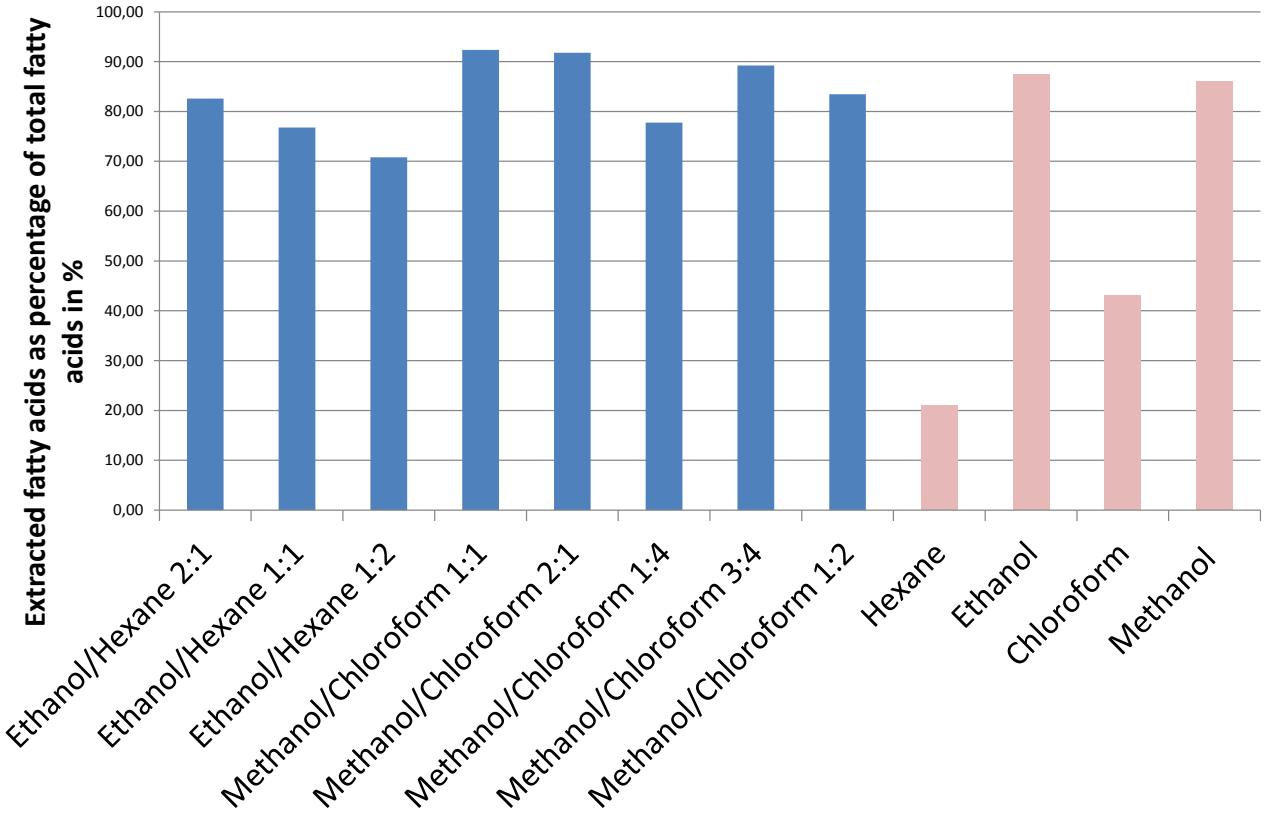


Figure 4.7 Solvent mixture screening (alga: *Selenastrum rinoi*) (HS Lausitz 2013)

A special form of solvent extraction is soxhlet extraction, which is used in particular on a laboratory scale. In a special apparatus (Figure 4.8) the FA and TAG are extracted by repeated washing (percolation) with the recycled organic solvent, such as hexane or petroleum ether (Oilgae 2011). As a result of the repeated evaporation and condensation of the solvent, uncharged solvent is always brought into contact with the material being extracted and the mass transfer equilibrium is by-passed.

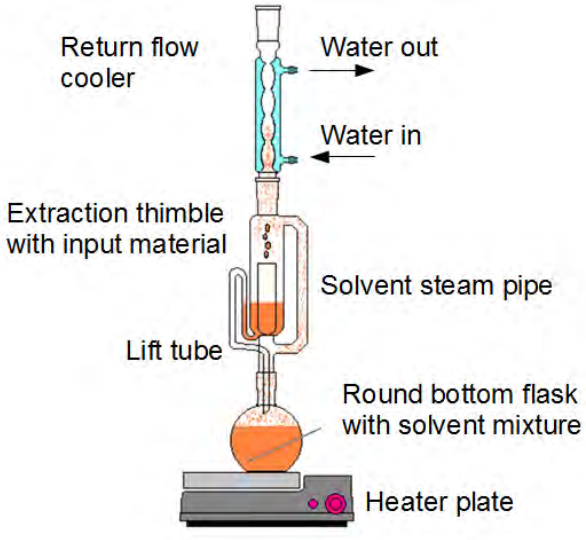


Figure 4.8 Soxhlet apparatus

Guckert et al. conducted a comparative study (using *Chlorella*) of various procedures featuring different solvents and techniques:

- Soxhlet extraction with methyl chloride/methanol,
- Extraction according to Bligh and Dryer (Bligh and Dyer 1959) with methyl chloride/methanol and
- Extraction with hexane/isopropyl/water (Guckert et al. 1988)

It was demonstrated that the procedures, as expected, led to differing lipid yields. They differ also, however, in their selectivity with regard to certain lipid classes (neutral lipids, glycolipids, polar lipids). Consequently, only the Bligh and Dryer method delivers maximum extraction for all lipid classes (Guckert et al. 1988). These results are less interesting for technical applications to produce FAs and TAGs, but certainly are of significance for biochemical analysis relating to lipid formation in the micro-alga *Chlorella*.

Frenz et al. subjected 18 organic solvents with differing polarity to screening for the production of hydrocarbons from a 'living' culture of the micro-alga *Botryococcus braunii* (Frenz et al. 1989). A key assessment criterion – alongside the highest possible hydrocarbon yield – was the so-called biocompatibility. This refers to the sustaining of photosynthesis activity in the extraction process. The studies showed that high hydrocarbon yields are attainable with strongly polarised solvents (alcohols, ketones). At the same time, however, the ability of the cells to regrow and again produce hydrocarbons following extraction is lost. By contrast, photosynthesis activity is largely retained when using weakly polarised or non-polar solvents (n-alkanes). Any water present inhibits the extraction process, so that only low oil yields are attained. If the algal biomass is dewatered (centrifuging, filtration) high extraction yields can be attained with weakly polarised solvents while at the same time maintaining biocompatibility (Frenz et al. 1989). Table 4.4 sets out the relevant results for seven solvents.

Table 4.4 Hydrocarbon yields and photosynthesis activity following extraction (Frenz et al. 1989)

Extraction agent	Yield[1)]	Activity[2)]
n-hexane	70.6 %	80.7 %
n-heptane	63.7 %	83.2 %
n-octane	64.8 %	86.7 %
n-dodecane	63.1 %	90.7 %
Dodecyl acetate	45.5 %	69.7 %
Dihexyl ether	61.5 %	68.3 %
Dodecanedioic acid-diethyl ester	47.5 %	29.2 %

[1)] Hydrocarbon yield referred to total hydrocarbon content (dry)
[2)] Photosynthesis activity (24 h after solvent treatment) referred to original activity

A procedure of the kind is interesting for continuous or periodic removal of products from living cell cultures and offers the following advantages:

- Improved cost-effectiveness and
- Fewer inhibiting influences on the product (Frenz et al. 1989).

4.4 Supercritical fluid extraction

The use of organic solvents does, however, pose a serious environmental hazard. For this reason, supercritical fluid extraction (SFE)[5] has been continually advanced in recent years.

In Table 4.5 conventional solvent extraction is compared with sc-CO_2[6] extraction.

Table 4.5 Comparison of supercritical CO_2 and conventional solvent extraction (Hosikian et al. 2010; Mercer and Armenta 2011)

Solvent extraction	Supercritical CO_2
Solvent use required Minor residues possible in the product	No solvent required High-purity products
Heavy metal and salt load	Extract is free of heavy metals and salts
Low selectivity Polar and non-polar dyes can be extracted	High selectivity Polar substances are not extracted
Removal of the solvent is necessary→Additional equipment = product loss + higher cost	No additional equipment required, high yields possible

When a fluid is in a state in which the pressure and temperature are above the critical values ($T > T_c$, $p > p_c$), it is designated as supercritical. Under these conditions liquids and gases can no longer be differentiated

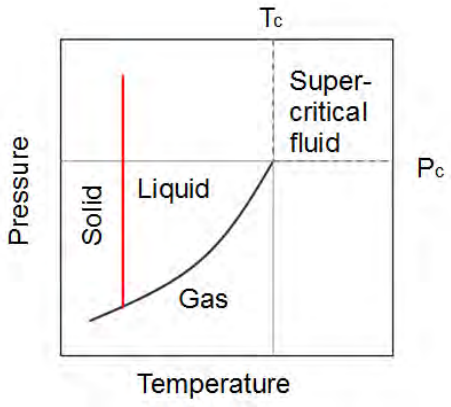


Figure 4.9 Phase diagram for a pure component (schematic according to Herrero et al. 2006)

[5] SFE: Supercritical Fluid Extraction
[6] sc-CO_2: Supercritical CO_2

Supercritical fluids have properties of both gases and liquids. As a solvent, they exhibit a number of special features which make them interesting compared to conventional solvents (Stahl et al. 1987):

- Low-cost mass transport (density like a liquid, low dynamic viscosity, high diffusion coefficient (Randall 1982)
- Simple phase separation of extract and solvent
- High selectivity based on adjustment of pressure and temperature

A characteristic of a supercritical fluid is that the density of the fluid can be changed by a change in pressure and/or temperature. Since density is directly correlated with solubility, the solvent strength of a fluid can be adjusted by changing the pressure. Further advantages of the method lie in its higher selectivity and shorter extraction times (Herrero et al. 2006).

Table 4.6 presents a selection of gases used.

Table 4.6 Physical-chemical data of some gases used for extraction (Stahl et al. 1987)

Gas	Tc (°C)	pc (MPa)	ρc (g/cm3)
CO_2	31.0	7.29	0.47
Propane	96.8	4.24	0.22
Ethane	32.2	4.82	0.20
CHF_3	25.9	4.69	0.52

The most important gas for industry is carbon dioxide (CO_2). Its use entails a number of advantages (Stahl et al. 1987):

- CO_2 is physiologically unharmful
- CO_2 is germ-free and bacteriostatic
- CO_2 is not combustible and not explosive
- CO_2 is available in large quantities

Owing to its low polarity, CO_2 is less effective for the extraction of strongly polarised natural materials. The polarity can be increased by adding so-called entrainers. Entrainers are usually strongly polarised substances which, when added to the CO_2 stream, contribute to a much changed solution characteristic of the supercritical CO_2 (Valcárcel and Tena 1997).

The main areas of application for extraction with supercritical fluids are the food industry (production of aromatics, oils and essential fatty acids) and agriculture (32 %), followed by the fuel industry with 24 % (Herrero et al. 2006). SFE is employed, among other applications, to decaffeinate coffee, to obtain hop extracts and for the extraction of spices (Igl and Schulmeyr 2006).

The extraction of value materials from algae by means of supercritical CO_2, in particular chlorophyll and carotenoids such as zeaxanthin, canthaxanthin and astaxanthin as well as beta-carotene, is described in the literature (Mendes et al. 1995; Hosikian et al. 2010; Valderrama et al. 2003; Char et al. 2011;

Grierson et al. 2012; Andrich et al. 2005; Herrero et al. 2006; Mendes 2003; Liau et al. 2010). The advantage of this method is that components can be selectively extracted from the biomass. The biomass must first be dried, however, which entails considerable energy input.

Mendes et al. (Mendes 2003) investigated supercritical fluid extraction with CO_2 on different micro-algae. In the case of *Botryococcus braunii*, the long-chained hydrocarbon components (alkadienes and trienes) could be extracted by means of supercritical CO_2 (at a constant temperature of 40 °C). Studies showed that the solubility of the components in CO_2 is dependent on the pressure and an optimum in terms of yield and extraction rate is attained at 30 MPa.

From freeze-dried *Chlorella vulgaris* Mendes and colleagues extracted carotenoids and other lipids with supercritical CO_2 at temperatures in the range from 40 °C to 55 °C and at pressures between 1 MPa and 35 MPa. They also conducted comparative studies at 35 MPa and 55 °C in order to determine the dependency of the yields on the comminution state – complete, slightly comminuted and fully comminuted alga cells (Mendes et al. 1995; Mendes 2003). The yield from the components increases as a result of the mechanical loading of the cells. At constant temperature and rising pressure, higher lipid and carotenoid yields are achieved. The temperature has little influence on the extraction yield in the analysed pressure range. An exception to this is to be noted at 20 MPa. There a rise in temperature leads to a fall in carotenoid yield. Two thirds of the extracted carotenoids comprise astaxanthin and canthaxanthin (35 MPa and 55 °C).

The species *Dunaliella salina* is rich in beta-carotene (up to 14 % of its dry matter), which is present as a mixture of cis- and trans-isomers. For pharmaceutical applications, especially, it is important no toxic organic solvents are used in the separation process. Extraction with supercritical CO_2 improved the cis/trans ratio as compared to extraction with acetone. An optimum beta-carotene yield was attained at 30 MPa and 40 °C (Mendes 2003).

The micro-alga *Arthrospira (Spirulina) maxima* , along with other lipids, is capable of producing high γ - linolenic acid (GLA) content. The studies by Mendes et al. (Mendes 2003) showed that the highest GLA yields are attained by supercritical extraction at 35 MPa and 60 °C with a mixture of CO_2 and 10 % ethanol (molar) (0.44 % GLA referred to dry matter).

Liau and colleagues (Liau et al. 2010) also studied supercritical extraction with CO_2 and the co-solvent ethanol (*Nannochloropsis oculata*). Adding a co-solvent improves the extraction rate of lipids and carotenoids. Table 4.7 presents examples of the use of other agents for supercritical extraction of micro-algae (Herrero et al. 2006).

Table 4.7 Examples of studies on supercritical extraction of micro-algae with different extraction agents

Extraction agent	Micro-alga used	Extracted component	Reference
Water	*Spirulina platensis*	*Anti-oxidants*	(Herrero et al. 2006)
Ethanol	*Haematococcus pluvialis and Dunaliella salina*	Carotenoids	(Denery et al. 2004)

4.5 Recovery of other compounds

Grima et al. investigated various solvents for the extraction of polyunsaturated fatty acids (PUFAs) from the micro-alga *Isochrysis galbana*. The extraction was carried out under nitrogen atmosphere, in order to avoid auto-oxidation and degradation of the PUFAs . Among the findings from this was that by adding KOH to the solvent the PUFAs can be obtained directly by way of saponification/hydrolysis. The highest yield, of 81 %, was attained for the hexane/ethanol mixture (at a volume ratio of 1 : 2.5) followed by 79.8 % with ethanol. The yields from extraction of the lipids with subsequent acid hydrolysis to recover the PUFAs are generally somewhat higher, though extraction with direct saponification can cut costs and shorten operating times (Molina Grima et al. 1994).

Medina et al. compare various methods of recovering and cleaning high-purity polyunsaturated hydrocarbons, particularly the ω-3 and ω-6 fatty acids. The two main concentration and cleaning methods are urea fractionation and HPLC. Other methods, including supercritical fluid extraction, are also described (Medina et al. 1998).

A simple downstream process for the recovery of high-purity PUFAs from micro-algae is described by Grima et al. The process comprises the following steps (Molina Grima et al. 1994):

- Concentration of the biomass in the culture medium
- Extraction of the fatty acids by direct saponification of the moist biomass with KOH-ethanol
- Extraction of the non-saponifiable components with hexane
- Concentration of the PUFAs by the urea method
- Isolation of the desired PUFAs by preparatory HPLC

In addition to the polyunsaturated fatty acids, other key constituents of the micro-algae can be obtained by means of solvent extraction, including the carotenoids astaxanthin or beta-carotene (Brennan and Owende 2010).

Tchorbanov and Bozhkova (Tchorbanov and Bozhkova 1988) investigated extraction with organic acids as a pre-treatment for the enzymatic hydrolysis of cell proteins for the alga species *Chlorella* and *Scenedesmus*. Removing the lipophile components improved the enzyme substrate contact, resulting in higher protein yields. A further advantage is that the lipophile extract in addition to chlorophylls contains a wide variety of biologically active components such as vitamin E, pigments, eicosapentaenoic acid (EPA), chondrilla-sterine, beta-carotene, etc. Extraction with more strongly polarised organic solvents results in better yields of hydrolysed proteins. The highest lipophile extract yield was achieved with ethanol as the extraction agent.

5 Hydrothermal liquefaction (HTL)[7]

5.1 Introduction

This section investigates the method known as hydrothermal liquefaction (HTL) in detail, and gives an estimate as to its potential for the processing of algal biomass.

HTL is regarded as a highly promising technology for treating a wide range of different biomasses and extracting valuable products from then, such as bio-oil. It was originally developed for coal liquefaction. Nowadays the term "hydrothermal liquefaction" is applied to all thermochemical processes which produce a tar or oil type product (Peterson et al. 2008; Kruse 2011).

In hydrothermal liquefaction, moist biomass is subjected to moderate temperatures (250 350 °C) and pressures of approximately 5 20 MPa (Peterson et al. 2008).

Close to the critical point (T_c = 374 °C; p_c = 22 MPa) water possesses a number of interesting properties (Table 5.1). Its low viscosity and high solubility for organic matter, in particular, makes near-critical water an excellent medium for fast, efficient reactions (Kruse 2011).

Table 5.1 Water properties under selected conditions (adapted according to Toor et al. 2011)

	Normal conditions	Near-critical water		Supercritical water	
Temp. (°C)	25	250	350	400	400
Pressure (MPa)	0.1	5	25	25	50
Density (g*cm-^{3})	1	0.80	0.6	0.17	0.58
Dynamic viscosity (mPa*s)	0.89	0.11	0.064	0.03	0.07

The biomass, primarily consisting of carbohydrates such as cellulose and hemicellulose, as well as other components including lignin, salts and proteins, is decomposed with the aid of the water. Highly molecular compounds are converted into short-chained carbon compounds. In this, a large number of different (chemical) reactions determine the composition of the product mix. They include solvolysis, depolymerisation, decarboxylation, hydrogenolysis and hydrogenation (Balat 2008; Demirbas 2000).

HTL offers a number of advantages over other thermochemical processes:

- Presence of water: Biomass often contains large amounts of water. It normally has to be removed at the expense of energy (such as for pyrolysis, extraction). In HTL water is usually the reaction medium (Peterson et al. 2008)
- Diversity of products: The conversion of biomass by HTL into products including biodiesel (Mata et al. 2010), bio-oil (Minowa et al. 1995; Duan and Savage 2011; Xu and Lad 2008), hydrogen (Ni et al. 2006; Kong et al. 2008) is described in the literature.

[7] This section was authored by the Hochschule Lausitz.

5.2 State of the art of hydrothermal liquefaction

In order to gain an overview of the current state of the art in HTL, various databases were analysed with regard to the topic. The terms "hydrothermal liquefaction", "hydro liquefaction" and "deoxy liquefaction" proved suitable filters. Based on these keywords, various literature sources were identified. The evaluation of the research results was presented in a table. Various parameters were applied to break down the individual results.

Bio-mass	Temperature-range (°C)	Pressure-range (MPa)	Cata-lyst	Reaction-time (min)	Atmo-sphere	Solvent	yield (% (m/m))				Com	Source
							Oil	Water soluble	Solidf	Gas		

Figure 5.1 shows the table header. The table is included as appendix Overview of HTL.

Bio-mass	Temperature-range (°C)	Pressure-range (MPa)	Cata-lyst	Reaction-time (min)	Atmo-sphere	Solvent	yield (% (m/m))				Com	Source
							Oil	Water soluble	Solidf	Gas		

Figure 5.1 Table header

The literature sources found were analysed with regard to the biomass used, the temperature and pressure range, the catalyst used and the reaction atmosphere. The research results were then broken down by bio-oil, water-soluble substance, solid matter and gas content. The values presented were either cited explicitly in the relevant publications or were taken from the diagrams depicted in them, so allowing for a degree of interpretation.

The following considers some of the key literature sources in detail. It must be stated as a general principle, however, that any direct comparison between individual research results is possible only to a limited extent. The reasons for this are (Behrendt et al. 2008):

- Differing definitions of liquid and solid yield
- The system pressure during the process is often not specified
- The product preparation prior to analysis varies between the individual research groups

Intensive research was conducted into hydrothermal liquefaction as far back as the late 1970s and 1980s, driven by the impending shortages of natural energy source materials such as petroleum, with the aim of developing alternative energies (Elliott 2007).

Various research groups have set out to investigate the influence of different parameters on the reaction products, from the input biomass, through the reaction temperature, to the use of a wide variety of catalysts.

One of the first studies conducted was by Kranich (Kranich and Eralp 1984), using so-called "municipal solid waste" (MSW). The study investigated the conversion of this biomass into bio-oil in the temperature range from 300 °C to 425 °C at 14 MPa. The reaction times ranged from 20 to 90 min.

The Itoh group (Itoh et al. 1994) discovered that, at 300 °C and 10 MPa, 48 % of the inputted sewage sludge was converted into heavy oil. According to the researchers' estimates, 1.5 t/d of the separated heavy oil might be recorded as energy production from 60 t/d of input biomass.

The hydrothermal treatment of MSW exhibits not only a high conversion rate, but also produces high bio-oil yields (Hammerschmidt et al. 2011; Goto et al. 2004; He et al. 1999; Vardon et al. 2011).

HTL bio-oil contains a large number of chemical compounds including branched and unbranched aliphatic components as well as aromatic structures such as phenols, furfurals etc. (Russell et al. 1983). In order to qualitatively and quantitatively characterise the compounds contained in it, it is necessary to identify degradation mechanisms of the biomass. Biomass is a complex material which primarily consists of lignin (10 to25 m/m %), cellulose (40 to 80 m/m %) and hemicellulose (15 to 30 m/m %) (Klass 2004; McKendry 2002). In principle this can be used to trace back the degradation of all biomass to one of the following processes, in order to derive appropriate reaction mechanisms:

- Liquefaction of cellulose/hemicellulose
- Liquefaction of lignin

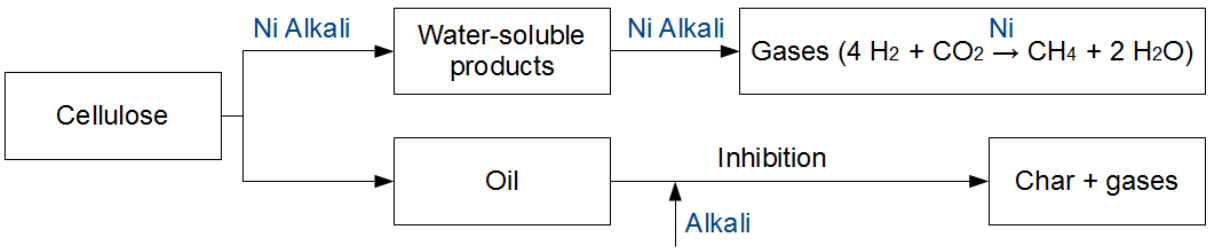


Figure 5.2 Proposed model of cellulose degradation and the role of various catalysts during HTL (adapted according to Minowa et al. 1998; Fang et al. 2004)

Minowa et al. (Minowa et al. 1998) and Fang et al. (Fang et al. 2004) conducted experiments with cellulose using alkali and nickel catalysts (Figure 5.2). The reaction was carried out in the temperature range 200 350 °C in a conventional autoclave with a magnetic agitator. To prevent the water from evaporating, the system was flushed with nitrogen and then a 3 MPa gas cushion was created. The results led to the conclusion that the cellulose degrades above a temperature of around 200 °C. The highest oil yield was attained at 280 °C. A further temperature rise resulted in increased tar and gas content, with a reduction in oil content. Using alkali catalysts resulted in a low tar content, implying that alkali salts prevent secondary degradation reactions of the oil. By contrast, nickel catalysts might favour gas formation based on the steam reforming reaction (Figure 5.2). The Kamio group (Kamio et al. 2008) confirmed by experimentation that above 240 °C the hydrolysis of cellulose dramatically increases.

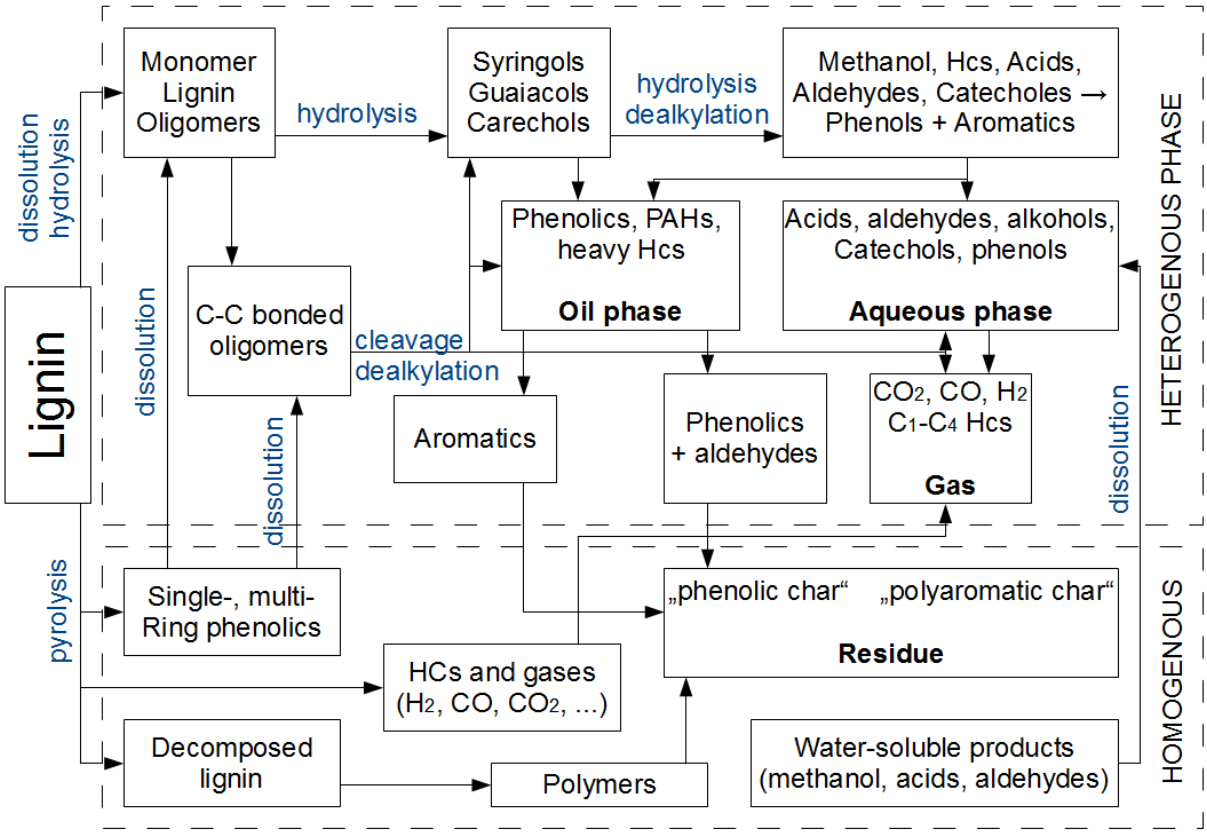


Figure 5.3 Proposed model for the degradation of lignin (adapted according to Fang et al. 2008)

Hydrothermal application to lignin in various solvents has been documented by a wide range of publications (Fang et al. 2008; Okuda et al.; Brebu and Vasile 2010). Fang et al. (Fang et al. 2008) investigated the degradation of lignin in a phenol-water mixture at 400 to 600 °C and approximately 57 100 MPa. Based on the analysed reaction products, they developed a model setting out possible reaction paths for the degradation of lignin (Figure 5.3).

Cellulose and other polymer structures are split into monomer units under hydrothermal conditions. The hydrolysis of glucose (and fructose) has already been investigated by numerous research groups (Knežević et al. 2009; Barbier et al. 2011).

They all found that under hydrothermal conditions the glucose was completely degraded (Table 5.3).

Table 5.2 shows a number of degradation products of glucose/fructose which may be produced under HTL conditions.

The investigation of model substrates such as cellulose, fructose and glucose with regard to their behaviour during hydrothermal treatment provides valuable information on possible degradation and conversion mechanisms. By contrast, applying the findings obtained to complex structured biomass entails some difficulties. Alongside the examples set out thus far, there have been numerous HTL studies into the reaction of various biomasses in near-critical water (Table 5.3).

Table 5.2 Possible degradation products of glucose/fructose under hydrothermal conditions

Degradation product	Literature source
Formic acid	(Lu et al.)
1.6-anhydroglucose	(Xiang et al. 2004)
o-, m-, p-cresol	(Russell et al. 1983), (Barbier et al. 2011)
Dihydroxyacetone	(Sasaki et al. 2000)
Erythrose	(Sasaki et al. 2000)
Acetic acid	(Xiang et al. 2004)
Fructose	(Antal et al. 1990), (Xiang et al. 2004)
Furfural	(Sasaki et al. 2000)
Glycolaldehyde	(Knežević et al. 2009)
Glyceraldehyde	(Knežević et al. 2009)
5-HMF	(Antal et al. 1990)
Levulinic acid	(Chang et al. 2006), (Knežević et al. 2009)
1-methyl-2-ethyl benzene	(Russell et al. 1983)
Lactic acid	(Knežević et al. 2009)
Phenol	(Russell et al. 1983)

Table 5.3 Results of hydrothermal treatment of various biomasses

Substrate	Reaction conditions	Oil properties	Literature source
Pig dung	250-305 °C 6.9-10.3 MPa 120 min Reducing atmosphere (CO)	Yield: 63 % HHV: 30.5 kJ/kg Composition: C: 65-68 %, H: 8-10 %	(He et al. 1999)
Eucalyptus	150-350 °C Atmospheric pressure	Yield: 1-38 %	(Sugano et al. 2008)
Wood	200-300 °C No pressure documented K_2CO_3 as catalyst	Yield: 6-36 %	(Karagöz et al. 2006)
sewage sludge	260-400 ° 7-13 MPa Solvent: Ethanol Alkali- and iron-containing catalysts	Yield: 28-57 %	(Li et al. 2010)
Switchgrass	235-260 °C 20 min K_2CO_3 as catalyst	Yield: 39-51 %	(Kumar and Gupta 2009)

5.3 Hydrothermal liquefaction with algae

In the report the focus of HTL applications is on the use of micro-algae as substrate. Owing to their high growth rate and efficient CO_2- fixing, algae are the focus of attention in relation to the production of propellants (Tsukahara and Sawayama 2005). There are only a few research works into the hydrothermal liquefaction of micro-algae however. Dote et al. (Dote et al. 1994) were among the first to apply HTL to *Botryococcus braunii* with and without catalyst (Na_2CO_3). At 300 °C bio-oil yields of 57 to 64 % were obtained. The quality of the oil roughly matched that of petroleum.

Dunaliella tertiolecta was treated by Minowa et al. (Minowa et al. 1995) under HTL conditions (300 °C, 10 MPa) likewise with and without Na_2CO_3 as catalyst. The average oil yield was 37 %. Altering the reaction parameters (temperature, time, Na_2CO_3 content) resulted in no significant increase in proportionate oil content. However, the chemical composition of the oil was heavily dependent on the reaction temperature. The gas phase mainly consisted of carbon dioxide. A further component of the reaction mixture was a tar-like substance which floated on the surface of the aqueous phase and so could be easily separated.

Yang et al. (Yang et al. 2011) investigated the liquefaction of *Dunaliella salina* with bifunctional Ni/REHY catalysts in ethanol and under H_2 gas in moderate conditions (200 °C, 2 MPa, 60 min) in order to determine the influence of the catalyst on the bio-oil yield and composition.

Dunaliella tertiolecta and *Botryococcus braunii* were likewise hydrothermally treated by Sawayama and colleagues (Sawayama et al. 1999) and subsequently investigated in terms of energy balance sheet and CO_2 reduction. They concluded from the results that micro-algae with higher lipid content are better suited than micro-algae with low lipid content.

Consequently, many research groups concentrated on the use of stocks with high lipid content. Lipid production is linked to stress situations, such as shortage of nitrates. This results in lower biomass productivity. The cultivation of such algae is usually more time-consuming and expensive, which is why Yu et al. see advantages in the use of algae with low lipid content in terms of large-scale industrial production (Yu et al. 2011).

The liquefaction of *Spirulina* has been the subject of several experiments (Jena et al. 2011a; T. Suzuki T. Matsui C. Ueda N. Ikenaga 2006; Ross et al. 2010; Huang et al. 2011).

The focus of the studies by Ross et al. (Ross et al. 2010) was on the use of alkali compounds and organic acids (formic acid, acetic acid) in the liquefaction of *Spirulina* and *Chlorella vulgaris*. Compared to the alkali catalysts, higher bio-oil yields were attained in the presence of the acids, and the yields increased as the temperature rose. The group likewise found higher bio-oil production in the case of *Chlorella* than with *Spirulina* algae which – similar to Sawayama previously (Sawayama et al. 1999) – led them to the conclusion that the bio-oil yield rises as the lipid content of the alga increases. Adding the organic acids caused the nitrogen content in the aqueous phase to decrease and the proportion of NH_3 and HCN in the gas phase increased. The nitrogen content of the oil phase remained unchanged.

Suzuki et al. (T. Suzuki T. Matsui C. Ueda N. Ikenaga 2006) investigated the hydrothermal liquefaction of *Spirulina* in various organic solvents (tetralin, 1 methyl naphthalene, toluene) or water under hydrogen, nitrogen or carbon monoxide atmosphere in a temperature range of 300 to 425 °C. The

uncatalysed reaction delivered a conversion rate of more than 90 % and 60 % oil content. Adding $Fe(CO)_5$ S as a catalyst increased the bio-oil yield from 52 % to 67 % at 350 °C with 60 minutes in tetralin. By comparison, hydrothermal treatment in water at 350 °C under hydrogen atmosphere without catalyst produced an oil yield of 78 %.

Since the reaction conditions in the liquefaction of biomass are similar to those in the hydrothermal liquefaction of coal, Ikenaga et al. (Ikenaga et al. 2001) conducted experiments in the combined liquefaction of various micro-algae (*Chlorella*, *Spirulina* and Littorale) with coal (Australian Yallourn brown coal and Illinois No. 6 coal) under H2 atmosphere in 1-methyl naphthalene at 350 to 400 °C for 60 min with various catalysts ($(Fe[CO]_5)$ S, $((Ru_3[CO]_{12})$, $(Mo[CO]_6)$ S). All three catalysts were suitable for the combined liquefaction of micro-algae and coal. At 400 °C and with a surplus of sulphur relative to iron (S/Fe = 4), using 1:1-*Chlorella* and Yallourn coal a conversion rate of 99.8 % and 65.5 % oil yield could be attained. A similar trend was observed in the liquefaction of Littorale and *Spirulina* in the presence of iron-containing catalysts.

A summary of all publications relating to the liquefaction of micro-algae discovered to date is presented in Table 5.5.

In some experiments macro-algae were also used (Anastasakis and Ross 2011; Zhou et al. 2010). The results of those experiments are not considered here.

Drawing up mass and energy balances for hydrothermal liquefaction poses a major problem. Only a small number of research groups are investigating the process with regard to its efficiency (Minarick et al. 2011). Sawayama et al. (Sawayama et al. 1999) applied hydrothermal liquefaction to *Botryococcus braunii* and *Dunaliella tertiolecta* and compared the respective oil yields and their calorific values. *Botryococcus* produced more oil with a lower calorific value compared to the micro-alga *Dunaliella* (Table 5.4). The researchers concluded from this that the energy inputs for the cultivation and separation of *B. braunii* must likewise be lower than for the comparative alga. Based on these calculations, *B. braunii* is more suitable for oil production than *D. tertiolecta*.

Table 5.4 Oil yield and energy consumption rate of oil production in hydrothermal liquefaction of micro-algae (Tsukahara and Sawayama 2005)

	Oil yield (%)	Energy for HTL/ Energy of produced oil
Botryococcus braunii	64	0.15
Dunaliella tertiolecta	42	0.34

The elemental composition and calorific value of the bio-oil HTL obtained is presented in Table 5.7.

Table 5.5 Overview of the hydrothermal liquefaction of various micro-algae

Micro-alga	**Reaction conditions**	**Oil properties**	**(Main) components of the oil**	**Literature reference**
Nannochloropsis sp.	200-500 °C 60 min	Max. yield: 43 % (at 350 °C) HHV = 39 MJ/kg	Phenol and alkyled derivative, heterocyclic N-compounds, long-chained fatty acids, alkanes, alkenes, derivatives of phytol and cholesterol	(Brown et al. 2010)
Chlorella pyrenoidosa	200-300 °C 0-120 min	Max. yield: 43 % (at 280 °C)	No data	(Yu et al. 2011)
Nannochloropsis sp.	350 °C Pd/C, Pt/C, Ru/C, Ni/SiO_2-Al_2O_3, $CoMo/\gamma$-Al_2O_3 and zeolite as catalysts	35-57 %	Fatty acids, phenol derivatives, long-chained alkanes	(Duan and Savage 2011)
Dunaliella salina	200 °C 2 MPa 60 min REHY/Ni-REHY as catalysts Ethanol as solvent	35-72 % HHV = 30.11 MJ/kg	Ester, glycerine,	(Yang et al. 2011)
Chlorella vulgaris ***Spirulina***	300-350 °C 60 min Alkaline, Na_2CO_3, K_2CO_3 and organic acids as catalyst	9-20 % HHV: 33.4-39.9 MJ/kg	Hydrocarbons, nitrogen-containing heterocycles, long-chained fatty acids, alcohols	(Ross et al. 2010)
Dunaliella tertiolecta	250-340 °C 5-60 min Na_2CO_3 as catalyst	37 % HHV = 36 MJ/kg	No data	(Minowa et al. 1995)
Spirulina platensis	200-380 °C 0-120 min	18-40 % HHV: 34,7-39.9 MJ/kg	C16-C17, phenol derivatives, carbon acids, esters, aldehydes, amines, amides	(Jena et al. 2011a)
Spirulina	300-425 °C Various solvents. $Fe(CO)_5$-S	60 %		(T. Suzuki T. Matsui C. Ueda N. Ikenaga 2006)

Micro-alga	**Reaction conditions**	**Oil properties**	**(Main) components of the oil**	**Literature reference**
Spirulina	260-400 °C 7-13 MPa Alkali- and iron-containing catalysts Ethanol as solvent	32-52 % HHV>32 MJ/kg	Mainly fatty acid ethyl esters and methyl esters	(Huang et al. 2011)
Chlorella Spirulina Littorale + coal	300-400 °C 60 min $Fe(CO)_5$-S, $Mo(CO)_6$-S, $Ru_3(CO)_{12}$ 1-methyl naphthalene as solvent	5-82 %	No data	(Ikenaga et al. 2001)

Table 5.6 Overview of the elemental composition and calorific value of the bio-oil HTL

Alga		*Spirulina*	*Nanno-chloropsis sp.*	*Spirulina*	*Chlorella vulgaris*	*Nanno-chloropsis sp.*	*Dunaliella salina*	*Botryococcus braunii*
Temp. (°C)		*350*	*350*	*350*		*350*	*300*	*300*
Press. (MPa)		*5*	*No data*	*No data*		*No data*	*No data*	*No data*
Elemental composition (%)	C	57.3	75.3	75.4	73.6	76.0	72.1	84.2
	H	7.4	10.2	10.8	10.7	10.3	8.3	14.9
	O	28.5	9.18	8.7	10.7	9.0	12.9	0.0
	N	6.8	4.18	4.6	4.9	3.9	6.7	0.9
	S		0.84	0.5	<0.2	0.89		
	K							
	P							
Catalyst		without	without	Na_2CO_3		without	without	without
Calorific value (MJ/kg)		26.0	38.5	34.8	37.1	39	34	50
Source		(Matsui et al. 1997)	(Duan and Savage 2011)	(Ross et al. 2010)		(Brown et al. 2010)	(Minowa et al. 1995)	(Dote et al. 1994)

5.4 Pilot and demonstration plants

The currently high investment costs and the economic unfeasibility of thermo-liquefaction pose a major problem in terms of the commercialisation of the process. Nevertheless, there are a number of scale up and demonstration plants (Table 5.7).

Table 5.7 Overview of HTL processes in pilot plants (Toor et al. 2011)

Process	Developer	Country	Biomass	Temp. (°C)	Press. (MPa)	Plant capacity	Oil yield (%)
HTU® (hydrothermal upgrading)	Shell Research Institute	Netherlands	Wood chips	300-350	12-18	100 kg/h	Unknown
LBL	Lawrence Berkeley Laboratory	USA	Wood chips	330-360	10-24	No data	33
TDP (Thermo-Depoly-merization)	Changing World Technologies Inc.	USA	Turkey innards and fats	200-300	4	250 t/d	Unknown
CatLiq® process	SCF Technologies (A/S)	Denmark	Distillers' dried grains with solubles (DDGS)	280-350	22.5-25	20 L/h	34
STORS process	Organo Corp.	Japan	Sewage sludge	300	10	5 t/d	38
PERC process	Pittsburgh Energy Research Center	USA	Wood chips	330-370	20	No data	53
STORS process	EPA's Water Engineering Research Laboratory	USA	Sewage sludge	300	Unknown	30 kg/h	Unknown
DoS process	HAW	Germany	Lignocellulose-containing biomass	350-500	8	5 kg/h Semi-continuous test plant	Unknown

One of the first pilot plants for the hydrothermal treatment of wood chips and oil was developed by Appel and colleagues at the Pittsburgh Energy Research Center (PERC). The PERC process converts the wood/oil mixture into anthracene oil at 330 to 370 °C in the presence of a catalyst (Na_2CO_3) under reduced atmosphere (CO/H_2). The oil yield was 45 to 55 %. However, technical problems led to the plant being shut down.

The prior problems led to a modification of the PERC process at the Lawrence Berkeley Laboratory (LBL). The LBL process is basically similar to the PERC process, except that no oil is recycled and water is used as the carrier medium. Additionally, the biomass is subjected to acidic hydrolysis with sulphuric acid (WHITE and WOLF∗ 1995; Elliot 2011; Toor et al. 2011). Following neutralisation with Na_2CO_3, the mixture is hydrothermally treated in a tube reactor at 330 to 360 °C and 10 to 24 MPa. The product is a viscous bitumen-like mass with a maximum calorific value of 34 MJ/kg (Behrendt et al. 2008).

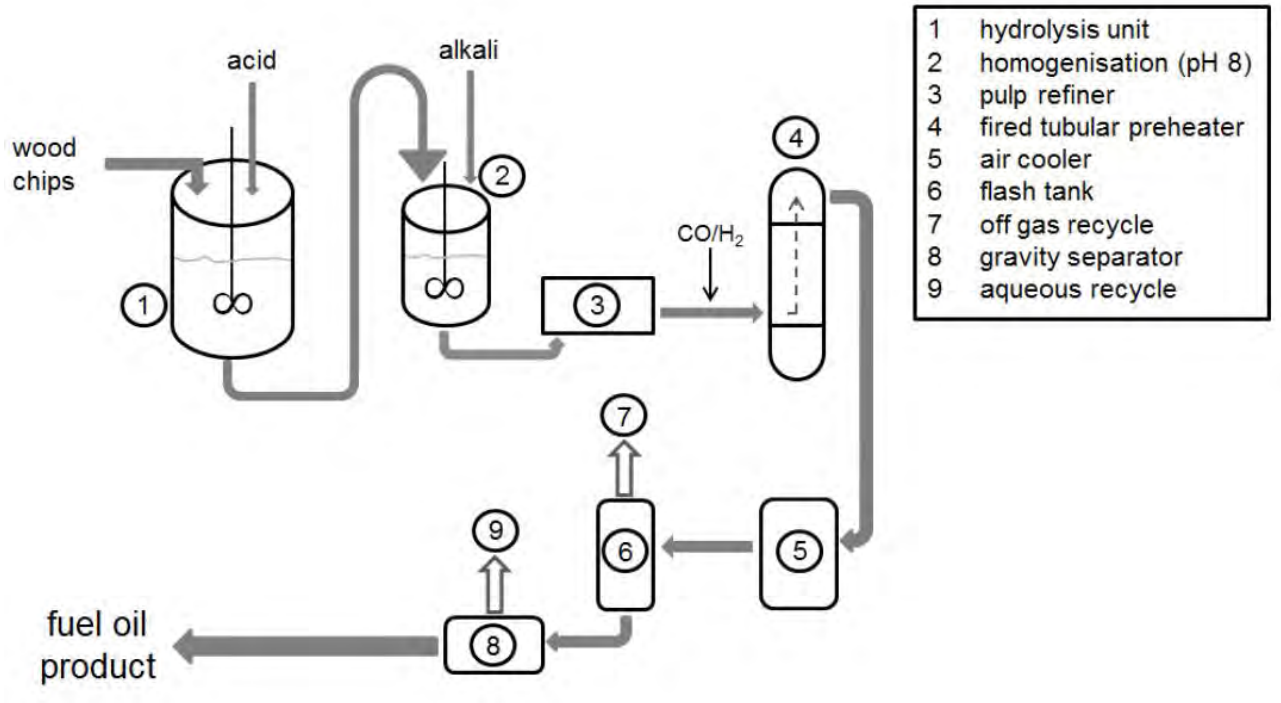


Figure 5.4 Schematic view of the PERC process (according to Elliot 2011)

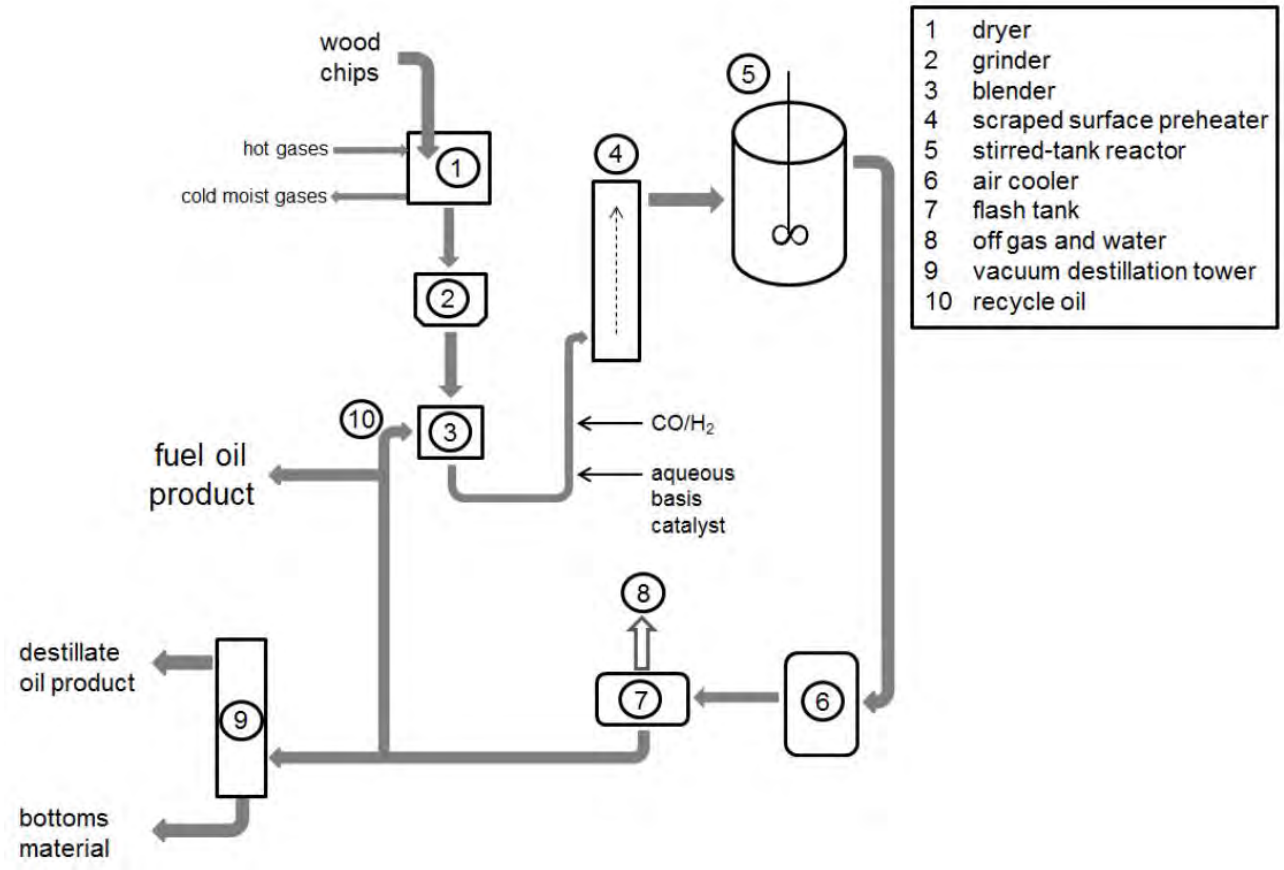


Figure 5.5 Schematic view of the LBL process (according to Elliot 2011)

A hydrothermal process for the conversion of industrial wastes into motor fuels (the TDP process) was likewise developed by Appel and colleagues at Changing World Technologies Inc. (CWT). The process was described by Roberts et al. (Roberts et al. 2004). Figure 5.6 shows the process flow chart with mass flows. The process is divided into two main stages. During the first, hydrothermal, stage the biomass is macerated and then brought to 200 to 300 °C at 4 MPa. The solids are separated off and the water is separated from the fluid phase. In the second stage the non-aqueous phase is processed further at approximately 500 °C (Peterson et al. 2008). The resultant minerals, nitrogen-containing liquid, carbon and diesel oil are marketable products.

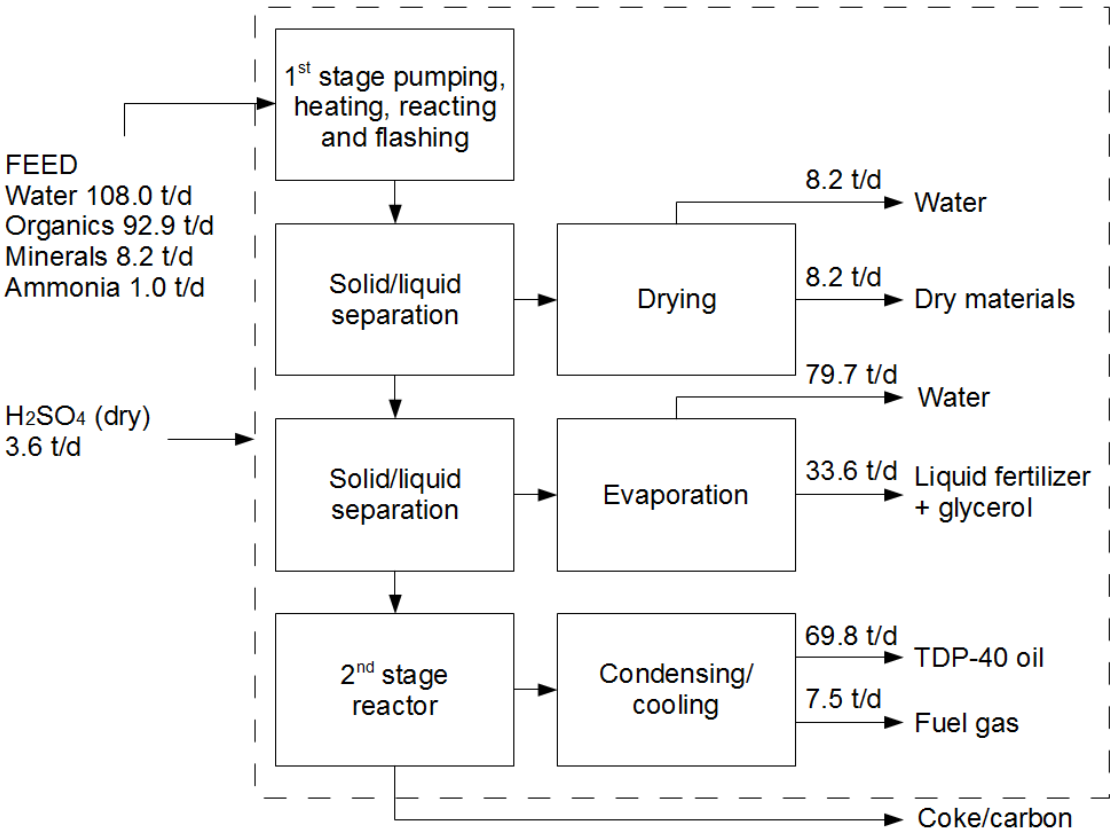


Figure 5.6 Mass balance of the TDP process (according to Roberts et al. 2004)

The energy efficiency for this process design is 85 %. A portion of the produced gas is needed to run the plant. The pumps, motors and heaters additionally need electric power (Roberts et al. 2004). The plant is so efficient because it uses internally generated steam to heat the feedstock.

In Germany, the Hochschule für Angewandte Wissenschaften (HAW) in Hamburg developed a process for the direct liquefaction of lignocellulose-containing biomass (straw, wood, etc.). In this, the biomass is treated at a pressure of 8 MPa and temperatures between 350 and 500 °C under H2 atmosphere (Behrendt et al. 2008). The efficiency of the plant is approximately 70 %, referred to the calorific value of the input material (Meier 2007).

The existing HTL processes have the potential to become leading-edge in the field of conversion technologies for moist biomass. Experiments on a laboratory scale and in pilot plants have shown that

the process is quite capable of functioning. However, the plants needed can only be operated with enormous safety and monitoring constraints, due to the high system pressures. It is vital to improve and optimise the technology with the aim of continuously conducting long-term industrial-scale trials.

5.5 Preliminary HTL experiments

Material and methods

The biomass used was the alga *Chlorella vulgaris* FHL132 cultivated at the Hochschule Lausitz (FH) Senftenberg (appendix “Algae data sheets“).

A 1.8 L agitator reactor (T316 stainless steel) from Parr Instruments was used to conduct the HTL experiments (Figure 5.7). The operating limits of the reactor are max. 34.5 MPa and 450 °C (Graphoil flat gasket). To protect the apparatus and operator from unexpected high pressure, the Parr reactor contains rupture discs. For continuous mixing of the suspension a magnetically driven agitator from Parr Instrument was used, controlled by a Heidolph agitator control unit. Figure 5.7 shows the set-up of the HTL plant at Hochschule Lausitz (FH) in Senftenberg.

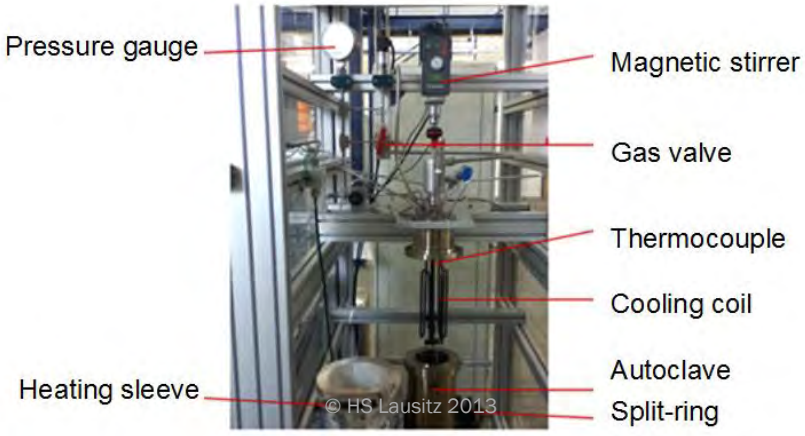


Figure 5.7 HTL plant

For a typical test, the amount of algal suspension necessary to attain the selected pressure at a specific temperature (3 to 20 % by mass) was inputted into the reactor. The "Wtherm" program was used to determine the fill quantity (Wagner and Kruse 1998). Then the reactor was sealed pressure-tight. The reactor was heated up to the desired temperature by means of an adapted heating cuff (source: HORST). This took two to three hours, depending on the final temperature. The temperature was then kept constant for the desired reaction time. At the end of the reaction time the reactor was cooled over a period of several hours with no external cooling. Once room temperature was reached, the resultant gas phase was carefully vented by way of a valve. No quantitative and qualitative analysis of the gas has yet been carried out. When the gas had been vented, the reactor was opened and the reaction mixture was transferred into a glass beaker. Tar residues were removed using a spatula. The liquid phase contained the process water as well as water-soluble and water-insoluble hydrocarbons.

Regeneration

The liquid phase was regenerated according to the scheme shown in Figure 5.9.

The primary aim of the regeneration was to define the quality of the components produced.

The distinct separation between the oil and aqueous phases described in the literature did not occur in the experiments conducted. Only a thin oily film was detectable on the surface of the aqueous phase. For regeneration, the liquid phase was forcefully shaken and then an aliquote of 50 ml was regenerated with dichloromethane. After distillation of the solvent, what remained was a browny-orange coloured mass. Regenerating the product again with acetone enabled two different fractions to be isolated: an acetone-soluble oily fraction and an acetone-insoluble solid (Figure 5.8 and Figure 5.9).

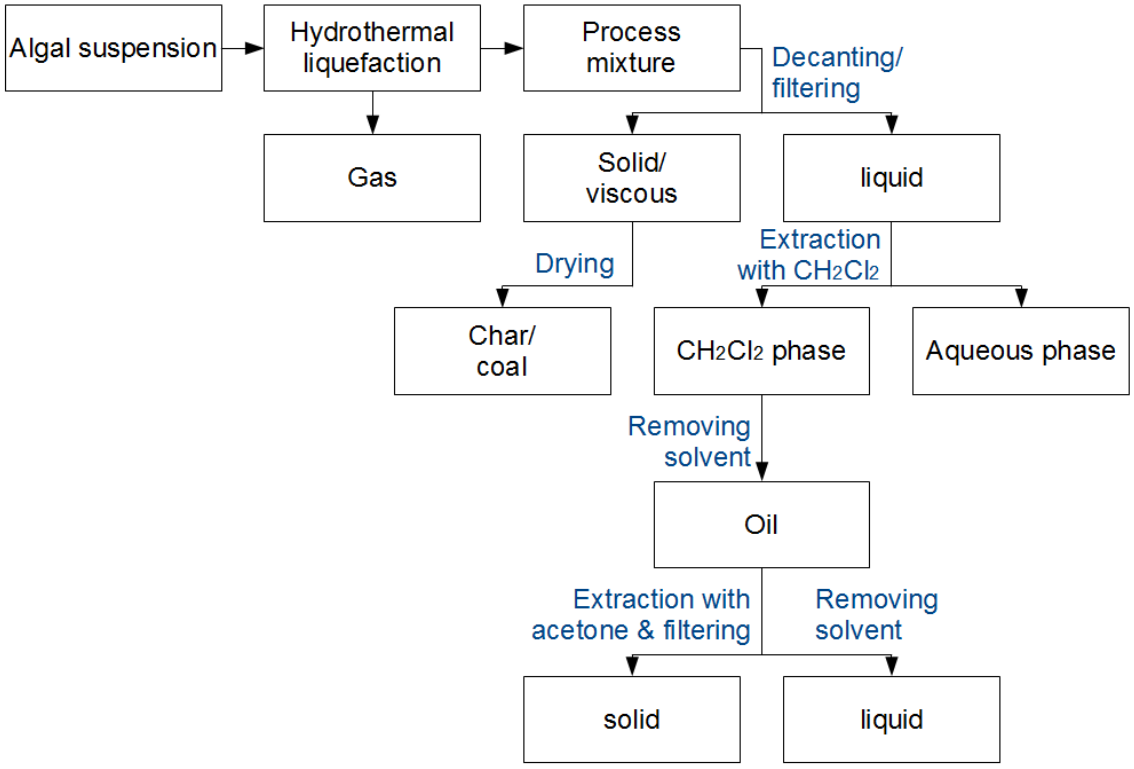


Figure 5.8 Regeneration scheme of the HTL reaction mixture

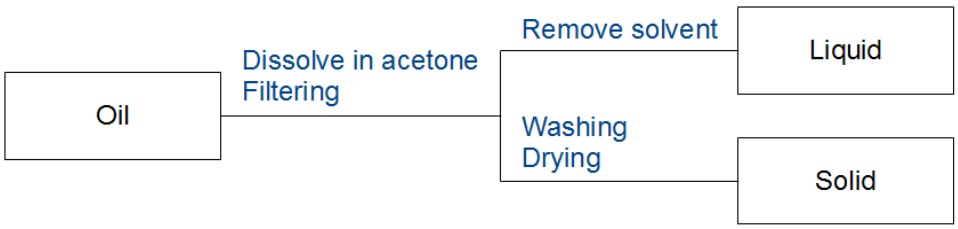


Figure 5.9 Regeneration scheme of the oil phase

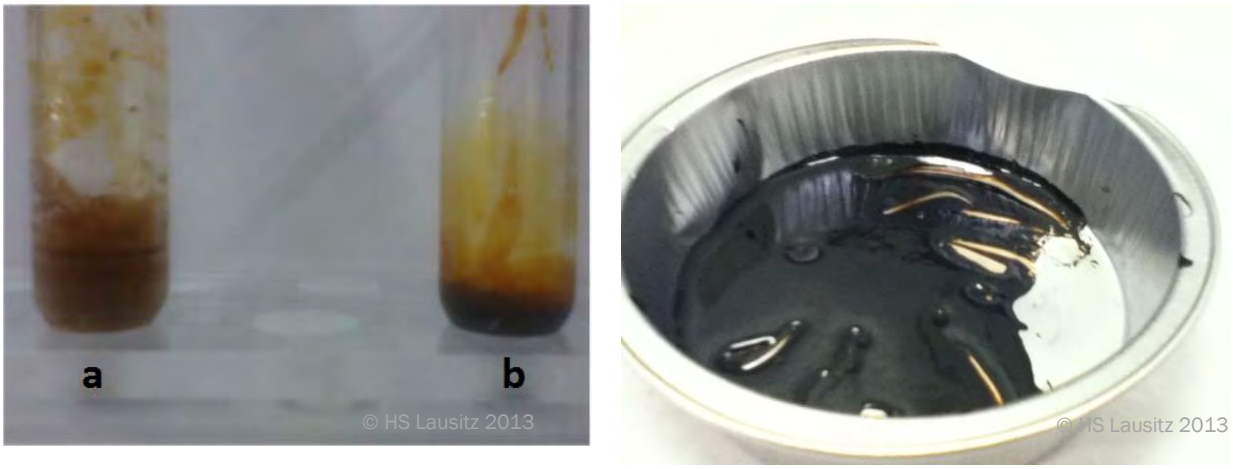


Figure 5.10 Left: a.) Acetone-insoluble solid; b.) Acetone-soluble oil. Right: Tar

Unambiguous identification of the individual components poses major problems at present. It is possible, however, by means of specific chemical reactions at least to define the classes of the components (ester, ether, ketone, acids, etc.).

It is planned to analyse the elemental composition of the individual phases in order to identify the form of the carbon and the nitrogen, for example after the reaction.

Experiments to date have also shown a need for optimisation with regard to the conducting of experiments. The aim is to obtain a clearly delimited oil phase which can then be analysed both quantitatively and qualitatively.

6 Hydrothermal carbonisation (HTC)[8]

6.1 HTC literature research

Hydrothermal carbonisation (HTC) can be regarded as the engineered simulation of the chemical processes which have been occurring beneath the Earth's surface for many millions of years in the formation of brown coal. In hydrothermal carbonisation the processes are accelerated by increased temperatures. Whereas the natural process of brown coal formation has taken a long time over the course of the Earth's history, in hydrothermal carbonisation it takes just a few hours. The aim in developing this process in the first half of the previous century (Bergius 1932) was to convert biomass into usable energy source materials. Owing to the widespread availability of fossil fuels, this technique was for many years not advanced further. As energy needs are increasingly focusing on renewable energy sources, HTC is once more attracting attention, as it can primarily be used to convert the biogenic residues occurring in agricultural food and energy production into a more usable form. The technique enables most of the fixed carbon to be converted into a form permitting further use.

The coal produced is a porous, brown coal-like substance. Firstly, it is possible to use this coal for energy purposes. Thanks to its higher energy density and its friableness compared to the original biomass, it cuts the expenditure on transportation systems (Funke and Ziegler 2011) as well as for crushing. Research is currently being carried out into various energy use paths. The include direct burning of the coal, as well as mixing it into conventional heating boilers, and also gasification it in synthesis gas and converting it further into petrol, such as by the Fischer-Tropsch process. Thanks to its porous structure and higher stability than the inputs, so-called bio-coal is also seen as having great potential for soil improvement (Koch; Ramke and et al 2010; AVA-CO_2 Hydrothermal carbonisation / Renewable energy - Energy balance).

6.1.1 Method

Hydrothermal carbonisation takes place in the temperature range from 170 to 260 °C (Lynam et al. 2011; Heilmann et al. 2010) in a closed system. The pressure follows the saturation vapour pressure of the corresponding temperature, which result in pressures above 4 MPa. At higher temperatures the range of hydrothermal liquefaction is reached; at the critical temperature of the water hydrothermal gasification begins. The transitions are not precisely defined, but fluid. Usually solid contents of 10 to 25 percent are used. The ability to convey and stir the educt is the key factor in this. The carbonisation itself takes place through several parallel and sequential hydrolysis depolymerisation and recombination reactions.

It can generally be stated that the carbon content of the solid phase increases as the temperature rises. The reaction is additionally influenced by the temperature, the retention time, the pH value and the composition of the biomass, and by the presence of catalysts (Leibnitz et al.; Jena et al. 2011b).

The chemical reaction of hydrothermal carbonisation is frequently modelled by the following formula:

[8] This section was authored by the Deutsches Biomasseforschungszentrum (DBFZ).

$C_6H_{12}O_6 \rightarrow C_6H_{20} + 5\ H_2O$

A purely glucose input is assumed here. The coal produced is represented in the chemical formula, though no direct conclusion can be drawn as to the structure of the product. An estimate of the reaction enthalpy based on Hess's law delivered a value of 780 kJ/mol (Titirici et al. 2007). However, theoretical calculation of the enthalpy from the stoichiometric formula normally delivers higher values than experimentation.

In the first large-scale HTC demonstration plant built in Germany, based on recycling of the process water with good heat insulation a large portion of the initial energy is drawn directly from the process (AVA-CO_2 Hydrothermal carbonisation / Renewable energy - Energy balance). Funke and Ziegler (Funke and Ziegler 2011) identified reaction heat exposures of the magnitude of one megajoule per kilogram biomass based on dynamic differential calorimetry of the hydrothermal carbonisation of various biomasses. The reaction can thus be considered to be exothermic.

Catalysts

The use of catalysts has been investigated by numerous researchers in order to improve conversion rates at low process temperature and shorter process times. Lynam et al. (Lynam et al. 2011) were able to improve the properties of the produced coal, primarily with regard to its calorific value, by the use of acetic acid and lithium chloride. However, this required large quantities of the chemicals in the range of 0.2 g to 2.4 g(catalyst)/g(feedstock), so that this method would only be economically viable if the catalysts were completely re-usable. Further experiments relating to catalysed HTC with the aid of citric acid (Cao et al. 2011), lithium chloride and acetic acid (Lynam et al. 2011), levulinic acid and formic acid (Funke and Ziegler 2011) were not, however, able to show a significant improvement in the process based on these auxiliary substances. These experiments were related to terrestrial biomass with high lignocellulose content. Experiments with micro-algae, which can differ widely in their composition from terrestrial biomass, were likewise unable to show any significant change in the products by the addition of $CaCl_2$ and $MgCl_2$, with otherwise unchanged parameters (Heilmann et al. 2010). In view of the small influence of the catalysts on the results achieved, therefore, catalysts are mostly not used, particularly with regard to large-scale technical applications.

6.1.2 Products

The basic products of hydrothermal carbonisation are a solid phase, the so-called bio-coal, the eluate – that is to say, the aqueous phase with dissolved organic degradation products – and the gas phase.

Coal

The solid phase, representing the produced coal, has specific properties. The carbon content of the coal is essentially higher than that of the feedstock. The most widely discussed possibilities for using the coal are for soil improvement and as energy carrier. Thanks to its porosity, the coal is likewise usable directly as an adsorbent, and owing to its composition it requires no activation, such as is necessary when producing activated carbon from fossil coals (Libra et al. 2011). The research also considers other areas of application, including as solid matter storage for hydrogen, as a catalyst (Serp and Figueiredo 2009), and as new-style cathodes for lithium-ion batteries (Libra et al. 2011).

Eluate

The decomposition of the macromolecular structure of the biomass also produces water-soluble constituents in addition to the solid coal. Owing to the composition of the biomass, these may be water-soluble organic substances such as short-chained organic acids. Inorganic substances such as alkali metals washed out of the biomass also collect in the eluate. The concentration of the various substances is often so high that the eluate can no longer be regarded as an easy-to-dispose of effluent. A variety of different processes are being investigated for disposal of the eluate and also in order to use the substances dissolved in them and so achieve an improved balance. The focus of studies is on use as a liquid biogas substrate and as a nutrient medium for micro-alga cultivation (Heilmann et al. 2011; Sawayama et al. 1999; Jena et al. 2011b).

Gas phase

The resultant gas phase is small compared to the other fractions (Libra et al. 2011). Experiments by the DBFZ on the hydrothermal carbonisation of organic residues showed that far less than one percent of the inputted carbon, primarily in the form of carbon dioxide, is transferred to the gas phase. Thus its percentage share of the energy and mass balance is negligible. This fraction does have to be taken into account in the case of an industrial-scale HTC, however, as it cannot be discharged unfiltered into the environment due to its composition and odour.

6.1.3 HTC coal from micro-algae

Hydrothermal carbonisation of micro-algae is a relatively new research field. A team of scientists at the University of Minnesota headed by Heilmann considered the topic directly (Heilmann et al. 2010; Heilmann et al. 2011). Other works touch on the field of HTC, though their focus is on other processes such as hydrothermal liquefaction (Jena et al. 2011a; Jena et al. 2011b; Brown et al. 2010). A major advantage of applying hydrothermal processes to micro-algae is that the biomass does not have to be completely dried in order to be suitable for the process. The produced coal is easier to dewater than the untreated micro-algae. The coal sediments in the aqueous solution, while algal suspensions often remain stable over long periods of time. The work of Heilmann et al. (Heilmann et al. 2010) showed that the application of hydrothermal carbonisation enables dewatering of the biogenic energy source material micro-alga with a positive energy balance. Six different micro-alga species were used in the experiments. Heilmann et al. showed that the use of algae for energy purposes by means of HTC is of interest in that the dewatering and drying properties of the coal are much better than those of the algal biomass. Contrary to the algae biomass, the HTC coal can be subjected to technically simpler filtration, thereby reducing both capital investment costs and operating costs. Other relevant findings relate to the process parameters. The dm content of the algae being carbonised and the reaction temperature were identified as key parameters in this, whereas the retention time had little influence on the results. Furthermore, the general assumptions relating to HTC could be confirmed. For example, the energy yield on the coal side of approximately 65 % was higher than the 40 % mass yield. There were less of the sulphur and nitrogen elements disadvantageous to incineration in the produced coal than in the input.

The available scientific works on the HTC of micro-algae (Heilmann et al. 2010; Heilmann et al. 2011) show that the calorific value can be increased from 18 to 21 MJ/kg of the algal biomass to over

30 MJ/kg of the produced coal. And more than 60 % of the input carbon can be recovered in the solid phase. Other constituents are to be found in dissolved and colloidal form in the liquid phase, with a small portion in the form of CO_2 in the gas phase and dissolved in the aqueous phase. The experiment results also showed that the nitrogen content of the dry matter could be reduced by HTC. This reduction was on average around 30 % referred to the dry matter. A large portion of the phosphate also remained in the liquid phase (Heilmann et al. 2011; Jena et al. 2011a), making the phase fundamentalky interesting as a nutrient supplier for ongoing alga cultivation. More detailed studies into the use of the eluate as a nutrient solution have yet to be undertaken however.

6.1.4 HTC market overview

HTC is a process which is currently in the development and demonstration stage. There are as yet no industrial HTC plants. Table 6.1 provides an overview of companies developing technical HTC plant in Germany based on the latest information available.

Table 6.1. Overview of HTC process developers in Germany

Developer	Reactor type	Operating mode
Artec Biotechnologie GmbH	Tubular reactor	Continuous
AVA-CO_2 Forschung GmbH	Batch reactor	Discontinuous
Brinkhege Engineering GmbH	Autoclave	Discontinuous
CS carbonSolutions GmbH	Tubular reactor	Continuous
Loritus GmbH	No data	No data
SunCoal Industries GmbH	Shaft reactor	Continuous
TerraNovaEnergy GmbH	Agitator reactor	Continuous
TFC Engineering AG	No data	Continuous

6.2 Preliminary HTC experiments

6.2.1 Objective

In addition to carrying out a literature search on HTC from micro-algae, the DBFZ performed some initial experiments. The aim of the experiments was to be able to assess the basic suitability of the micro-algae for hydrothermal carbonisation, to identify favourable operating parameters such as dm content, temperature and retention time, and to estimate the expected quantities and qualities of the bio-coal.

6.2.2 Material and methods

The feedstock was algae of the genus *Scenedesmus obliquus*, cultivated by GMB GmbH in the FPA-PBRs at the Senftenberg location during the summer months of 2011. These were present in a concentration of 0.35 % dm. Owing to the small amount of available algal biomass (10 L of algal medium) only two hydrothermal carbonisation experiments could be conducted. With regard to the process parameters of carbonisation (temperature, time, input concentration), empirical values were taken as the basis from other studies. The algal biomass content was 10 wt.%(dm). For the purpose,

following centrifugation it was diluted with distilled water to approximately 10 % dry matter. The hydrothermal carbonisation was carried out for three hours at a time at a temperature of 180 °C and 220 °C respectively. The final temperature was reached by continuous heating at 2 K/min. The cooling was carried out without external cooling over a period of several hours. After cooling, the resultant solid matter was separated off by means of filtration and dried in the drying cabinet at 105 °C until a constant weight was attained.

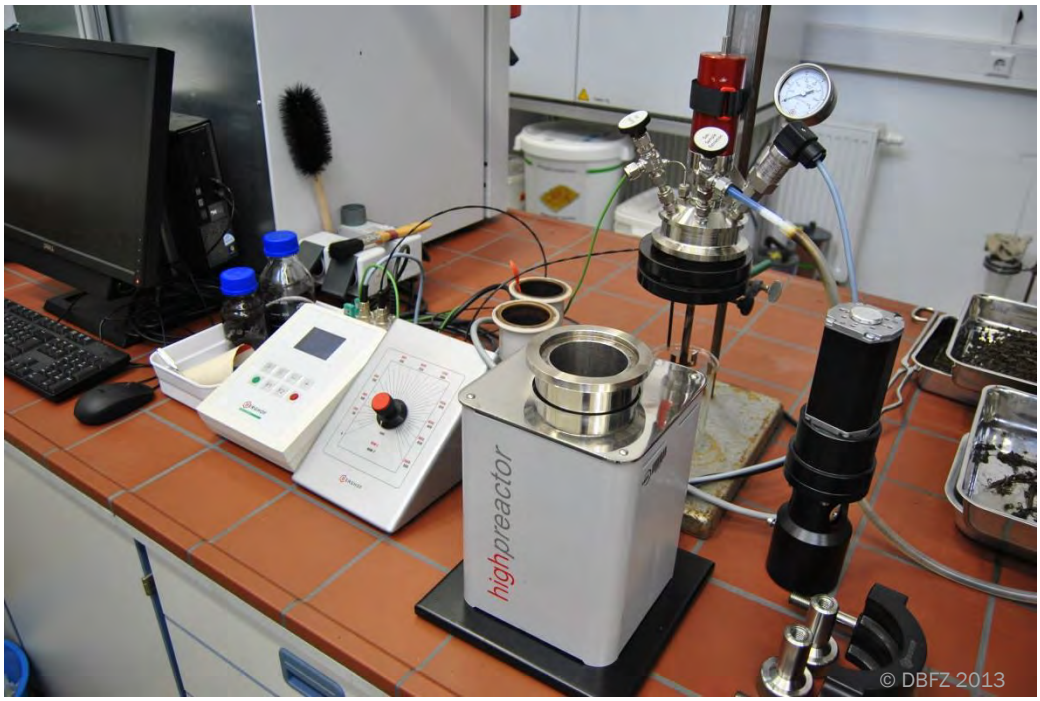


Figure 6.1 HTC test stand

The experiments were conducted in the "highpreactor BR 300" autoclave with data logger, magnetic agitator and BTC 3000 temperature controller from Berghof. The reactor volume was 450 ml. Additionally, the temperature, pressure, heat output and agitator speed parameters were automatically recorded at 30 second intervals. The laboratory analyses carried out are presented in the following Table 6.2:

Table 6.2 Analysis methods

Analysis	Method	Educt	HTC coal	Eluate
Elemental analysis	DIN EN 15104	X	X	X
Total content (chlorine, fluorine, sulphur)	In-house method	X		
Total content (bromine, chlorine, fluorine, sulphur)	DIN EN 15289 DIN EN ISO 10304-1		X	
Total content (bromide, chloride, nitrate, nitrite, phosphate, sulphate)	DIN EN 12457-4			X
Calorific value	DIN EN 14918	X	X	
Primary and secondary constituents CEN total decomposition	DIN EN 15290 DIN EN 15297	X	X	X
pH value	DIN 38404-5			X
Water content	DIN EN 14774-1 DIN EN 14346	X	X	X
COD/ BOD	External			X

The produced gas could not be reliably analysed due to its low quantity.

6.2.3 Results

In contrast to the algal biomass, which is fully homogeneous at 10 wt. % (dm), meaning no separation due to sedimentation or coagulation is detectable, the coal was deposited on the floor of the reactor after the reaction. The water content of the coal after filtration was 47 wt .% at 220 °C and 77 wt. % at 180 °C. The value after the reaction at 180 °C, especially, cannot be attained by mechanical methods with fresh algae. After drying, the two coals had a loose, porous consistency. The colour was dark brown, though in different shades. The coal produced at 180 °C was of a lighter colour than that produced at 220 °C.

Solid phase

After hydrothermal treatment, the analyses listed in section 6.2.2 were conducted. Their results are presented here and compared against the analysis results for the untreated algae.

Table 6.3 Analysis results for the solid matter before and after hydrothermal treatment

Material	Mass (%)	Calorific value in kJ/kg (dm)	Elemental analysis in wt.% of dm						
			C	H	O*	N	K	P	S
Algae	9.98 g (100%)	21600	48.3	6.50	33.7	6.5	1.7	2.7	0.6
Coal 180 °C	6.32g (63%)	23200	51.2	6.8	33.4	5.2	0.6	2.4	0.4
Coal 220 °C	7.80 g (78%)	27890	61.9	7.2	24.4	4.0	0.2	2.0	0.3

* Calculated from difference

The following diagram illustrates the temperature dependency. The experiments conducted indicate a trend as to which elements are enriched and depleted in the solid phase.

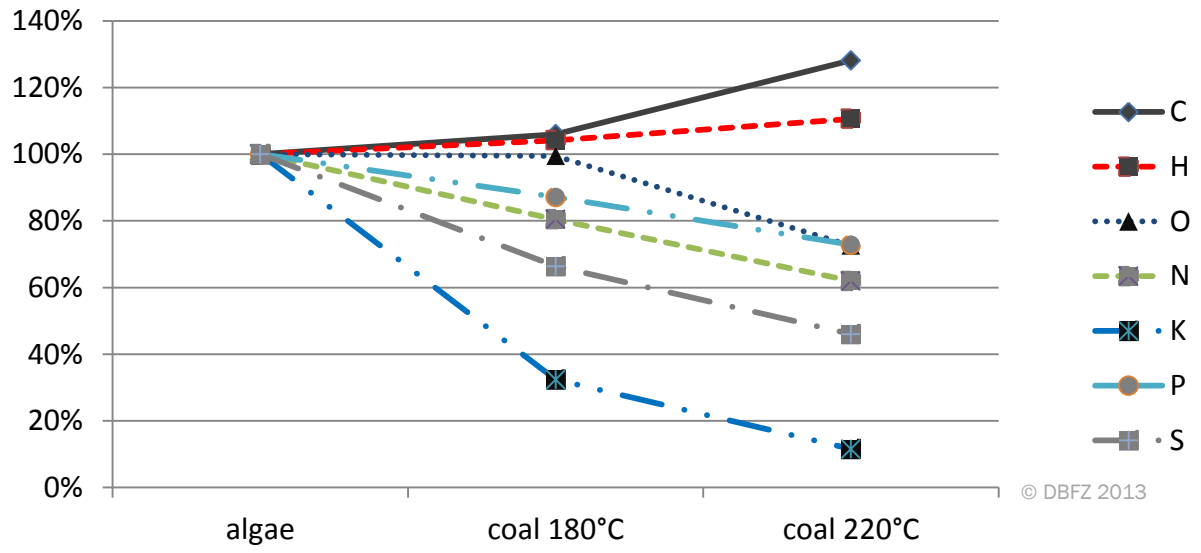


Figure 6.2 Change in elemental composition dependent on process temperature

Liquid phase (eluate)

The eluate was a dark brown, almost black liquid with a pH value of 6.5 and 6.8 respectively. 7 dm.% at 180 °C and 6 dm.% at 220 °C were dissolved organic products of the carbonisation. Of that total, approximately 27 dm.% was carbon. It is noticeable that the substances dissolved in the eluate have high oxygen content. Experiments with other input materials such as greencut or biowaste exhibited similar concentrations. The reason for the high concentration may be low-molecular organic acids, or carbonate formation due to the high alkali metal content. For detailed clarification further investigation would be required, but this could not be carried out within the project. The results of the analyses of the liquid product are presented in Table 6.4 It should be noted that part of the liquid phase was dried with the coal.

Table 6.4 Analysis of the liquid products

Parameter	Eluate 180 °C	Eluate 220 °C
General parameters of the complete liquid:		
Water content (% total)	93.9	92.7
pH value	6.52	6.78
COD (BOD_5) in mg/l	42500 (10968)	56500 (10968)
Elemental analysis:		
Carbon (% dm)	26.4	27.2
Hydrogen (% dm)	4.1	4.1
Oxygen (% dm)*	60.4	61.4
Nitrogen (% dm)	3.5	3.92
Sulphur (% dm)	0.34	0.37
Secondary constituents (total decomposition):		
Calcium (mg/kg dm)	3180	Not detectable
Potassium (mg/kg dm)	24100	21000
Magnesium (mg/kg dm)	8480	906
Sodium (mg/kg dm)	1150	873
Phosphor (mg/kg dm)	16200	7340

* Calculated from difference

Gas phase

The experiments showed that the gas phase formed represents a small portion of the mass balance. At 180 °C, 60 ml of additional gas was produced; and at 220 °C, 310 ml. With the composition identified, this corresponds to a mass of just under 0.1 g or 0.5 g respectively. More precise measurements could not be performed. It is assumed, however, that the major portion of the gas formed is CO_2 .

Assessment of results

The results of the experiments confirm the assumptions made regarding hydrothermal treatment. The carbon content and, proportional to it the calorific value, of the solid matter was significantly increased. As was the hydrogen content. An oxygen reduction occurs only in the case of the 220 °C coal. Only smallish amounts of nitrogen, potassium, phosphor and sulphur are to be found in the solid reaction product. Only smallish amounts of nitrogen, potassium, phosphor and sulphur are to be found in the solid reaction product. The results obtained from experimentation by Heilmann et al. can be confirmed with regard to the properties of the coal, the location of the nutrients and the temperature dependency of the reaction in these preliminary experiments. While the calorific value of the algae is comparable to that of brown coal, the latter could be increased especially at 220 °C.

With regard to the eluate, the basic expectations as to the constituents can be confirmed on the basis of the analysis data. So in addition to carbon, nitrogen, potassium and Phosphor are to be found in this fraction. The proportionate nitrogen and sulphur contents after the reaction increase as the experiment's temperature rises. The analysis also shows that disposal or indeed use of this liquid

without further treatment might be problematic. Owing to its high nitrogen and phosphor content, further use should be targeted. The high proportion of dissolved organic matter in the eluate requires further investigation however.

The results permit the conclusion that a large portion of the chemical energy which the micro-algae – in this case *Scenedesmus obliquus* – contain is transferred to the coal by hydrothermal carbonisation. It would also be conceivable to recover the nutrient content from the educt in this way.

The temperature dependency of the reaction is likewise underpinned by the experiments. This is confirmed primarily by the composition of the coal dependent on the process temperature (Figure 6.2). Significant differences are also discernible in the analyses of the liquid product and the gaseous products. These must be traced back to the reaction temperature, as other parameters, such as the retention time or the solid matter concentration, were not changed in the experiments.

Since the experiments conducted serve only as a guide, it not having been possible to carry out a sufficient number to form a sound scientific framework, further experiments need to be performed in order to verify the observations made. Moreover, a comparison with other alga species is necessary in order to check whether the results are possibly transferable.

Nevertheless, the experiments did essentially fulfill the expectations arising from the literature reviewed. Consequently, the use path of carbonisation of micro-algae is a promising approach, also in view of current development trends in HTC technology in other application areas.

7 Motor fuel production processes[9]

7.1 Fuel requirements

Liquid motor fuels will continue to be the key energy source materials for mobility over the coming decade based on their advantages (high energy storage density, mature technology, existing infrastructure). In the aeronautical sector there are currently no discernible alternatives at all.

However, the finite nature of mineral oil resources and the climate change linked to the use of fossil fuels demand increasing substitution of conventional motor fuels by products made from renewable raw materials. In this, both the use of pure biofuels and the admixture of biofuel components is conceivable. In both cases the fuel brought onto the market must meet the minimum requirements stipulated in standards. This relates in particular to the "hard" criteria dictated by the propulsion technique and the operating conditions. Table 7.1 provides an overview of important motor fuel properties and of the typical components of conventional fuels (Wauquier et al. 1995)

Table 7.1 Requirements for motor fuels and typical composition (ARAL 1995; ARAL 2000)

	Gasoline	fuel	Jet fuel JET A-1
Parameter	Volatility Knock resistance Stability	Combustibility Cold resistance Stability	Cold resistance Purity (water) Clean combustion (no sooting)
Composition	i-alkanes, aromatics BR[10]: 25-200 °C C number: 4 - 10	n-alkanes, i-alkanes BR: 200 – 350 °C C number: 12 – 20	i-alkanes, naphthene BR: 180 – 250 °C C number: 9 -13

Gasoline:

In a spark ingnition engine, an ignitable fuel vapour/air mixture must be able to form under all operating conditions (cold-starting through to hot-running). This demands a certain *volatility* which ultimately is dictated by the boiling behaviour of the fuel. To avoid uncontrolled ignition of the mixture in the cylinder, the fuel must have good *knock resistance*, which is characterised by the octane number. Particularly n-alkanes with C-chain lengths > 5 are highly reactive, and so little suited to use in gasoline. Conversely, iso-alkanes, naphthene and aromatics, as well as oxygen-containing compounds such as alcohols and ether, have high knock resistance. Also, the proportionate amount of unsaturated components (e.g. olefins as products of cracking) is limited, because they cause ageing of the fuel based on the formation of so-called gum (polymerisation) and impair its *storage stability* (DIN EN 228:2008-11, standard - Beuth.de).

[9] This section was authored by the TU Bergakademie Freiberg.
[10] BR: Boiling range

Fuel:

Diesel engines, as self-igniting units, require highly reactive fuels. A measure of *ignition quality* is the cetane number. Particularly long-chained n-alkanes exhibit good ignition properties. However, these substances tend to crystallise at low temperatures. Consequently, *cold resistance* is a very important criterion. This can be characterised, for example, by the cold filter plugging point (CFPP) or the cloud point. The boiling range is less important than in the case of gasoline, as diesel is injected in liquid form into the hot air filled cylinder (DIN EN 590:2010-05, standard - Beuth.de)

Jet fuel Jet A-1

The fuel is combusted in gas turbines. Properties such as ignitability or knock resistance are of lesser importance. The widely differing temperature conditions impose extremely high demands in terms of *cold resistance*. The fuel must be liquid and pumpable both at an altitude of 10 kilometres and on the ground. The fuel components must neither evaporate nor crystallise. So only a very tight boiling range comes into question. Since the turbine blades may be destroyed by solid matter, a clean and above all *soot-free combustion* is required. Particularly suitable as jet fuels are iso-alkanes and naphthene (DSTAN 2008).

According to estimates by EADS (Stuhlberger 2012), the global kerosene market will increase by 160 billion Dollars by 2050. It estimates that approximately 50 % of the jet fuel then in use will originate from regenerative resources.

So-called first-generation biofuels are currently used as fuel substitutes based on renewable energy sources:

- Fatty acid methyl ester (FAME, biodiesel) for fuel and
- ethanol for gasoline.

Owing to the technical disadvantages linked to application, the maximum addition percentages are limited to 7 % and 10 % respectively. Higher mix rates can only be achieved if the fuel from alternative raw materials is chemically fundamentally identical to conventional motor fuels and exhibits the same or better motor properties.

The use of algae to produce energy is not an entirely new idea. In fact, it was the subject of scientific research back in the 1950s (Meier 1955). In the period from 1978 to 1996, research work relating to the production of motor fuels from micro-algae was funded as part of the US Department of Energy's Aquatic Species Program (ASP) (Sheehan et al. 1998). The research works considered three possibilities:

- the production of methane by biological and thermal-chemical processes;
- the production of ethanol by fermentation; and
- the production of biodiesel.

It was above all the high proportion of natural oils which some micro-alga species contained which made them interesting as a potential raw material source for the production of motor fuel (Sheehan et

al. 1998). There have to date been numerous publications relating to the production of biodiesel and biofuels from micro-algae.

The following sections focus especially on the production of liquid products which can be used as substitutes for conventional motor fuels.

7.2 Transesterification

Some micro-algae contain large amounts of lipids which, owing to their low polarity, are soluble in organic solvents such as hexane (Table 7.2). Constituents of the lipids are triacylglycerols (for short: triglycerols). These are formally esters of fatty acids and the trivalent alcohol glycerol. Algae also contain quantities of free fatty acids. Thus transesterification is a practicable method for the production of motor fuels from algal biomass, as for the production of biodiesel from vegetable oils.

Table 7.2 Lipid contents of some alga species (Demirbas 2010)

Species	Lipid content (% dm)
Ankistrodesmus TR-87	28–40
Botryococcus braunii	29–75
Chlorella sp.	28–32
Cyclotella DI-35	42
Cylindrotheca sp.	16–37
Dunaliella tertiolecta	36–42
Hantzschia DI-160	66
Isochrysis sp.	7–33
Nannochloris	20–63
Nannochloropsis	31–68
Nitzschia sp.	45–47
Nitzschia TR-114	28–50
Phaeodactylum tricornutum	31
Scenedesmus TR-84	45
Schizochytrium sp.	50–77
Stichococcus	33 (9–59)
Tetraselmis suecica	15–32
Thalassiosira pseudonana	(21–31)
Chlorella vulgaris)*	21
Scenedesmus obliquus)*	23

dm: dry matter *) source: I. Petrick (Hochschule Lausitz)

In transesterification, triacylglycerols are converted with methanol to fatty acid methyl esters (FAME), i.e. biodiesel, and glycerol. In this process – as shown in Figure 7.1 – one molecule of triglycerol reacts

with three molecules of alcohol in stages by way of diglycerol and monoglycerol to form one molecule of glycerol and three molecules of FAME. This is an equilibrium reaction which takes place under ambient pressure and dependent on the catalyst used at temperatures between 30 and 100 °C. By using of suitable reaction conditions (methanol excess, FAME withdrawal) triglycerol conversion rates of 99 % are attained. The reaction can be base-catalysed or catalysed acidically.

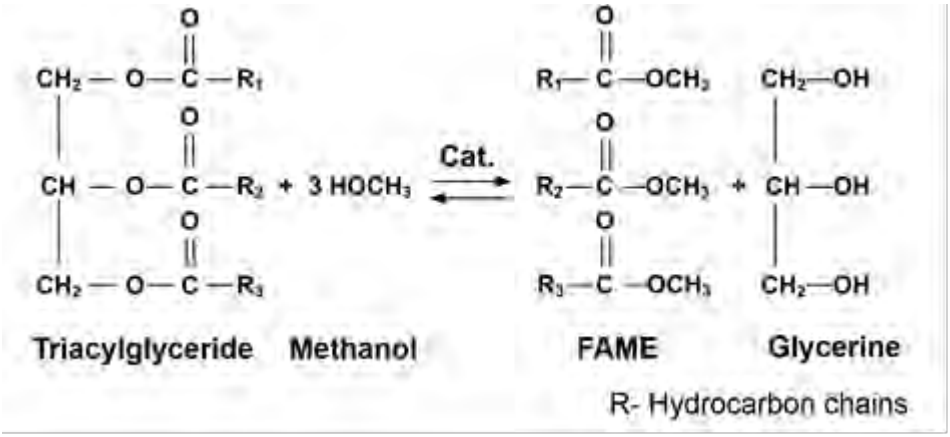


Figure 7.1 Transesterification reaction

The alkaline conversion with sodium or potassium hydroxide as the catalyst takes place at milder temperatures and requires shorter retention times than the acidically catalysed reaction. It has therefore established itself as the technically preferred method for the production of biodiesel from vegetable oils. It takes place by way of methylate anions formed in a preliminary reaction. In some cases sodium methylate (sodium methoxide) itself is used as the catalyst. The disadvantage of alkaline conversion is that the free fatty acids possibly contained in the raw material are preferentially converted to sodium or potassium soaps. This entails catalyst loss. An even more problematic effect, however, is the tenside action of the soaps, which makes the necessary separation of the oily ester phase from the aqueous glycerol phase more difficult.

For acidic conversion, organic sulphonic acids such as p-toluene sulphonic acid or sulphuric acid can be used. The reaction takes place by way of a carbo-cation mechanism. Advantageously, under these conditions the free fatty acids are also converted to esters; no saponification is possible.

Suckow and Petrick report on comparative studies of the direct transesterification of algal mass with a basic a acidic catalyst. As opposed to purely basic transesterification with potassium hydroxide, using sulphuric acid can significantly increase the ester yield. Combining the two procedures even doubles the yield relative to base catalysis (Suckow et al. 2011).

Other research groups also found that alkaline catalysts are not suitable for the transesterification of lipids with high free fatty acid contents (Miao and Wu 2006). In these studies the micro-alga *Chlorella protothecoides* was cultivated under both autotrophic and heterotrophic conditions. When glucose was added (10 g/l), a significant increase in lipid content soluble in n-hexane accompanied by a decrease in protein content was recorded. These lipids could be transesterified with sulphuric acid to a product comparable to biodiesel. At 30 °C and over a 4 h reaction time, biodiesel yields of almost 80 dm. % referred to total lipid content (approximately 55 %) were attained in heterotrophic cultivation.

With an enzymatically catalysed transesterification, Li, Xu and Wu presented a new and very interesting means of producing FAME from algal biomass. The work describes the influence of key operating parameters such as methanol content, solvent quantity, pH value, temperature, and many more, on the speed of conversion of a soxhlet extract (n-hexane) of the heterotrophically cultivated micro-alga - *Chlorella protothecoides*. Under optimal conditions, conversion rates of over 98 % of the extract could be achieved with one lipase. The composition of the algal diesel obtained is shown in Table 7.3 (Li et al. 2007).

Table 7.3 Composition of the biodiesel (Li et al. 2007)

	Relative content of fatty acid methyl ester (%) in different cultivation sizes		
Component	5 l	750 l	11,000 l
$C_{15}H_{30}O_2$ Myristic acid	1.31	-	-
$C_{17}H_{34}O_2$ Palmitic acid	12.94	9.71	10.1
$C_{18}H_{36}O_2$ Margaric acid	0.89	0.62	0.71
$C_{19}H_{34}O_2$ Linoleic acid	17.28	18.62	18.33
$C_{19}H_{36}O_2$ Oleic acid	60.84	66.67	65.75
$C_{19}H_{38}O_2$ Stearic acid	2.76	2.64	2.85
$C_{21}H_{38}O_2$ Nonadecanoic acid	0.36	1.05	1.01
$C_{21}H_{40}O_2$ Eicosenoic acid	0.42	0.56	0.67
$C_{21}H_{42}O_2$ Eicosanoic acid	0.35	0.48	0.59

Similarly to the case of rape-seed oil methyl ester, the esters of the C18 fatty acids predominate with over 75 %. The algal diesel is thus comparable to the quality of biodiesel from rape-seed oil or other vegetable oils (Li et al. 2007).

As an alternative to a multi-stage process (extraction followed by transesterification), direct transesterification was also investigated. In this, the entire algal mass – in the present case the heterotrophic micro-alga *Schizochytrium limacinum* – is used (Johnson and Wen 2009). Simultaneously using solvents such as chloroform, hexane or petroleum ether, up to 20 % higher raw biodiesel yields were attained as compared to the two-stage process. Interestingly, however, only the use of chloroform results in high FAME contents in the biodiesel (63 % as opposed to 66 % in the two-stage procedure). With the other solvents, other oil constituents not specified in more detail are mainly obtained. The work also describes the influence of algal mass moisture. In the two-stage process the biodiesel yield is impaired when the algal mass is moist. By contrast, in direct transesterification with chloroform higher biodiesel yields are obtained, though with only a very low FAME content (8 %).

Lewis et al also investigated direct, acidically catalysed transesterification with a mixture of methanol/chloroform/hydrochloric acid (10:1:1) in comparison to the conventional method, in which the transesterification was preceded by an ultrasound-assisted sequential extraction with chloroform, methanol and water. In the case of the direct method, under the same transesterification conditions (90 °C, 60 min) the fatty acid methyl ester yield was increased by 18 % to 400 mg/g (dry algal mass) (Lewis et al. 2000).

Li et al. report on comparative studies of conventional two-stage transesterification with single-stage production of biodiesel from the micro-alga *Nannochloropsis sp*. using a base Mg-Zr catalyst in a packed bed (Li et al. 2011). The advantages of the single-stage process are seen as being a simplification of the process and a reduction in process and product cost. The experiments were conducted in small round-bottom flasks with a magnetic agitator and a reflux condenser, using a methanol/dichloromethane mixture. Compared to conventional transesterification, the two-stage procedure delivers the higher extract yields: 28 % as opposed to 22 %. Moreover, the biodiesel produced by the single-stage process has a lower oxygen content and a higher calorific value (Li et al. 2011). The authors relate these advantages to the continual disturbance to the solubility equilibrium due to the transesterification taking place simultaneously with the extraction, as a result of which the seed viability for extraction is increased.

Homogeneous catalysts (strong acids or bases) are usually used for the transesterification of native oils and fats. Reddy et al. report on the use of calcium oxide as a heterogeneous catalyst for the transesterification of soya bean oil etc. Whereas with ordinary calcium oxide at room temperature no conversion was recorded, or only very low conversion rates, with nanocrystalline CaO a virtually quantitative conversion rate could be attained (Venkat Reddy et al. 2006).

The transesterification of triglycerols with methanol is a heterogeneous liquid phase reaction which requires the most intensive possible intermingling of the two not mutually soluble phases for the creation of large phase boundaries. The researchers report on laboratory-scale experiments in which the transesterification of triglycerols was carried out with and without ultrasound assistance (Gole and Gogate 2012).

Table 7.4 Comparison of speed constants in 10^{-2} l/(mol min) with and without ultrasound assistance (Gole and Gogate 2012)

	Temperature in °C			Cat. concentration in g cat. /g oil			
	40	50	60	0.5	1.0	1.5	2.0
Conventional	2.8	7.2	9.6	4.3	8.9	9.0	9.0
With ultrasound	25.6	27.7	27.6	11.0	21.8	22.1	22.1

Thanks to the high energy input when using ultrasonic sound, a very high dispersion rate is obviously attained, which was reflected in much faster reaction rates, particularly in mild temperature conditions. A second-order reaction was applied as the basis for a kinetic evaluation. When using ultrasonic sound, a doubling or even as much as quadrupling of the speed constant was recorded.

In studies relating to the transesterification of fatty acid distillates from palm oil, Deshmane and his colleagues were able to show that the positive influence of the ultrasonic sound is brought to bear primarily at higher conversion rates. Above a conversion rate of approximately 30 % the reaction mixture becomes heterogeneous and the mass transfer between the phases impedes the progress of the reaction. This resistance is overcome by the cavitation caused by the ultrasonic sound and the associated micro-turbulences. For example, agitation without ultrasound produces a 93 % conversion rate at 40 °C after 300 minutes, while activating the ultrasonic sound (22 kHz) increases the conversion rate to 95 % after just 150 minutes (Deshmane et al. 2009).

Overall, the following advantages can be achieved by using ultrasonic sound in the transesterification of triglycerols (Gole and Gogate 2012):

- Lower reaction temperatures
- Shorter reaction times
- Higher final conversion rates under otherwise identical conditions
- Less methanol surplus
- Easier separation of the glycerol and the catalyst

The transesterification process can also be positively influenced by the use of microwaves. Azcan and Danisman report on relevant studies relating to rape-seed oil transesterification with NaOH or KOH in the temperature range between 40 and 60 °C. Microwaves are used to speed up the introduction of heat, enabling the reaction times to attain a specific conversion rate to be significantly shortened. At the same time, they recorded high purity levels of the biodiesel obtained (Azcan and Danisman 2008).

Conclusion:

- Particularly heterotrophically cultivated micro-algae contain proportionately large amounts of lipids which can be transesterified with methanol to produce biodiesel (fatty acid methyl ester).
- Owing to the in part high free fatty acid content, the use of acidic catalysts is preferable. Combined procedures (base and acidic catalysation) may be advantageous.
- Studies into enzymatic transesterification proved promising.
- Cost savings relative to the normally applied multi-stage transesterification can be achieved by single-stage direct transesterification in the present of solvents (such as chloroform) and by omitting the biomass drying procedure.
- The transesterification produces biofuels consisting of fatty acid methyl esters. These FAMEs have a disadvantageous effect on storage stability and engine running when added to diesel in larger proportions.
- For the production of other motor fuel types (kerosene, gasoline) or of high-quality diesel, further conversion processes must follow on from the transesterification (hydrocracking, hydrogenation).
- With the assistance of ultrasound, or by using microwave technology, the reaction times and temperatures can be lowered or the conversion rate increas

7.3 Pyrolysis / Cracking

Pyrolysis refers to the thermal decomposition of organic compounds under exclusion of air. At temperatures between 350 and 1,000 °C bonds (C-C, C-S, C-O, C-H) in large molecules are broken up. Following saturation of the free valences, the resultant fractions deliver liquid and gaseous products. Additionally, an intramolecular hydrogen rearrangement creates a solid residue: the pyrolysis coke. This also contains the mineral components of the raw material.

Pyrolysis (in the crude oil processing sector also termed cracking) is able to operate purely thermally by way of a radical reaction mechanism, or with the aid of acidic catalysts by way of an ionic mechanism.

In contrast to transesterification, pyrolysis is able to convert all the algal material. The liquid product yield is dictated, firstly, by the composition of the feedstock (Antonakou et al. 2006, Miao and Wu 2006) and, secondly, by the pyrolysis conditions. As Table 7.5 shows, high yields are promoted by short retention times accompanied by relatively high temperatures.

Table 7.5 Overview of pyrolysis conditions and product yields (Brennan and Owende 2010)

Pyrolysis type	Temperature	Retention time (medium)	Oil (%)	Gas (%)	Coke (%)
Flash	500 °C	Very short, approx. 1 s (vapour)	75	13	12
Fast	500 °C	Short, approx. 10-20 s (vapour)	50	30	20
Slow	400 °C	Very long (solid matter)	30	35	35

As well as the highest possible liquid product yield, the composition of the liquid products is also of great interest in technical terms. Numerous studies relating to the pyrolysis of other feedstocks, such as coal or wood, have shown that the quality of the liquid products deteriorates as the yield increases.

Miao, Wu and Yang investigated the fast pyrolysis of micro-algae (*Chlorella prothothecoides* and *Microcystis aeruginosa*) in a laboratory fluidised bed reactor at 500 °C and with vapour retention times of 2-3 s (heat-up velocity 600 K/s) (Miao et al. 2004). The micro-algae were freeze-dried prior to use and crushed in a mortar down to < 0.18 mm. Despite inadequacies of the apparatus (algal dust adhered to the reactor wall due to electrostatic forces), the bio-oil yield was 17.5 % (Chlorella) and 23.7 % (Microcystis) respectively. The yields are thus higher than the lipid content in the original algae (14.6 and 12.5 % respectively). The bio-oils obtained had a calorific value of 29 MJ/kg and a density of 1.16 kg/l. In the view of the authors, pyrolysis oil from micro-algae is better suited to use as fuel oil than fast pyrolysis oil from wood (Miao et al. 2004). Table 7.6 gives the compositions of the algal bio-oils:

Table 7.6 Composition of fast pyrolysis oil from algae (Miao et al. 2004)

Bio-oil from	Saturated (wt.%)	Aromatic (wt.%)	Polar (wt.%)	Asaphaltene (wt.%)
Chlorella prothothecoides	1.31	0.75	53.84	64.10
Microcystis aeruginosa	0.96	0.53	28.50	70.01

As Table 7.6 shows, the pyrolysis oils are charactered by extraordinarily large high-molecular asphaltene contents and very small amounts of saturated and aromatic components. The very high oxygen contents of the oils, of 19.4 and 21.0 % by mass respectively, also correspond to this (Miao et al. 2004).

The same authors also investigated the influence of the cultivation (heterotrophic/autotrophic) on the bio-oil yields in fast pyrolysis of *Chlorella prothothecoides* (Miao et al. 2004). As expected, much higher bio-oil yields were achieved for the heterotrophically cultivated alga than for the autotrophic alga culture (57.2 % as opposed to 16.6 %). Furthermore, the chemical composition of the oil was evidently much better, as reflected in the significantly higher calorific value (41 MJ/kg as opposed to 30 MJ/kg).

The influence of the pyrolysis temperature was likewise investigated by Miao et al and by Demirbas. The optimum in terms of oil yield is at 450 °C (Miao et al. 2004) up to 500 °C (Demirbas 2009).

Miao quotes a potential oil yield of 68 % for fast pyrolysis of micro-algae in an optimised reactor (Miao et al. 2004).

Generally, pyrolysis oils are mostly highly viscous, acidic and unsaturated. They also contain solid matter as well as dissolved water. To enable the products to be used as regular fuels, their oxygen content must be lowered and the impeding substances removed. This can be done by means of hydrogenation processes for example (Wildschut et al. 2009).

Catalytic cracking is used particularly to improve the quality of oils produced in an upstream stage from micro-algae, such as by means of solvent extraction. Thus, researchers report on the catalytic cracking of different natural oils on form-selective zeolite HZSM-5 catalysts as an alternative to conventional transesterification (Milne et al. 1990). In addition to vegetable oils, oils from four micro-alga species (*Chaetoceros muelleri, Monoraphidium minutum, Naviculus saprophilla, Nannochloropsis sp.*) were also investigated. These were obtained by mechanical methods (centrifuging, compacting) followed by solvent extraction (methanol/chloroform/water or butanol). The results are high yields of high-octane, aromatics-rich naphtha as well as diesel components with good cold properties. The coking rate is between 3 and 10 %. By-products are carbon oxides and water. The authors speculate that the process would also be suitable for processing of the complete alga (Milne et al. 1990).

Kitazato and colleagues also presented studies on the catalytic cracking of lipids. The feedstock was the oil of the alga *Botryococcus braunii* , which primarily consists of long hydrocarbon chains with C-numbers between 30 and 36. With the correspondingly high boiling temperatures (around 400 °C), it is not suitable for use as gasoline or diesel fuel. The experiments were conducted in a micro-packed bed reactor, evidently using a zeolithic catalyst, at approximately 500 °C. With an oil conversion rate of 85 %, a naphtha yield of approximately 62 % (octane number 95) was achieved. These results are comparable to those relating to the catalytic cracking of vacuum gas oils originating from crude oil in the commercial fluid catalytic cracking process (FCC) (Kitazato et al. 1989); see Table 7.7:

Table 7.7. Results of catalytic cracking of oil of the alga *Botryococcus braunii* compared to FCC of a heavy crude oil fraction (Kitazato et al. 1989)

Feedstock	FA and TAG from *Botryococcus braunii*	Vacuum gas oil
Reaction temperature	497 °C	470 – 540 °C
Yields (wt %)		
H_2S	0	1.0
C2-C4	17.2	21.4
Petrol	61.7	52.3
Coke oil	14.8	13.2
Slurry	0	6.6
Coke	5.3	5.6
Conversion rate	85.2	80.2
Naphtha octane number	95	92

Figure 7.2 shows the reaction sequence proposed by Kitazato et al. for the formation of aromatic structures from C34-botryococcene, a typical compound of the oil of *Botryococcus braunii*, by catalytic cracking.

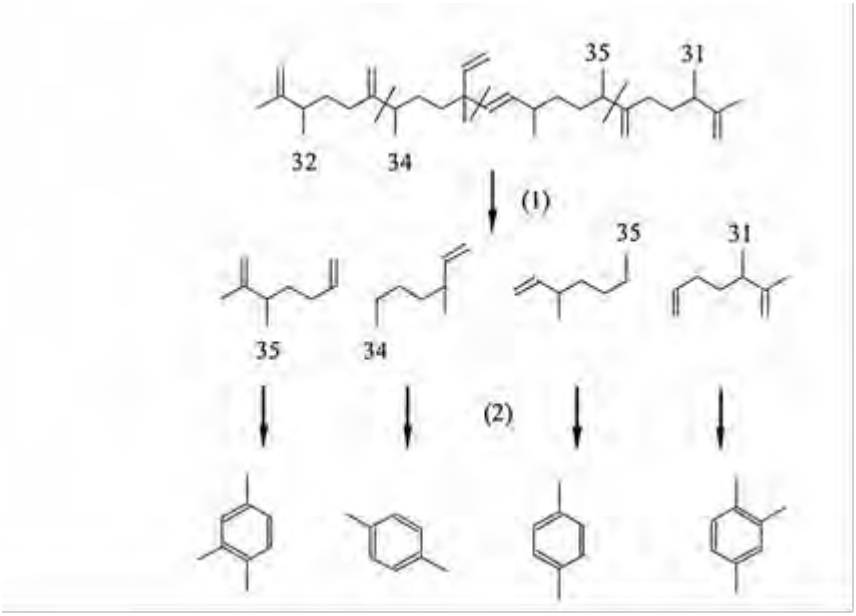


Figure 7.2 Reaction sequence for catalytic cracking of C34-botryococcenes according to Kitazato et al. (Tran et al. 2010)

Conclusion:

- Based on pyrolysis at high temperatures accompanied by very short reaction times (fast or flash pyrolysis), high yields of liquid, oleaginous products can be obtained from micro-algae.
- The products are of very poor quality (containing large amounts of high-molecular unsaturated compounds and oxygen-containing components) and require chemical upgrading.

- Catalytic cracking on solid acidic catalysts comes into consideration mainly to improve the quality of primary oils. For triglyceridic oils, catalytic cracking can be seen as an alternative to conventional transesterification.

7.4 Hydrocracking

In pyrolysis (cracking) processes the formation of a solid residue is unavoidable. The products are always characteristically unsaturated. These negative symptoms can be mitigated if the split is overlaid by a hydrogenation. So-called hydrocracking requires high hydrogen partial pressures (up to 20 MPa) and the use of usually bifunctional catalysts. These are supported catalysts, whereby the hydrogenation components in the form of noble metals or metal sulphides are deposited on a porous, acidic carrier (zeolites, chlorinated aluminates).

The principle of hydrocracking can be traced back to the Bergius-Pier process, which was employed on a large technical scale during the Second World War for the liquefaction of coal. It is in widespread use nowadays for the processing of heavy distillation residues in crude oil refineries.

Hillen and colleagues (HILLEN et al. 1982) investigated the hydrogenating splitting of lipids of the micro-alga *Botryococcus braunii* in a continuous pilot plant reactor (throughput 230 ml/h) using a cobalt molybdate catalyst. At 400 °C and 20 MPa, 80 % by mass of the lipid was converted into an oil product, primarily consisting of naphtha (66 %), diesel (16 %) and kerosene (14 %) (Figure 7.4). The naphtha fraction obtained is low in aromatics and has an octane number (RON) of 82 (HILLEN et al. 1982). This very high value results from the very high iso-paraffins content. Olefins are present in the products only in traces.

The input oil was obtained by an acetone extraction of the freeze-dried alga, and accounted for 30 % of the total algal mass.

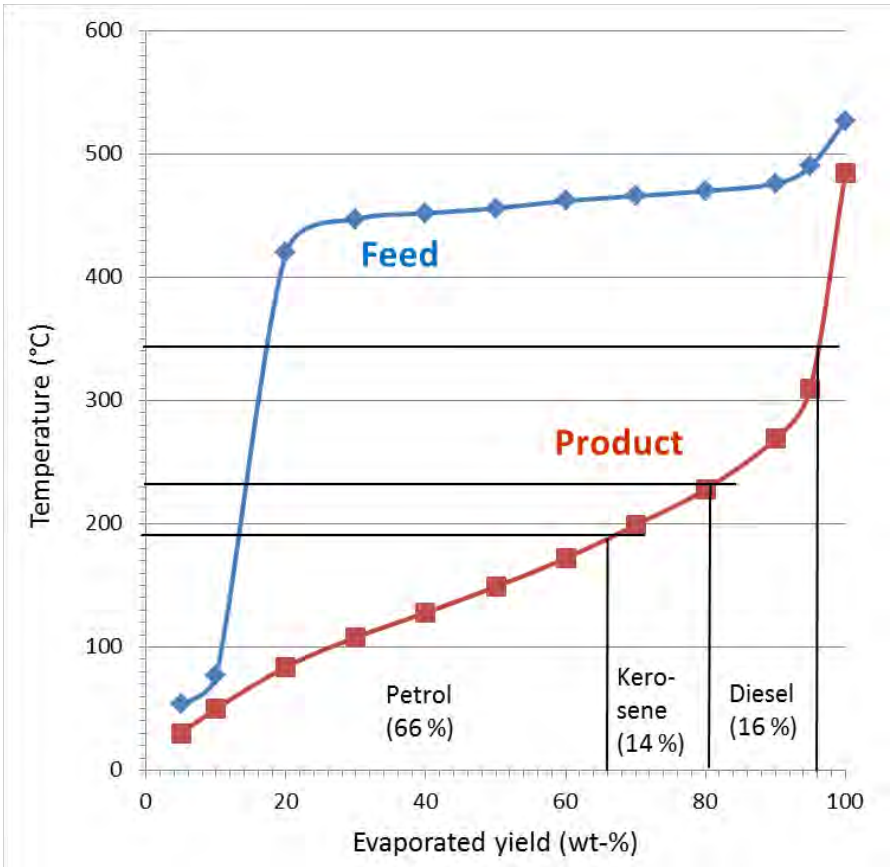


Figure 7.3 Boiling behaviour of the oil of the alga *Botryococcus braunii* before and after hydrocracking (according to HILLEN et al. 1982).

Conclusion:

- Hydrocracking, like catalytic cracking, is also suitable as a means of improving the quality of liquid products obtained from algae, especially for the long-chained hydrocarbons of *Botryococcus braunii*.
- In contrast to catalytic cracking, hydrocracking products are low in aromatics and olefins. No coking occurs.
- Disadvantages are that the raw material can obviously not be used moist, and very high hydrogen partial pressures are required.

7.5 Hydrogenation

Hydrogenating processes (hydrotreating, hydrorefining) are used in the oil industry to remove hetero-atoms and to saturate cracking products. The C-O, C-S and C-N bonds are broken by the application of increased temperatures (300 – 450 °C). The hetero-atoms are catalytically transferred to the corresponding hydrogen compounds (water, hydrogen sulphide and ammonia) under high hydrogen pressure, thereby passing into the gas phase. The remaining hydrocarbon residues are saturated with hydrogen. Depending on how the hetero-atoms were bound into the molecular structure, the hydrogenation entails a reduction in the original molecule size. Additionally, the implementation of

sharp conditions can be used specifically to initiate the splitting of C-C bonds (splitting hydrogenation). The transition to hydrocracking is seamless.

Wildschut and colleagues report on the hydrogenation of fast pyrolysis oil obtained from lignocellulose-containing biomass. For the discontinuous experiments in small autoclaves both noble metal catalysts (ruthenium, platinum, palladium on different carriers) and typical hydrotreating catalysts (nickel-molybdenum, Cobalt-molybdenum on alumina, sulphided) were used. In terms of product yield and oxygen removal, the noble metal catalysts delivered somewhat better results than the sulphidic hydrotreating catalysts. With ruthenium on carbon, at 20 MPa (H_2) and 350 °C an oil yield of 60 % and an oxygen removal rate of 80 % were achieved (Wildschut et al. 2009)

Grange and colleagues also researched the hydrotreating of pyrolysis oils obtained from biomass. Pyrolysis products from wood are thermally very unstable, and may decompose into a coke-like substance when being heated up to the required hydrogenation temperatures. The authors therefore propose a two-stage procedure whereby the oil is first stabilised at mild temperatures by hydrogenation of the reactive components (olefins, alcohols and ether). It can already be used as heavy fuel oil even in this form, or be fully hydrogenated under sharper temperature conditions (350 to 425 °C) (conversion of the phenols and furanes). The works are based on thermodynamic and kinetic studies relating to the hydrogenation of model components on sulphidic cobalt-molybdenum catalysts (Grange et al. 1996).

The chemical composition of pyrolysis oils from lignocellulose (wood, straw) and from algae differs fundamentally however. The same applies also to the feedstock materials. The results of the aforementioned works are thus only applicable to the hydrogenation of algal materials to a very limited extent.

At present the hydrogenation of vegetable oils is under consideration as an alternative route to transesterification for the production of biofuels from natural oils and fats. The NESTE Oil corporation offers a process of this kind, and the first industrial plants already exist in Porvoo (Finland), Singapore and Rotterdam (Lindfors 2010).

The lipids extractable from micro-algae contain triglycerols and free fatty acids which should respond similarly to vegetable oils under hydrogenation conditions.

Figure 7.4 shows the structure of a triglycerol typical for native oils and fats. In the transesterification described in section 4.3, the trivalent alcohol glycerol is replaced by three methanol molecules. The resultant product (FAME) retains the double bonds and the ester groupings. Conversely, under hydrogenating conditions a mixture of pure n alkanes is produced (Endisch et al. 2008). The triglycerol molecule is split at the locations marked in brown and the free valences are saturated with hydrogen – as are the double bonds in the fatty acid residues.

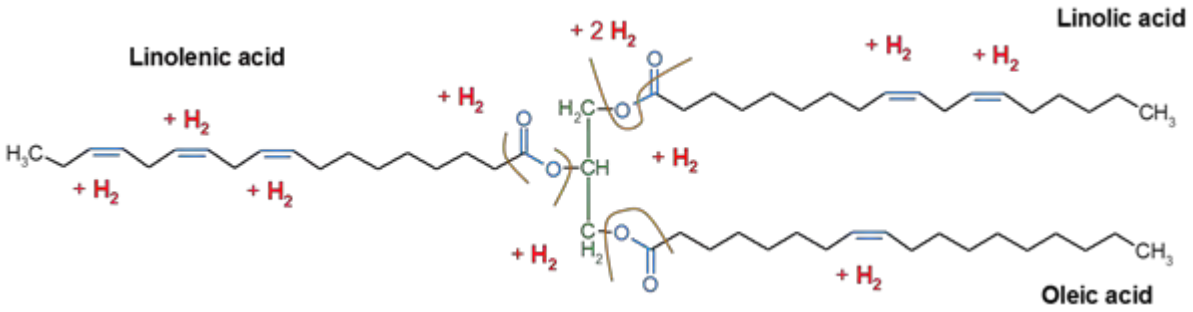


Figure 7.4 Triglycerol structure and reaction paths in hydrogenation (Kuchling et al. 2010)

The hydrogenation of vegetable oils was investigated experimentally in a continuous test plant at 320 °C and 6 MPa (H_2) using a commercially available nickel/molybdenum catalyst. Figure 7.5 presents the results for the hydrogenation of rape-seed oil and jatropha oil in comparison. Approximately 85 % of the raw material can be transferred to a completely oxygen-free diesel fraction. By-products are propane, carbon oxides and water, corresponding to the postulated reaction sequence (Kuchling et al. 2010).

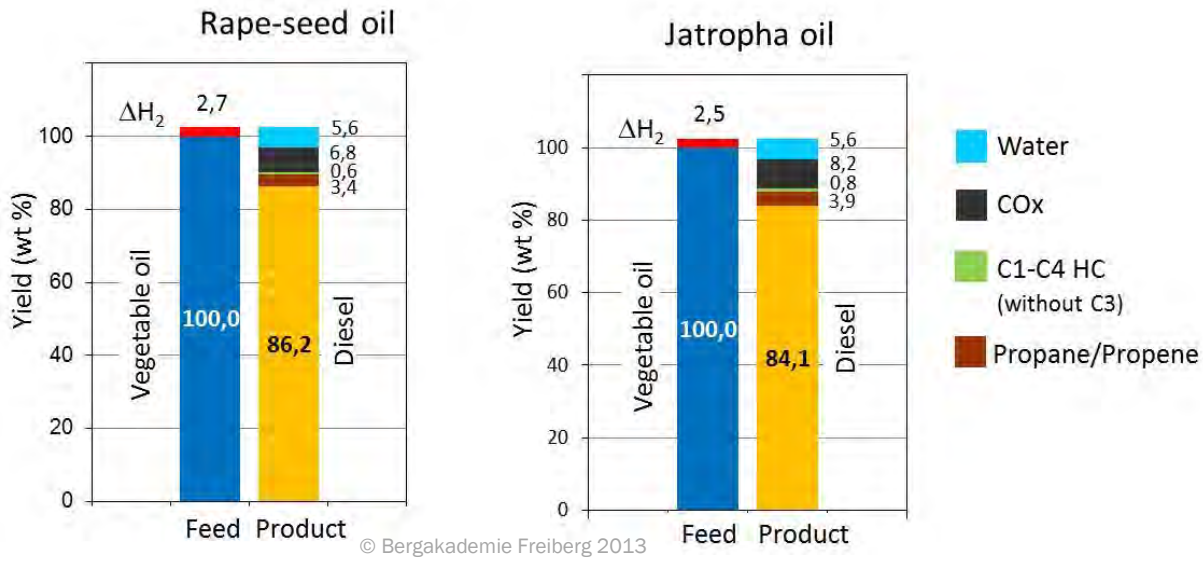


Figure 7.5 Results of hydrogenation of vegetable oils (Kuchling et al. 2010)

The dissertation by Chin (Chin 1979) reports on the hydrogenation of the micro-alga *Chlorella pyrenoidosa*. In this, the entire algal mass was converted under hydrogen atmosphere both discontinuously and continuously in agitator autoclaves on typical hydrogenating catalysts (Ni/Wo on alumosilicate and Co/Mo on alumina).

The pre-dried algae were introduced into the reactor with a carrier oil/solvent, in order to support the hydrogen transfer from the gas phase to the algal biomass. Table 7.8 shows the composition of the micro-alga used.

Table 7.8 Composition of the micro-alga Chlorella pyrenoidosa (wt.%) (Chin 1979)

C	H	N	O (diff.)	Ash	Moisture	Total
49,6	6.3	10.8	24.5	6.3	2.5	100

Proteins	Carbohydrates	Lipids	Ash	Moisture	Total
62,9	16.4	10.9	6.3	2.5	100

At temperatures of 400 to 430 °C and pressures of 7 to 14 MPa, oil yields of up to 46.7 % by mass (referred to the input algal mass) could be attained. The conversion rates were approximately 95 %. By-products were approximately 10 % water and up to 30 % gaseous products (mainly hydrocarbons and carbon oxides). Table 7.9 shows the product distribution for a test point (400 °C, H2 pressure 8.3 MPa, retention time 210 min) as well as the elemental composition of the main products (Chin 1979).

Table 7.9 Product distribution and elemental composition of products in hydrogenation of algae (wt.%) (according to Chin 1979)

Product	Percentage	C	H	N	O (diff.)	Ash
Oil	43.8	82.0	8.9	4.6	3.7	1.8
Asphaltenes	12.3	79.7	7.7	6.9	5.3	4.1
Residue	6.6	28.0	5.3	6.8	22.7	37.2
Water	9.6					
Ammonium carbonate	5.2					
Gas	22.5					

Generally, higher temperatures and longer reaction times result in an increase in conversion rate and a decrease in asphaltene formation. The oil yield and conversion rate increase likewise proportionally to the hydrogen pressure up to a maximum at 8.3 MPa (cold). The oil product then contains only relatively small amounts of oxygen and nitrogen, and consists of approximately 40 to 45 % pure hydrocarbons (Chin 1979). In addition to the process parameters temperature, pressure and residence time, the influence of the catalyst type and quantity as well as the type and quantity of the carrier oil were also investigated. Finally, a simple kinetic model was presented postulating a step-by-step degradation of the algal biomass to form asphaltenes and oils, accompanied by splitting-off of carbon oxides, water and hydrocarbon as well as the formation of ammonium carbonate (Chin 1979).

Conclusion:

- Hydrogenation is a suitable process for improving the quality of liquid products (saturation of double bonds, removal of hetero-atoms) and is in widespread use in refineries in cleaning of the primary crude oil products.
- A number of research groups are looking into the hydrogenation of wood pyrolysis products.
- For the hydrogenation of triglycerols, which form the main constituents of the extractable lipids in many micro-algae, experiences has been gathered from in-house experiments.
- The complete algal mass can also in principle be subjected to hydrogenation. The results of a study presented as part of a dissertation are promising.

- As in the case of hydrocracking, high hydrogen pressures as well as drying of the raw material are required for the reaction.

7.6 Gasification

Gasification refers to the conversion of carbon-containing substances with water vapour and/or carbon dioxide at high temperatures to form mixtures of gaseous and combustible gas components.

The process has been implemented for a wide variety of feedstock materials (coal, distillation residues, wastes, natural gas) and is state of the art (Krzack 2008). The processes usually operate in the temperature range between 600 and 1000 °C. Target products under these conditions are CO/H_2 mixtures, the so-called synthesis gas. Demonstration plants are also already operating for the gasification of biomass like wood and straw. (Kaltschmitt 2001, Brown 2011). A large number of procedures for its technical implementation.

The key gasification reactions

- $C + H_2O \rightarrow H_2 + CO$ (water gas reaction) and
- $C + CO_2 \rightarrow 2\ CO$ (Boudouard reaction)

are endothermic. The required heat can be supplied externally (allothermic gasification) or can be generated by partial incineration of the feedstock in the reactor itself (autothermic gasification, partial oxidation).

For the production of synthesis gas from micro-algae a number of research groups have investigated catalytically assisted low-temperature gasification (Tsukahara and Sawayama 2005, Elliott and Sealook 1999), in which the moist algal mass can be used directly. The nitrogen bound into the algal biomass is released in the process as ammonia. Thermodynamically, exothermic methane formation is promoted at lower temperatures, so the gasification of algae is suitable primarily for the production of biogenic methane (Amin 2009).

Minowa and Sawayama investigated the gasification of *Chlorella vulgaris* with 87.4 % moisture on nickel catalysts at 350 °C and 18 MPa. For each of the experiments 30 g of the moist algal mass was used, with a varied quantity of catalyst (Sawayama et al. 1999). The highest gas yield (70.1 %) was obtained by adding 15 g of catalyst. As the carbon conversion rate rises, the methane content increases and the hydrogen content decreases (Table 7.10).

Table 7.10 Gasification of *Chlorella vulgaris* – Conversion rate and gas composition (Sawayama et al. 1999)

Catalyst quantity	Carbon conversion	Gas composition (vol.%)			
		CH_4	**H_2**	**CO_2**	**Others**
5 g	35.0 %	15.6	34.9	46.2	3.3
10 g	62.0 %	27.0	25.5	43.5	4.0
15 g	70.1 %	37.5	10.0	48.8	3.7
Equilibrium	100.0 %	49.7	5.9	44.4	-

The pathway to motor fuel production also remains fundamentally open by way of the familiar methods: Fischer-Tropsch synthesis and methanol synthesis. This does, however, demand highly complex conditioning of the synthesis gas (gas purification, adjustment of the CO/H_2 ratio).

Conclusion:

- The process of (autothermic) gasification is in principle suitable for the production of combustion and synthesis gases from micro-algae.
- An upstream drying stage is not necessary, as water vapour is required in the process as a reaction partner. If the water content is too high, however, the necessary reaction temperatures cannot be reached.
- For micro-algae, a catalytically (nickel) assisted conversion at low temperatures to form a methane-rich biogas is a practicable option.
- The production of synthetic motor fuels by way of gasification is a highly complex and costly process, so the supply of combustion gas appears more reasonable.

8 Biogas production[11]

8.1 Introduction

The aim of this section is to set out the current state of knowledge relating to the use of micro-algae in biogas production. To that end, a literature search was conducted in relation to its general suitability, to the gas yield potential and to parameters relevant to the process biology which might influence the use of micro-algae as a biogas substrate. The section makes reference to the alga species used at the Senftenberg facility. Negative characteristics in terms of substrate composition, degradability and attainable gas qualities are described. It also investigates the possibilities for use of an intact algal cell as well as for biogas production from residual fractions of other production chains.

The first subsection provides a general overview of biogas production. The next subsections consider the suitability of micro-algae, their advantages and disadvantages for this procedure, and the possible biogas yields. Finally, the possible forms of use of micro-algae in biogas production are described.

8.2 Overview of biogas production

In a biogas plant a substrate is degraded by means of anaerobic processes primarily to produce methane, carbon dioxide and water. Substrates used are effluent (sewage; waste water), organic residue and by-products, as well as – especially in recent years – regrowable resources. Anaerobic degradation itself can be divided into three usually simultaneous steps. Different bacteria groups are responsible for each step. In a first step, called hydrolysis, polymer organic compounds are transferred into low-molecular compounds such as fatty acids and alcohols. The next step, acetic acid formation, converts those compounds into acetic acid. The biogas itself is produced in the final step, methane formation. Most of the gas is formed by the splitting of the acetic acid into methane and carbon dioxide ($CH_3COOH \rightarrow CO_2 + CH_4$). The smaller portion of the biogas, around 30 %, is produced by the reaction of carbon dioxide and hydrogen to form water and methane ($CO_2 + 3H_2 \rightarrow 2H_2O + CH_4$). In order to provide the bacteria with optimum living conditions, enabling a high biogas yield, the digesters are kept at a constant temperature. Based on this temperature, biogas plants are classified either as mesophilic systems, with an operating temperature of approximately 37 °C, or thermophilic systems, which operate at around 57 °C. While the intrinsic energy demand of mesophilic plant is lower, thermophilic plants offer advantages in terms of productivity and hygiene. Mesophilic processes are more widespread in practice.

A key parameter of a biogas plant is the hydraulic retention time (HRT), which indicates the average time the substrate spends in the biogas plant. It is calculated from the volume of the digester and the continuously or semi-continuously inputted (and simultaneously outputted) volumetric flow:

[11] This section was authored by the Deutsches Biomasseforschungszentrum (DBFZ)

$$HRT = \frac{V}{\dot{V}}$$

HRT	Hydraulic retention time in days
V	Digester volume in m³
$\dot{V}$	Flow rate in m³/day

Another important variable is the volume load of the digester. This indicates the quantity of substrate converted in the digester per day referred to the volume. It is calculated as follows:

$$B_R = \frac{F}{V}$$

B_R	Volume load in kg/(m³ d)
F	Solid matter flow into the digester in kg/d
V	Digester volume in m³

Common values for the hydraulic retention time and the volume load for various digester types are presented in the following Table 8.1:

Table 8.1. Hydraulic retention time and volume load of selected digester types according to (Kaltschmitt 2009)

Digester	Hydraulic retention time in days	Volume load in kg/(m³d)
Stirred tank	10…40	2…4
Contact method	3…10	5…15
UASB (sludge bed digester)	0.2…1	5…15
Anaerobic filter	0.5…8	5…15

Other parameters of the biogas plant are dependent on these two variables. Among others, they specify the necessary dry matter content of the substrate. Although increasing volume load and reducing retention time are targets in terms of economic viability, any non-conformance to these guideline values in most case impacts negatively on the plant's productivity. If the retention time is shortened, for example, there is a risk that too many bacteria will be flushed out of the digester and reduce biogas production. Increasing the volume load results in acidification of the digester. The hydrolytic bacteria produce a surplus of acids, which in turn hinder methane formation.

In practice, mainly semi-continuous plants are encountered. That is to say, the substrate is not added continuously but periodically, such as every four hours. The process itself can thus be regarded as

continuous. The only exceptions are the so-called dry digestion plants, in which stackable substrates are batch-digested.

8.3 Suitability of micro-algae for biogas production

Biogas production plays an important role in terms of utilising the energy in algae. Whereas oil extraction only uses the lipids from the algae, biogas production also enables the proteins and polypeptides to be converted into a generally usable energy source material. Based on that fact, there are a large number of research works looking into biogas production from the algae themselves, or from the residues of upstream productions steps (Weissman and Goebel 1987; Lundquist et al. 2010; Harun et al. 2011). Against this background, digestion experiments with micro-algae have already been conducted. These involved micro-algal suspensions containing 0.8 % (Mussgnug et al. 2010) to 2.5 % (Liu 2010) organic volatile solids (VS). These concentrations are quite common for batch-digestion experiments. They accurately simulate the situation in the digester. For these conditions to be maintained in a continuous plant without too many bacteria being flushed out of the digester, higher solid matter concentrations of the input materials are required. The results should thus be evaluated primarily in terms of biogas yield, as the experiment conditions differ substantially from those of biogas production in practice. Commercial plants operate with much lower water content in the substrate. The biogas measurement programme II (Weiland et al. 2010) determined an average dry matter content in the substrates of German biogas plants of 25 %. This percentage is kept constant by an appropriate mixture of the available materials. In this way the reachability of the substrate for the hydrolytic bacteria and the resultant productivity can be optimised. Moreover, using substrates with lower dry matter content would mean that more energy would have to be expended to heat the substrate, and the heat losses via the discharge of the digested materials would be increased. Against this background, it becomes clear that, as in existing biogas plants, micro-algae for anaerobic digestion must also be present in concentrations comparable to those of conventional substrates.

The real biogas yield depends primarily on the retention time and on how well the hydrolytic bacteria in the digester are able to break down the cell structures and render hard-to-degrade constituents water-soluble, and so make them available. Generally applicable statements regarding biogas yield do not withstand stringent testing, as not only the selection of an alga species but also the conditions under which it was cultivated have a major influence on the composition.

Green algae, which frequently have cell walls containing hemicellulose, are able to withstand degradation by the bacteria even over lengthy retention times, as the works of Mussgnug et al. (Mussgnug et al. 2010) show. Figure 8.1 indicates that the cells in some cases withstood 28 days' digestion in the biogas reactor without harm, depending on the alga species. This can either be countered by thermo-chemical pre-treatment (Chen and Oswald 1998), or the entire digestion is carried out under thermophilic conditions, by which the high resistance to bacterial degradation especially of living alga cells can be reduced (Golueke et al. 1957). This problem might be countered with complete cell decomposition. Doucha and Lívanský (Doucha and Lívanský 2008) achieved virtually complete cell decomposition (99 %) in a ball mill (agitator ball mill). This process on a laboratory scale consumed 0.66 kWh of power per kilogram of dry algal biomass.

By contrast, a positive factor in terms of digestibility is the small particle size of the micro-algae. Thanks to its high surface to volume ratio, this substrate is very reachable for the hydrolytic bacteria.

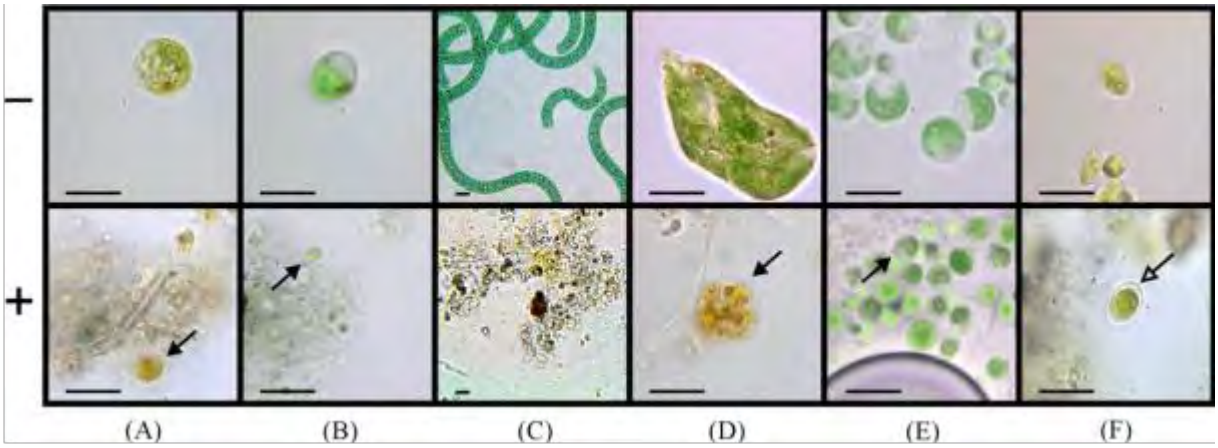


Figure 8.1 Various micro-algae as substrate before (-) and after (+) a 28-day digestion in the biogas reactor under mesophilic conditions; A: Chlamydomonas reinhardtii; B: Dunaliella salina; C: *Spirulina* platensis; D: Euglena gracilis; E: Chlorella kessleri; F: *Scenedesmus obliquus* from (Mussgnug et al. 2010)

Owing to their high nitrogen content, micro-algae as biogas substrate do not offer the optimal C/N/P ratio of 100...200/4/1 (Kaltschmitt 2009; Salerno et al. 2009). The nitrogen, which is mainly converted into ammonium and ammonia, may hinder biogas formation (Yen and Brune 2007). The extent to which adaptation of the micro-flora to increased ammonium concentrations in the digestion of micro-algae is possible cannot be specified in advance. The data relating to tolerance of high ammonium concentrations vary very widely, primarily due to the adaptability of the micro-organisms. With correspondingly long adaptation times (up to one year), this hindrance can be counteracted (Fachagentur Nachwachsende Rohstoffe 2005). Analysis of the micro-algae of the genus *Scenedesmus obliquus* provided by GMB GmbH revealed a nitrogen content typical for micro-algae of 6.5 % (dm). The C/N/P ratio could likewise be determined from the analyses, and is 40/5/2. This shows that the algae differ substantially from the conventional biogas substrates. Digestion of the algae thus requires considerable effort in terms of adaptation of the biocoenosis of the biogas plant.

There have been a number of experiments in the past aimed at optimising the C/N/P ratio in biogas production from algae. The admixture of additional substrates with a correspondingly low nitrogen and phosphor content plays a key role in this. Yen and Brune (Yen and Brune 2007) mixed the micro-algae with paper waste in order to increase biogas productivity. The highest productivity was achieved with a substrate comprising 40 % micro-algae and 60 % paper waste. Thus productivity could be almost tripled in comparison to the use of algae as a monosubstrate. Ehimen et al. (Ehimen et al. 2011) mixed glycerol into the algae as a co-substrate. After an initial adaptation phase, an increase in productivity could also be observed here. The method adopted by Salerno et al. (Salerno et al. 2009) of increasing biogas productivity by additionally feeding soy-bean oil into the digester likewise resulted in the desired higher yield. The addition of oil must be carefully judged, however, as it is in itself already a high-value energy product, and so it may be that there is no further benefit to using it in digestion.

A further hindrance on increasing the concentration may be the detected potassium concentration of just under 17 g/kg (dm) in the micro-algae of the genus *Scenedesmus obliquus* cultivated by GMB GmbH. Above a concentration of 3 g/l, potassium impedes biogas production (Kaltschmitt 2009) and at higher concentrations results in disturbances to the process extending even to the complete stoppage of biogas production (Hölker 2008).

8.4 Yield prediction

A variety of methods exist to estimate the digestibility of substrates for creating biogas. Feinberg (Feinberg 1984) assigns specific biogas yields to the individual fractions of the organic volatile solids (VS) of micro-algae. Other authors pursue the same approach. In contrast to Feinberg, the yields set out in Table 8.2 according to Weißbach and VDI guideline 4630 consider only the yield of conventional energy crops and biogas substrates. Consequently, as the comparison with the yields from the digestion experiments with micro-algae in Table 8.2 also shows, Feinberg's method is preferable to that of Weißbach and to VDI guideline 4630. Buswell's formula, enhanced by Boyle, which is frequently used for predicting biogas yield, delivers excessively high yields due to its purely stoichiometric approach. Consequently, it is not considered further here.

Table 8.2. Prediction of methane and biogas yields in each case as m³ in standard conditions

	Proteins Biogas (methane) m³/kg	Lipids Biogas (methane) m³/kg	Carbohydrates Biogas (methane) m³/kg
Yield according to Feinberg (Feinberg 1984)	0.65 (0.39)	1.37 (0.82)	0.50 (0.30)
Yield according to Weißbach (Weißbach 2009))	0.78 (0.40)	1.35 (0.96)	0.79 (0.40)
Yield according to VDI guideline 4630 {VDI - Gesellschaft Energietechnik #436}	0.80 (0.48)	1.39 (1.00)	0.75 (0.38)

In order to provide a more detailed picture of yields from anaerobic digestion, the following in Table 8.3 presents the yields from digestion experiments with various micro-algae. Here it should be noted that none of the experiments was initiated by time-intensive adaptation of the micro-flora to the substrate.

Table 8.3. Published biogas and methane yields (m³ in standard conditions) per kilogram VS (volatile solids) of digestion experimentsn with micro-algae

Substrate	Biogas yield in m³/kg	Methane yield in m³/kg	Comments	Source
Chlorella vulgaris		0.240 (0.147)	28 (16) days retention time	(Ras et al. 2011)
Scenedesmus obliquus	0.287	0.178		(Mussgnug et al. 2010)
Arthrospira (Spirulina) platensis	0.481	0.293		(Mussgnug et al. 2010)
Chlorella kessleri	0.335	0.218		(Mussgnug et al. 2010)
Chlorella sorokiniana		0.258 (0.319 with nitrogen limitation)		(Liu 2010)
Tetraselmis suecica		0.236 (0.337 with nitrogen limitation)		(Liu 2010)
Micro-algae mixed cropping	0.350…0.600		Linked production system: PBR + digestion	(de Schamphelaire and Verstraete 2009)
Mixed crop from sewage treatment	0.600	0.365		(Salerno et al. 2009)
Chlorella sp.	0.341	0.219		(Schmack et al. 2008)
Spirulina maxima	0.370	0.260	33 days retention time	(Samson and Leduy 1982)
Mixed crop from sewage treatment		0.340 (0.450)	without (with) thermo-chem. pre-treatment	(Chen and Oswald 1998)
By comparison: Biogas yields of the most commonly used conventional substrates				
Maize silage	0.809	0.422		(Weißbach 2009)
Whole-crop cereal silage	0.813	0.425		(Weißbach 2009)
Swine manure	0.400	0.210		(Weißbach 2009)

8.5 Recovery paths

The conceivable potential uses of micro-algae in biogas production are briefly set out at this point. The possible method of recovering biogas from algal biomass can be roughly divided into the following categories based on form of use (material, energy) and classification as main product or by-product:

8.5.1 Digestion of the complete algal biomass

The simplest recovery path is conversion of the complete biomass to biogas, so that the algae are used as regrowable resources with all the advantages and disadvantages. Figure 8.2 shows a schematic view of a vertical heated digester. Over 90 % of the German plants registered in the Biogas Measurement Programme (Weiland et al. 2010) are of the vertical wet digester design.

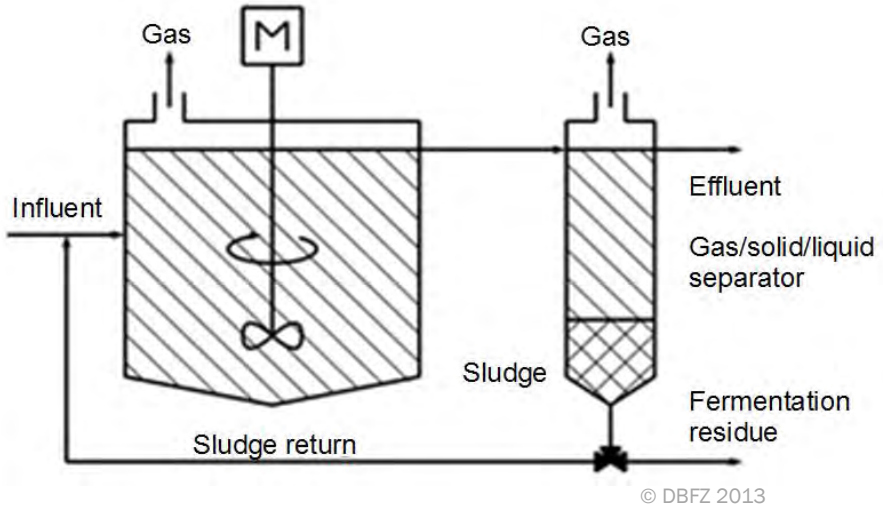


Figure 8.2 Schematic view of the contact method (DBFZ 2013)

Alongside digestion in conventional biogas plants, mention must be made also of the "covered lagoon" method. These were the preferred plant for the digestion of micro-algae and the residual fractions in the large-scale research projects on the use of micro-algae for energy (Weissman and Goebel 1987). The advantage of such a plant lies primarily in its simple design and uncomplicated mode of operation, with no intermingling. The only heat input is uncontrolled sunlight.

Figure 8.3 "Covered lagoon". Left: Schematic layout. Right: Plant (DBFZ 2013)

These plants are dependent on the ambient conditions. In Central Europe, year-round production is not possible because of the weather. The retention time and land requirement are therefore much greater than in the typical German plants. As a result, they offer an alternative to heated digesters especially for regions with little seasonal fluctuation in weather conditions. This favours a linked micro-alga production since, here too, large plants rely on moderate and as far as consistent ambient conditions.

Micro-algae can in principle also be used as co-substrate in biogas plants. As opposed to the forms of use described above, in this case the algae perform a kind of balancing function. If a substrate has a

lower nitrogen content than normal, for example, it can be adjusted by mixing-in defined quantities of micro-algae. This technique is commonly applied in biogas plants, where an attempt is made to establish as consistent a C/N/P ratio as possible by mixing various substrates.

Owing to the quite small quantities of micro-algae cultivated as raw material worldwide, and their resultant price, they have not to date been digested to produce biogas on a scale permitting direct transferability of the results to currently existing biogas plants and processes.

8.5.2 Digestion of individual fractions

An alternative to digestion of the complete biomass is the use of the residual products from upstream process steps. Ehimen et al., for example, demonstrated in experiments that approximately 0.25 m^3 of methane per kilogram (dm) could be recovered from the residues after oil extraction from *Chlorella vulgaris* (Ehimen et al. 2011). The same team of scientists also digested micro-algae from which the lipids had first been removed by an in-situ process (Ehimen et al. 2009). The specified yield indicates that oil extraction alone is not capable of fully or largely recovering the chemical energy contained in them.

Following a hydrothermal treatment, the portions of the chemically bound energy are to be found in the liquid phase, and can be made accessible by digestion. The basic suitability of the liquid phase of the product of a hydrothermal treatment could be demonstrated, for example, by Wirth and Mumme (Wirth und Mumme 2011). Since the digestible substances in this eluate are largely in dissolved form, it is opportune to use a digester specialising in substrates with low solid matter content. In temperate climate zones, stable and reproducible conditions for the biogas-producing micro-organisms can be created by so-called fixed film digestion (anaerobic filters). The functional principle of the anaerobic filter is based on the fact that the substrate flows through a fixed carrier material, on which the bacteria have been grown, and the organic components are degraded in the process. In order to achieve higher degradation rates, circulation is possible. Another variant of the anaerobic filter is the dynamic anaerobic filter. In this, the carrier material is slowly moved around in the liquid by a motor so as to ensure contact between the substrate and the bacteria.

8.5.3 Linkage with biogas cleaning

Alga cultivation and biogas production can also be linked by way of the carbon dioxide. In this, the carbon dioxide in the biogas is made available to the algae to construct the biomass.

The aim of the EBSIE project (Improved efficiency of biogas use based on solar energy) (Schmack et al. 2008) was to investigate this possibility on a pilot plant scale. For the purpose, the cultivation medium was fed through a gas scrubber tower into which the raw biogas was blown. This approach achieved a method of treating the biogas based on the differences in solubility of carbon dioxide and methane in water. The EBSIE project employed open ponds for cultivation, as a result of which small quantities of methane are also discharged to the atmosphere. No measurements of the methane losses were taken, but they were estimated from the solubility equilibrium at around 5 %. The positive climatic effect of carbon dioxide avoidance may be negated by the greenhouse gas potential of the methane, which is more than 20 times that of carbon dioxide. The scientists have as yet seen no economic potential in

this process. The main reasons for this were the high expense on nutrients for the micro-algae and the investment costs.

At the University of Rostock, a closed alga production system for biogas treatment was investigated. In closed systems no methane is discharged to the atmosphere. Instead, the biogas is enriched with the oxygen produced by the micro-algae. While on average 95 % by volume of the carbon dioxide could be removed from the digester gas, the oxygen content of the biogas rose from 1.0 % by volume to an average of 21 % by volume (Mann; Mann et al. 2009). Since the carbon dioxide was only replaced by oxygen as a result, the methane content of the biogas treated in this way increased only slightly.

Studies at the TU Dresden confirm these results. In experiments there involving removing the carbon dioxide from the digester gas of a sewage treatment plant by means of phototropic micro-organisms, the methane content (vol. %) of the gas could not be increased, because the oxygen produced was enriched in the digester gas. Separation CO_2 introduction and oxygen removal would pose a risk of explosive mixtures forming (TU Dresden - AG Abwasserbehandlung 2005).

The potential of the methods set out in this section remains low as long as no satisfactory solution is found for the problems of methane degassing from open systems and oxygen enrichment of the biogas in closed systems.

8.6 Summary

Biogas production plays a major role in the energy yield from alga production. Only by integrating this step into a system of process control, of whatever kind, for energy use will it be possible to make the chemical energy of the algal biomass usable (Harun et al. 2011).

It is assumed that biogas production from the algal suspension cannot be successful without prior concentration, as this would increase the volume of the biogas plant and extend retention times to an excessive extent. Studies cite necessary concentrations of 3 % dm to 5 % dm for biogas production in anaerobic ponds (Lundquist et al. 2010; Collet et al. 2011). For the wet digestion plants typical in Germany, it is to be expected that the target water content of the algae needs to be in the range of that of the substrates normally used. Another advantage of biogas production is the possibility to recover nutrients. As in the case of agricultural biogas plants, losses must be expected from the nutrient cycle (LfL 2009), as a result of which a closed nutrient circle is not possible.

An optimum operation point, at which the energy input for dewatering and heating is minimised relative to the amount of primary energy generated in the form of methane, is achieved specific to individual plants based on criteria including the given volume load, the dewatering method and the available waste heat. Here the existing plants would need to be investigated individually.

9 Algae as animal feed[12]

Algae are used in a wide range of commercial applications nowadays. They are used, among other things, to increase the nutritional value of human and animal foods, and they also play an important role in fish farming (Spolaore et al. 2006).

In order to be able to use algae for human and animal foods at all, a number of preconditions must be met (Becker 2004).

- Chemical composition
- Biogenic toxins
- Non-biogenic toxins
- Studies on protein quality
- Biochemical food studies
- Health analyses
- Short- and long-term safety studies
- Clinical studies
- Acceptance studies

According to estimates, around 30 % of global alga production is sold as animal foodstuffs or animal foodstuff additives (Belay et al. 1996). A series of feeding experiments with rats (Janczyk 2006), mice (Janczyk et al. 2006), hens (Halle et al. 2009) and pigs (Hintz et al. 1966) have proved that algae such as Chlorella, *Spirulina* and Scenedesmus have a positive influence on the animals (DELANOUE and DEPAUW 1988).

9.1 Poultry

The use of (micro-)algae in conventional poultry feed has already been investigated in the past and has been implemented. The replacement of conventional protein sources by algae (such as *Chlorella, Euglena, Oocystis, Scenedesmus* and *Spirulina*) has been investigated in a number of feeding studies (Becker 1994).

Saxena et al. (Saxena et al. 1983) replaced the protein source peanut cake by *Spirulina maxima* (alga content from 5.6 to 16.6 %) in the feed of white laying hens. They documented the weight gain and intake of food over a period of six weeks. The control group exhibited little difference in terms of weight gain compared to the feed group with a 5.6 % *Spirulina* content, while at higher alga content levels a much more marked weight gain was recorded. The amount of food intake showed little difference. No toxic effects of *Spirulina* were detected during the experiment phase. *Spirulina* can thus fully replace peanut cake as a protein source. The group headed by Saxena (Saxena et al. 1983) likewise

[12] This section was authored by the Hochschule Lausitz.

investigated the pigmentation effect on the egg yolk as compared to conventional carotenoid sources (yellow maize, cracked wheat, rice). Increasing percentage levels of *Spirulina* (3 – 21 %) resulted in a much more intensive colouring of the egg yolk than in the case of yellow maize as a carotenoid source.

Toyomizu et al. (Toyomizu 2001) conducted experiments to study the influence of increasing *Spirulina* content (0, 4, 8 %) on the growth and muscle pigmentation of broilers. These found no significant differences in body weight, liver and kidney weight and abdominal fat. The animals fed with *Spirulina* did, however, exhibit more marked coloration of the breast muscles. 4 % *Spirulina* resulted in the highest red tones, while *Spirulina* content of 8 % produced the highest yellow tones.

Raach-Moujahed and colleagues also came to similar results(Raach-Moujahed et al. 2011). In their studies, they considered the influence of *Spirulina* on the performance capability, meat quality, colour and sensory attributes of broilers (initially 81 days old). The diet with *Spirulina* content of 0, 1, 2.5 and 5 % lasted 38 days. No significant differences could be detected in the studies with regard to body weight and food intake. Except for its colour, the quality of the meat was unaffected. The sensory attributes also remained unchanged.

Only a small number of studies consider the influence of algae on the laying behaviour of hens. Halle et al. (Halle et al. 2009) conducted a study feeding laying hens with *Chlorella vulgaris* (2,5, 5 and 7.5 % content). They were able to show that *Chlorella vulgaris* as food additives positively influenced the laying capacity of hens, the number and quality of the eggs laid. Here, too, no negative effects on the health of the hens could be detected, so the alga can be used as a food additive for laying hens.

As well as replacing conventional protein sources (soya bean meal, fish meal etc.), *Spirulina* can potentially also help to stimulate the immune system. Qureshi et al. (Qureshi et al. 1996) fed domestic hens and broilers with different percentage contents of *Spirulina* (0, 10, 100, 1000 and 10000 ppm) from hatching. With this study the scientists were able to demonstrate that the *Spirulina* additive strengthened multiple immunological functions such as macrophage function, antibody response and phagocytosis. Based on those findings, they assumed that using 10000 ppm *Spirulina* might also enhance potential resistance to pathogens.

9.2 Pigs

In order to study the influence of algae on pigs, Hintz and Heitmann (Hintz et al. 1966) employed a mixture of *Scenedesmus* and *Chlorella*. Soya bean and cotton seeds were replaced by 2.5, 5 or 10 % algae. The extent to which bone meal might be replaced as a protein additive by algae was also investigated. The results showed that algae had approximately the same protein content as cotton seeds and a similar protein quality to meat and bone meal. However, algal proteins are less digestible than conventional protein deliverers. This must be taken into account in feed rationing.

9.3 Aquaculture

As the lowest link in the food chain of aquatic systems, it is inevitable that algae should play a key role especially in aquaculture. They are used, among other purposes, to feed larvae over a short period of

time. The fresh biomass is, firstly, used directly as foodstuff to feed various mussel species (Rosello Sastre and Posten 2010) and, secondly, as live bait for small fish larvae (Muller-Feuga 2004). The most frequently used alga species are *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema* and *Thalassiosira* (Borowitzka 1997; Muller-Feuga 2004; Yamaguchi 1997). For algae to be used in aquaculture, they must meet certain requirements (Brown et al. 1999), (Spolaore et al. 2006), (Rosello Sastre and Posten 2010):

- Easy to cultivate
- Non-toxic
- Suitable form and size to be digested
- Easily digestible cell wall
- High nutritional value

A combination of various alga species offers more balanced nutrition and improved growth compared to feeding with only one alga species (Spolaore et al. 2006). The protein and vitamin content is decisive in determining the nutritional value of the micro-algae (Hemaiswarya et al. 2011). The content of polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA) is likewise of major importance (Reitan et al. 1997). Some fatty acids are essential for many aquatic animals (Sargent et al. 1997). They particularly play a major role for the growth of crustaceans, fish and molluscs (Becker 2004). Similar requirements also exist for the growth and metamorphosis of larvae in aquaculture.

Micro-algae are also used in enhancing aquaculture products. Artificial food sources lack a natural source of pigments which, for example, given salmon and trout their characteristic muscle colour (Spolaore et al. 2006). Astaxanthin and canthaxanthin, primarily chemically synthesised, are the only pigments which can be used to colour the fish. The demand for natural product is rising steadily, making the search for natural sources of those pigments ever more important (Becker 2004). Nowadays natural sources of astaxanthin are extracted oil from fresh-water crabs and krill (Becker 2004), the yeast *Xanthophyllomyces dendrorhous* (formerly Phaffia rhodozyma) (Sanderson, et al., 1994) and *Haematococcus pluvialis* (Lorenz and Cysewski 2000). In studies, *Haematococcus pluvialis* was fed to rainbow trout (Sommer et al. 1991; Sommer et al. 1992; Choubert and Heinrich 1993). The effect of synthetic carotenoids, in particular astaxanthin, on the colouring of the trout's flesh was stored higher than in the case of carotenoids from algae. The cause of the better pigmenting efficiency lies in the free form of the synthesised astaxanthin, while in algae the carotenoid is present in esterified form and only in small quantities (Choubert and Heinrich 1993; Gouveia et al. 2008).

Despite the advantages of using live micro-algae in aquaculture, the current trend is not to do so, because production of the micro-algae entails high cost as well as difficulties in cultivation, concentration and storage (Becker 2004; Borowitzka 1997). To make the use of algae for aquaculture economically viable, it is necessary to cut the cost of producing the biomass.

9.4 Legal aspects

The use of marine algae and of calcified seaweed for individual feeding of agricultural livestock is regulated by EU law (98/67/EC). In Germany, they may also be used by QA certified concerns, because they are on the whitelist of the Standards Commission for individual feeding (Lognone 2003).

Since 01.08.2011, *Spirulina* and *Chlorella* algae have been officially licensed as animal foodstuffs in Germany (DLG e. V. 2011).

As well as the alga as a whole, algal products are also used as additives (emulsifiers, stabilisers, etc.) in animal foodstuffs. These are regulated by the EU Directive 70/524/EEC (Lognone 2003).

10 Assessment of product lines

Based on the available data, we present an initial estimate of the mass and energy balance of selected processes. This section thus contains definitions of essential parameters for the process chains cited by the project partners as promising, together with rough balances for them.

10.1 Fundamentals

10.1.1 Aims of the assessment

In order to make the individual process chains comparable, a unified basis must be defined. Based on the results of the balance analysis, some initial estimates can be given as to how promising the various lines are in terms of energy use. The cited literature sources relating to individual process chains are often linked to different preconditions (dm content, energy content, alga species,...). To still ensure comparability, the input conditions for the various lines are specified by reference to the same variables.Figure 10.1 presents a schematic view of the balance analysis.

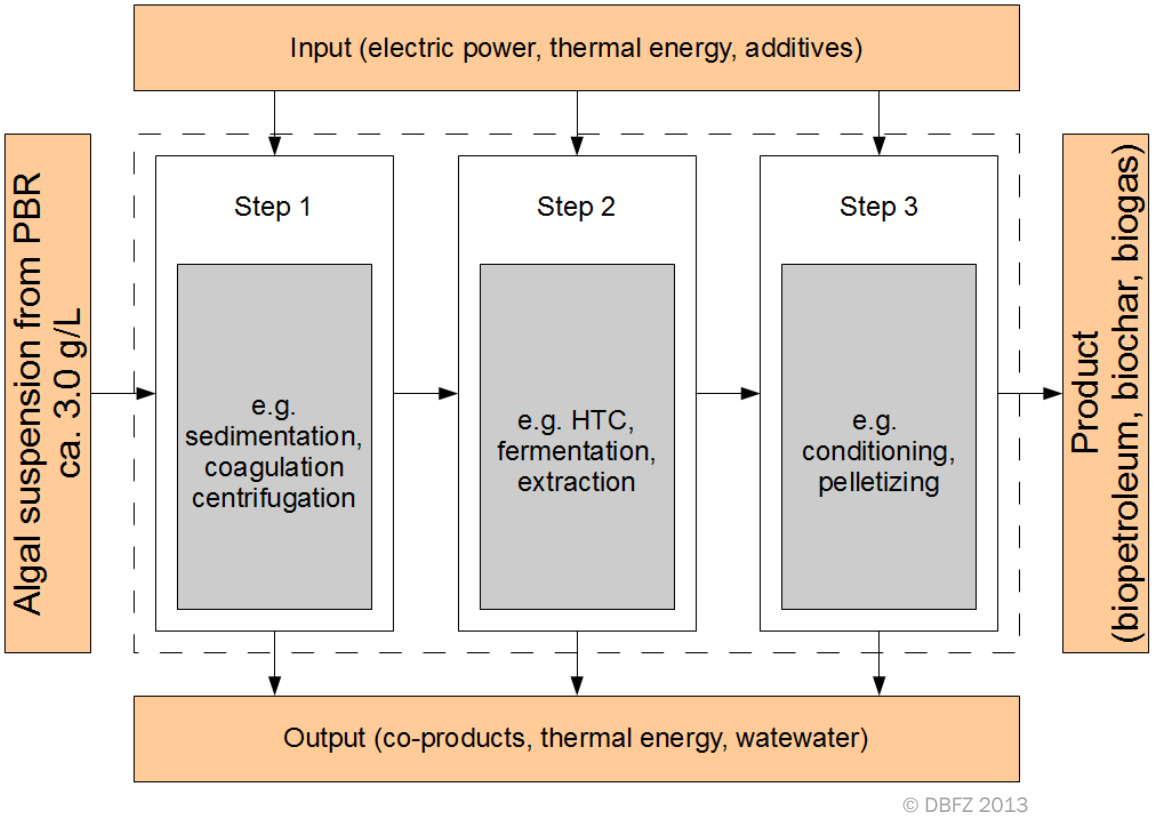


Figure 10.1 Schematic of assessment system

10.1.2 System constraints

Various process chains for the use of algal biomass as material and energy are investigated. As the same input conditions for all lines are assumed, the actually alga production in the photobioreactor

does not need to be considered. The study starts with the algal suspension, as it is harvested from the production plant. Consequently, for the following assessment based on the results of the literature search the following initial premises are defined:

1. Downstream of the photobioreactor the algal suspension attains a dry matter (dm), or total solid content of 3 g/l.
2. It is assumed that the energy represented by the alga and its production is factored into the assessment as ZERO.

The mass and energy balance is determined from the known values researched and from data obtained in prior experiments.

The calculations are founded on the following basic assumptions:

1. Production of 1 t algal biomass per day with a calorific value of 23.00 MJ/kg[13] (the functional unit for balance analysis of the biomass is thus 1000 kg).
2. At a productivity rate of 50 g m^{-2} d^{-1}, a land area of at least 2 ha (20,000 m^2) is required.
3. For a biomass content of 3 g/l, 13.89 m^3/h of algal suspension is required.

The following criteria are applied as benchmarks for assessing the energy balance:

$$Thermal\ efficiency = \frac{output\ energy}{chemical\ energy\ of\ algae + process\ ernergy}$$

$$Energy\ factor = \frac{output\ energy}{process\ ernergy}$$

$$Utilization\ rate = \frac{output\ energy}{chemical\ energy\ of\ algae}$$

For the mass balance, alongside the product yields

$$Mass\ balance = \frac{product\ mass}{algae\ mass}$$

the carbon efficiency:

$$Carbon\ efficiency = \frac{carbon\ output}{carbon\ in\ algae}$$

is used.

Detailed data for the assessment is presented in the appendix “Base data of assessment”

[13] DBFZ analysis

10.2 Process chains

In the course of the project a variety of process paths and products were defined (Figure 10.2) . From the large number of possible paths, five were considered in more detail.

The base case is the classic production of biodiesel by drying the algal biomass, solid-liquid extraction and transesterification of the fatty acids. It serves as a reference case. Secondly, the digestion of the biomass to form biogas is considered. The third path involves the hydrothermal conversion of the micro-algae to form biocoal (HTC), whereby only the coal is used for energy purposes. The fourth path reflects a hydrothermal liquefaction of the algae; and the fifth path demonstrates the possibility of hydrogenation.

As Figure 10.2 shows, dewatering of the algal suspension from the photobioreactor is first specified for all paths. This is to be done using only a centrifuge, as an upstream sedimentation cannot be modelled with sufficient accuracy. The centrifuge produces an algal paste with 15 % total solid, which is subjected to hydrothermal treatment. Algal biomass losses occur at the overflow, and total 10 % by mass. This process step is assumed to be the same for all defined lines.

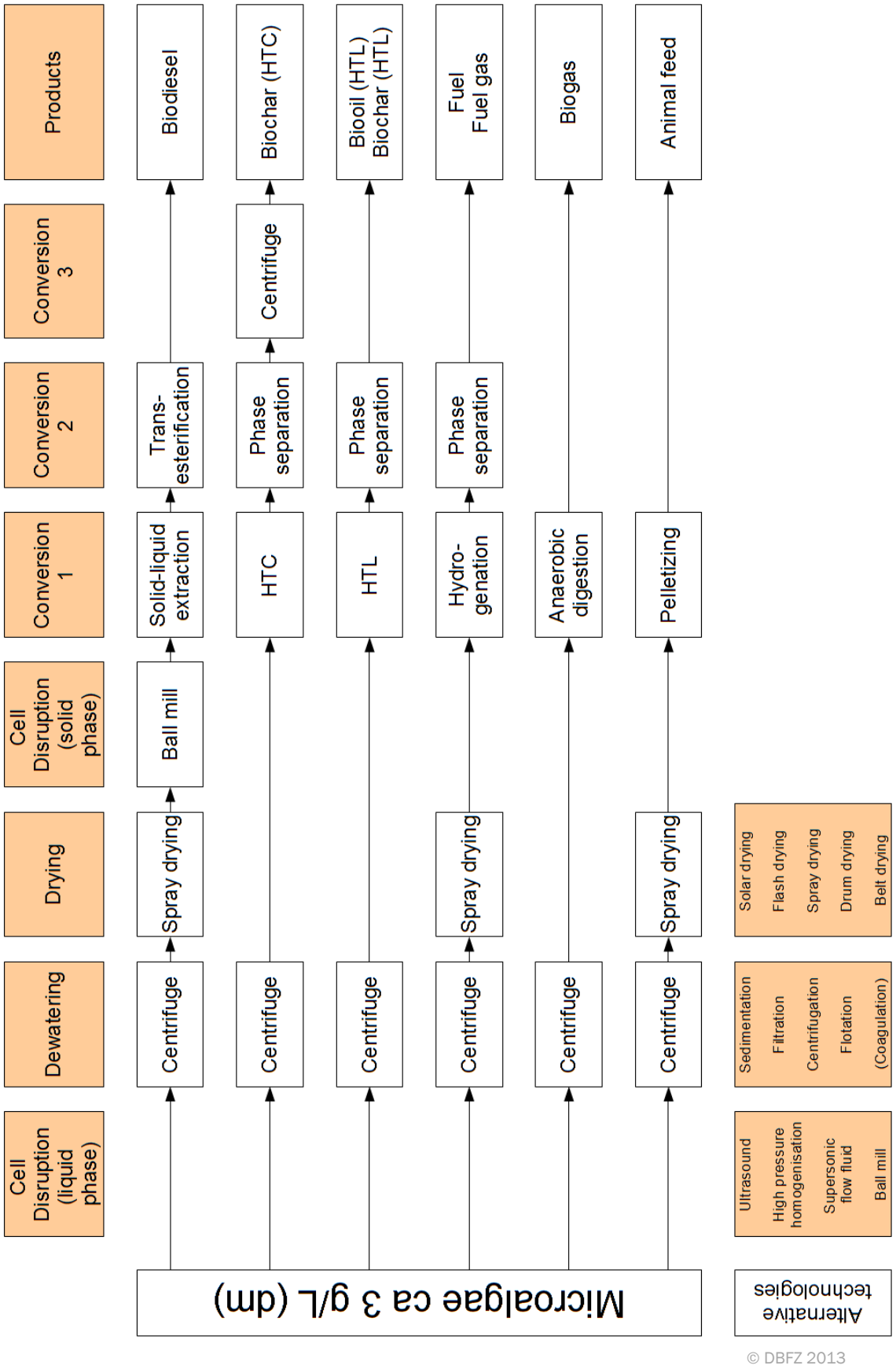


Figure 10.2 Overview of possible process paths

10.2.1 Base case

The base case involves the production of fatty acid methyl esters (FAME) by acidically catalysed (H_2SO_4) transesterification/esterification of the fatty acids bound in the extractable components and of the free fatty acids. The dewatering, drying, cell decomposition and extraction steps take place upstream of the transesterification stage (Figure 10.3).

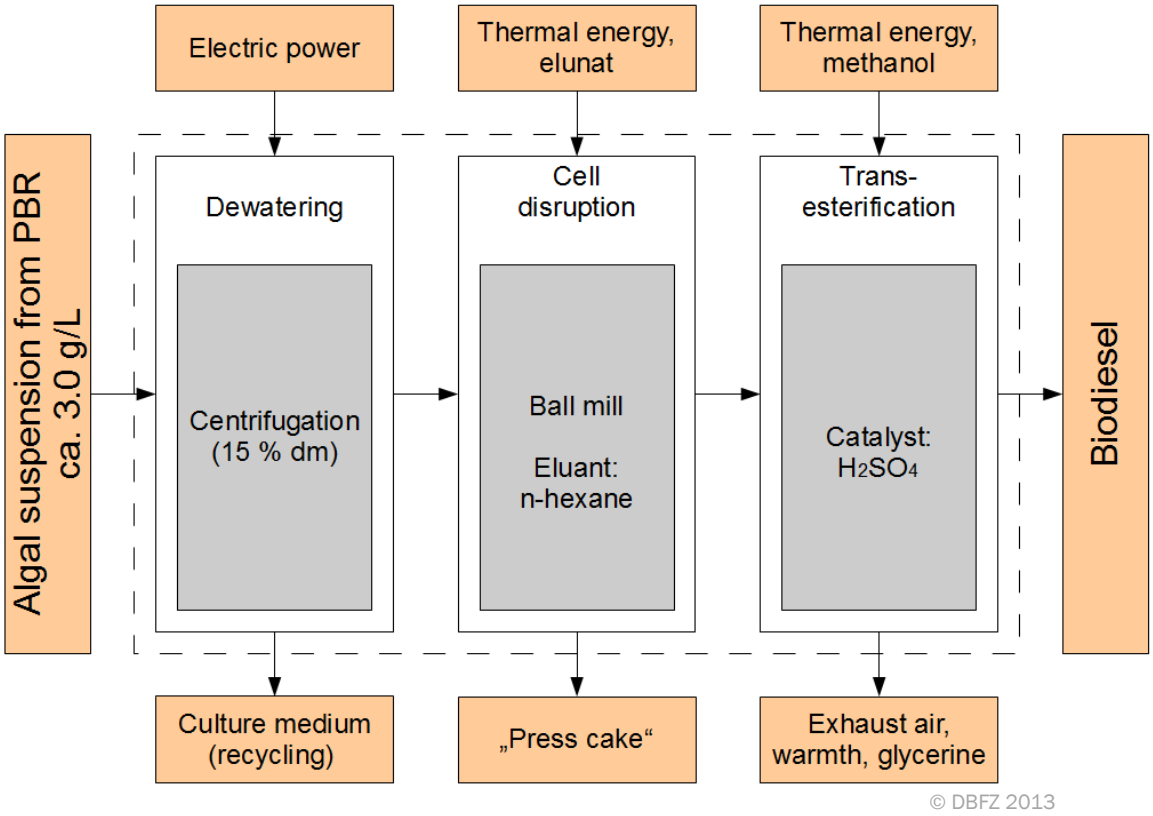


Figure 10.3 Schematic of biodiesel recovery path

The balance analysis was based on an extraction with n-hexane with an extraction agent/alga ratio of 1:1. For the solvent recovery, it is assumed that 50 % of the necessary heat can be provided by recovery of product heat. The rest must be provided by external energy supply. The methanol required as the raw material for transesterification is included as thermal energy consumption in the energy balance.

Reaction products in addition to the FAMEs are glycerol (from the triglycerols) and water (from the free fatty acids). For the quantity balance analysis, it was assumed that the free fatty acid content in the transesterifiable component of the lipids is 50 %. For simplification purposes, it is assumed that the lipids in the algal mass can be fully recovered by the extraction.

Balance of materials

The micro-alga under discussion *Scenedesmus obliquus* contains only relatively small proportionate amounts of extractable lipids, of which in turn only a partial amount consists of triglycerols and free fatty acids. The total fatty acid content accessible to transesterification/esterification is only just under

5 % by mass referred to the algal total solid. The amount of recoverable biodiesel is correspondingly small. The product distribution is as follows:

Biodiesel	5.1 wt.%
Residual lipid	17.7 wt.%
Water	0.2 wt.%
Glycerol	0.3 wt.%
Extraction residue (incl. K,P)	77.3 wt.%

The carbon efficiency is 7.3 %.

For the likewise considered micro-algae *Chlorella vulgaris*-, with a total fatty acid content of 6.6 %, the ratios are not substantially more favourable.

Energy balance

Table 10.1 shows the results of the energy balance.

Table 10.1 Energy balance of biodiesel path

Step		
Algal biomass energy consumption	(chemically bound)	23000 MJ 11657 MJ
Energy input		34657 MJ
Energy yield (biodiesel)		1848 MJ
Thermal efficiency	5 %	
Usage rate	8 %	
Energy factor	0.16	

Owing to the low biodiesel yield the resultant energy efficiency is extremely poor if only biodiesel is considered as a value product. This process chain can therefore only be implemented in an economically viable way if residues of the extraction and the transesterification can be used for material or energy purposes.

10.2.2 Biogas path

Biogas production is initiated by the dewatering step defined for all paths. The algal paste is fed into the digester. Before being fed in, it may be diluted with digested liquid. Since the liquid is taken out of the digester and immediately fed back in, however, it is not included in this balance analysis. The energy consumption of the digestion process results from the heating of the substrate to the digestion temperature of 37 °C and from the average internal power demand of German biogas plants specified in Biogas Measurement Programme II (Weiland et al. 2010). The yield and composition of the biogas was determined using the Feinberg model (Feinberg 1984).

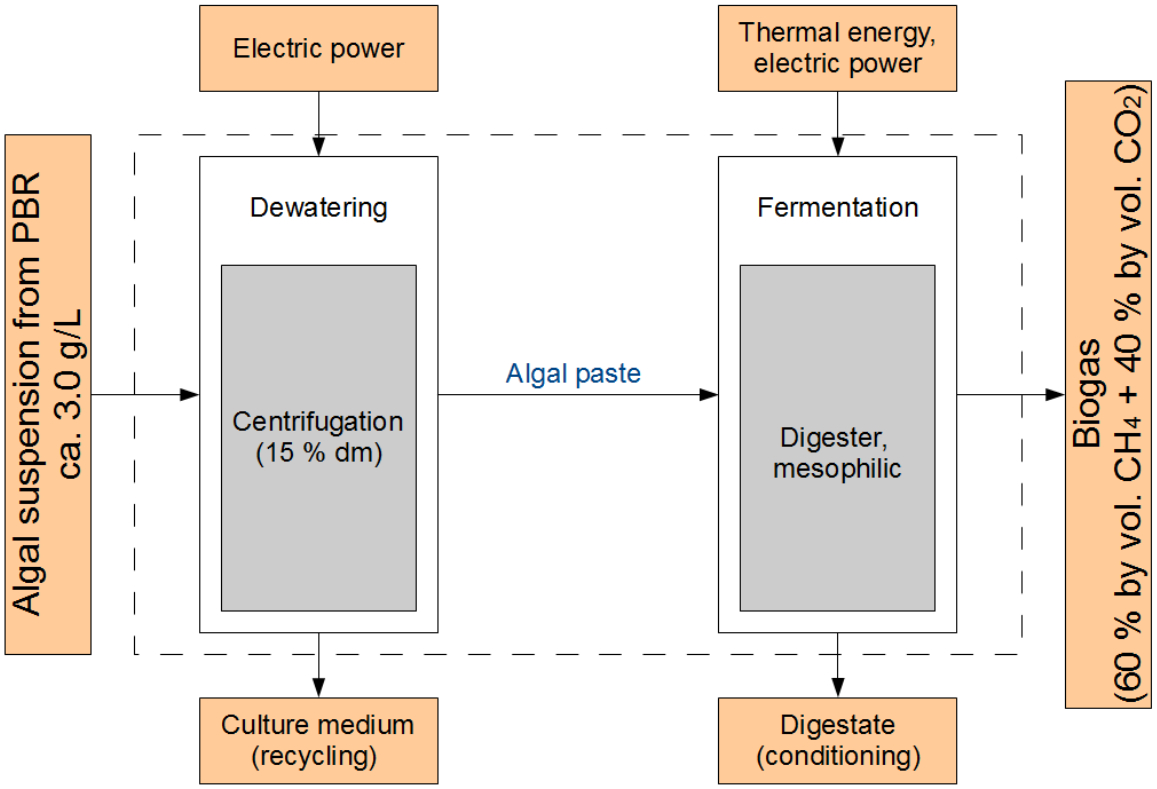


Figure 10.4 Schematic of biogas recovery path

The biogas thus consists of 60 % (by volume) methane and 40 % (by volume) carbon dioxide. The calorific value is 23700 kJ/m³.

Energy balance

With the assumptions made, the results presented in Table 10.1 and Table 10.2 are obtained. The distribution of the individual elements across the various fractions, main products or by-products provides initial indications as to the re-usability of the secondary flows. Nutrients such as nitrogen, potassium and phosphor should wherever possible be recycled, for example, in order to reduce the input – and thus the consumption – of new fertiliser. On the other hand – especially with a view to avoiding climate-damaging CO_2 – as much as possible of the carbon in the algae should be contained in the product.

Table 10.1. Energy balance of biogas

Step	Produced material	Mass in kg (dm)	Energy content in MJ	Energy consumption in MJ
Cultivation	Algal suspension	1000	23000	0
Dewatering	Algal paste	900	20700	1200
Digestion	Biogas	700	13831	958
Total energy consumption			2158	MJ
Thermal efficiency			55	%
Usage rate			60	%
Energy factor			6.4	

Elemental balance

Table 10.2. Elemental balance of biogas

Element	Percentage of algal biomass, absolute, in kg	Percentage of product (biogas), absolute, in kg	Difference in kg
C	483.0	313.1	170
H	65.0	62.3	3
O	336.3	336.6	0
N	64.9	0.0	65
S	6.7	0.0	7
K	16.9	0.0	17
P	27.2	0.0	27
Total	1000.0	712.00	288,0

The carbon efficiency is 64.8 %.

10.2.3 HTC path

In this process the algal biomass is converted into biocoal. For this, the algal paste from the centrifuge with 15 % total solid is subjected to hydrothermal treatment.

The actual hydrothermal treatment is carried out at a temperature of 220 °C over three hours, because the relevant prior experiments revealed these parameters to be most suitable. This is done in a continuous or semi-continuous process. The energy consumption results from the energy expended on increasing the pressure of the algal paste to the operating pressure of the HTC. This is determined at a corresponding pump efficiency rating, as well as from the heat capacity of the algal paste. Since the HTC process is fundamentally aimed at recovering heat, it is assumed that 20 % of the energy must be expended as is necessary to bring the algal paste from room temperature up to the operating

temperature of 220 °C. A downstream filtration stage separates the solid and liquid phases. The solid components are then present in the form of coal slurry, with a dm content of 50 %.

Following carbonisation, the biocoal is dried by a flash dryer. This was selected because it is the most favourable drying process in energy use terms. The energy demand of the drier was derived from the data set out in section 0.

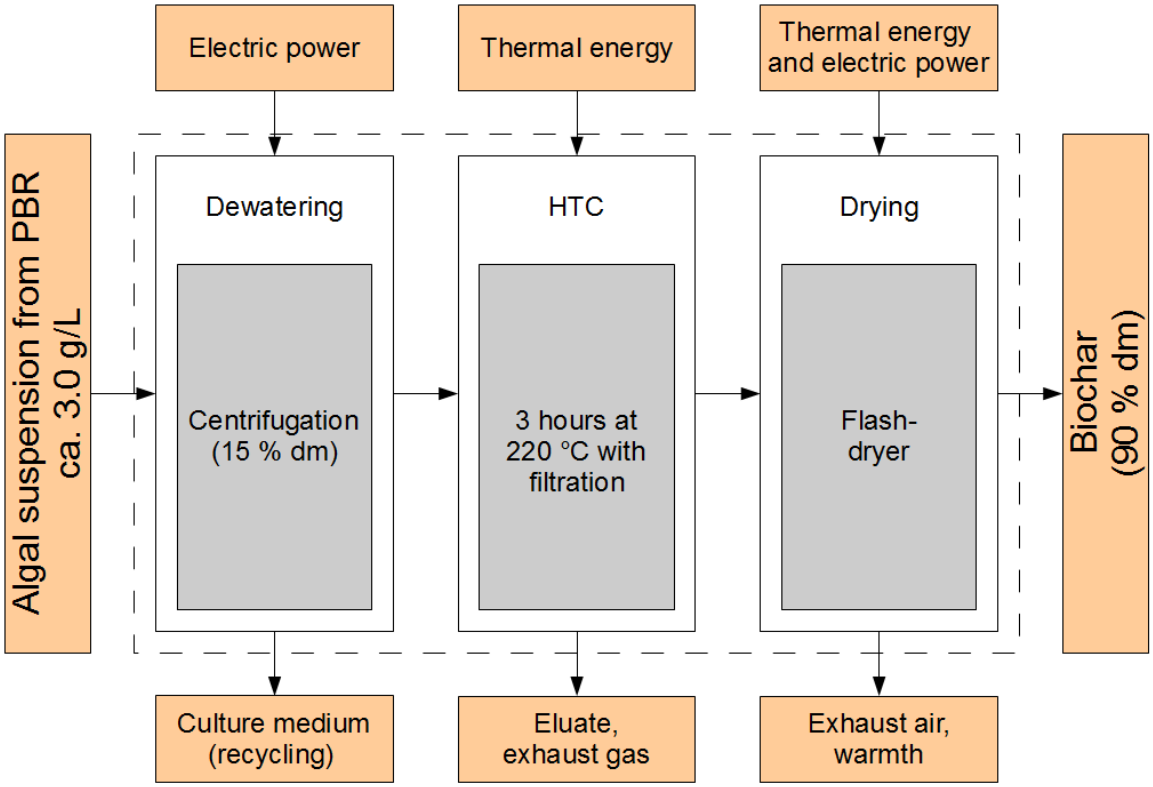


Figure 10.5 Schematic of HTC recovery path

The individual flows of material and post-treatment of the by-products additionally consume energy, though the influence of those factors on the total balance remains marginal. Thus, a biological fixed-bed filter can be specified for treatment of the exhaust air from the flash dryer and the resultant gas phase of the HTC. This is not included in the balance analysis due to its long operation time and low energy demand (when using an active filter with blower) or even zero demand (when using a passive biofilter).

The produced coal can either by used directly in this form or can be converted by further processes into solid, liquid or gaseous primary energy source materials.

Energy balance

With the basic assumptions already defined, the values presented in Table 10.3. are obtained.

Table 10.3. Energy balance of HTC

Step	Produced material	Mass in kg (dm)	Energy content in MJ	Energy consumption in MJ
Cultivation	Algal suspension	1000	23000	0
Dewatering	Algal paste	900	20700	1200
HTC	Coal slurry	700	19523	945
Drying	Biocoal	700	19523	3091
Total energy consumption			5236	MJ
Thermal efficiency			69	%
Usage rate			85	%
Energy factor			3.7	

Elemental balance

Based on the assumptions made for the balance analysis and the results of the prior experiments in hydrothermal carbonisation, the elemental balance set out in Table 10.4 can be derived.

Table 10.4. Elemental balance of HTC

Element	Percentage of algal biomass, absolute, in kg	Percentage of product (biocoal), absolute, in kg	Difference in kg
C	483.0	433.3	49.7
H	65.0	50.3	14.7
O	336.3	170.7	165.6
N	64.9	28.1	36.8
S	6.7	2.2	4.5
K	16.9	1.4	15.5
P	27.2	13.9	13.3
Total	1000.0	700.0	300.0

The carbon efficiency is 89.7 %.

10.2.4 HTL path

In this process the algal biomass is converted into bio-oil and biocoal. For this, the algal paste from the centrifuge with 15 % total solid is subjected to hydrothermal treatment.

As in the case of the recovery paths described above, downstream of the PBR the algal suspension is concentrated to approximately 15 % by mass by means of centrifuges.

For assessment of the hydrothermal liquefaction the following assumptions were made:

Mass balance (see Table 5.5):

- Bio-oil yield 30% of algal biomass
- Biocoal/tars yield 10% of algal biomass
- Gases and aqueous phase were not included
- The elemental analysis was taken from (Biller and Ross 2011)
- The elemental analysis corresponds to the values for biocoal (HTC) (Table 10.4)

Energy balance

- Compression of 0.1 to 15 MPa with an efficiency of 70%
- Heating utilising the waste heat with 20 % of the necessary thermal energy to heat up from 20 to 300 °C (as per HTC)
- Estimated demand for treatment/phase separation with 1044 MJ per functional unit
- No other energy inputs included
- The calorific value of the bio-oil (HTC) was assumed at 35 MJ/kg as approximately 10% below the values cited in the literature (Biller and Ross 2011; Duan and Savage 2011; Brown et al. 2010)

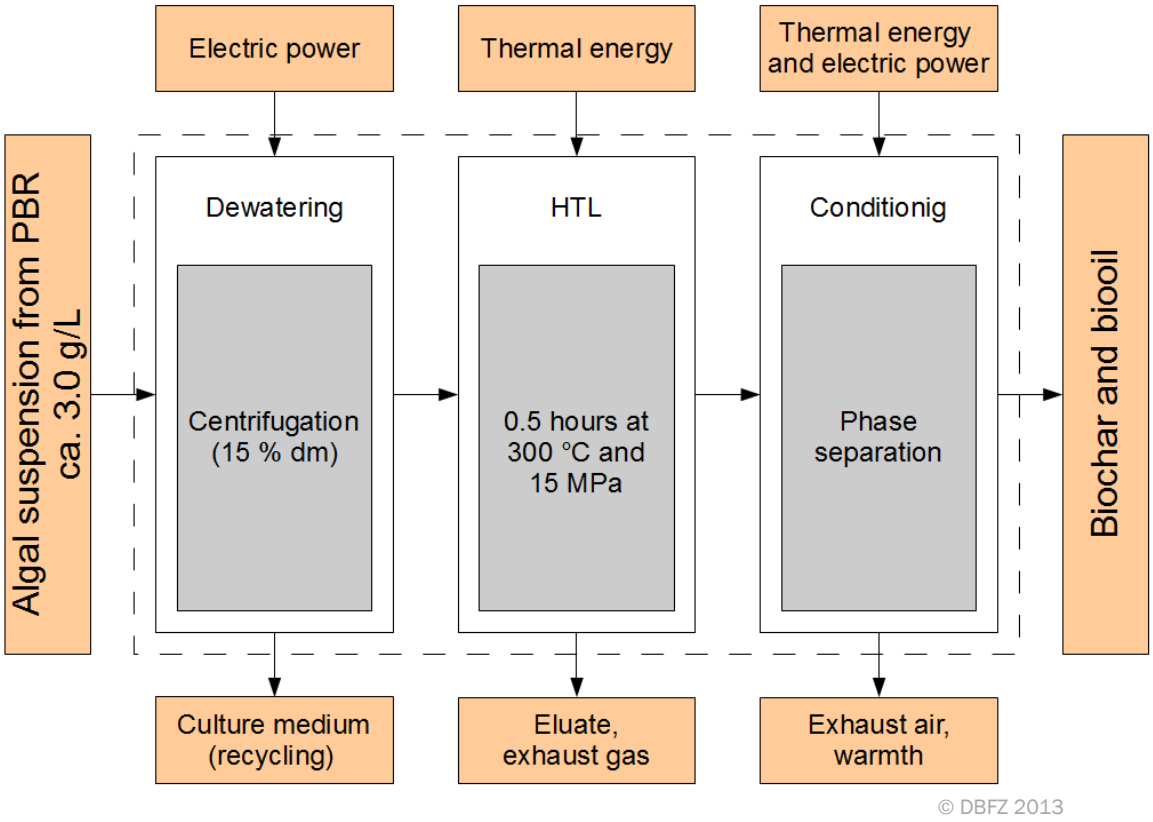


Figure 10.6 Schematic of HTL recovery path

The schematic for the assessment is presented in Figure 10.6

With the above basic assumptions, the simplified energy balance presented in Table 10.5 is obtained.

Table 10.5. Summary of HTL energy balance

Step	Produced material	Mass in kg (dm)	Energy content in MJ	Energy consumption in MJ
Cultivation	Algal suspension	1000	23000	0
Dewatering	Algal paste	900	20700	1200
HTL		700		1710
Treatment	Bio-oil	270	8100	1045
	Biocoal	90	2510	
Input with alga				26955
Input without alga				3955
Output			10610	
Thermal efficiency		44	%	
Usage rate		52	%	
Energy factor		3.0		

Balance of materials

Based on the assumptions set out above, the following mass balance is obtained.

Table 10.6. Elemental balance of HTL

Element	Percentage of algal biomass, absolute, in kg	Percentage of product (bio-oil), absolute, in kg	Percentage of product (biocoal), absolute, in kg	Difference in kg
C	483.0	198.7	55.7	228.6
H	65.0	28.9	6.47	29.6
O	336.3	28.9	21.95	285.5
N	64.9	13.0	3.62	48.3
S	6.7	0.5	0.29	5.9
K	16.9		0.2	16.7
P	27.2		1.8	25.4
Total	1000.0	270.0	90	640

For the carbon efficiency a value of 53 %.

10.2.5 Direct hydrogenation

For direct hydrogenation of the complete algal biomass, it must first be dewatered and dried. Together with a circulating oil as the carrier fluid and the necessary hydrogen, the feedstock is fed into the hydrogenating reactor, which is operated at 400 °C and 10 MPa. In addition to a hydrogenation

residue, which also contains the mineral components, the process delivers the product oil and a combustion gas. This gas can be used to provide the process heat and, in principle, also to generate the hydrogenating hydrogen. The processing of the hydrogenated oil to produce marketable fuels (distillation, or where appropriate hydrorefining) is dependent on the desired target product and on the composition of the oil, and is not included here.

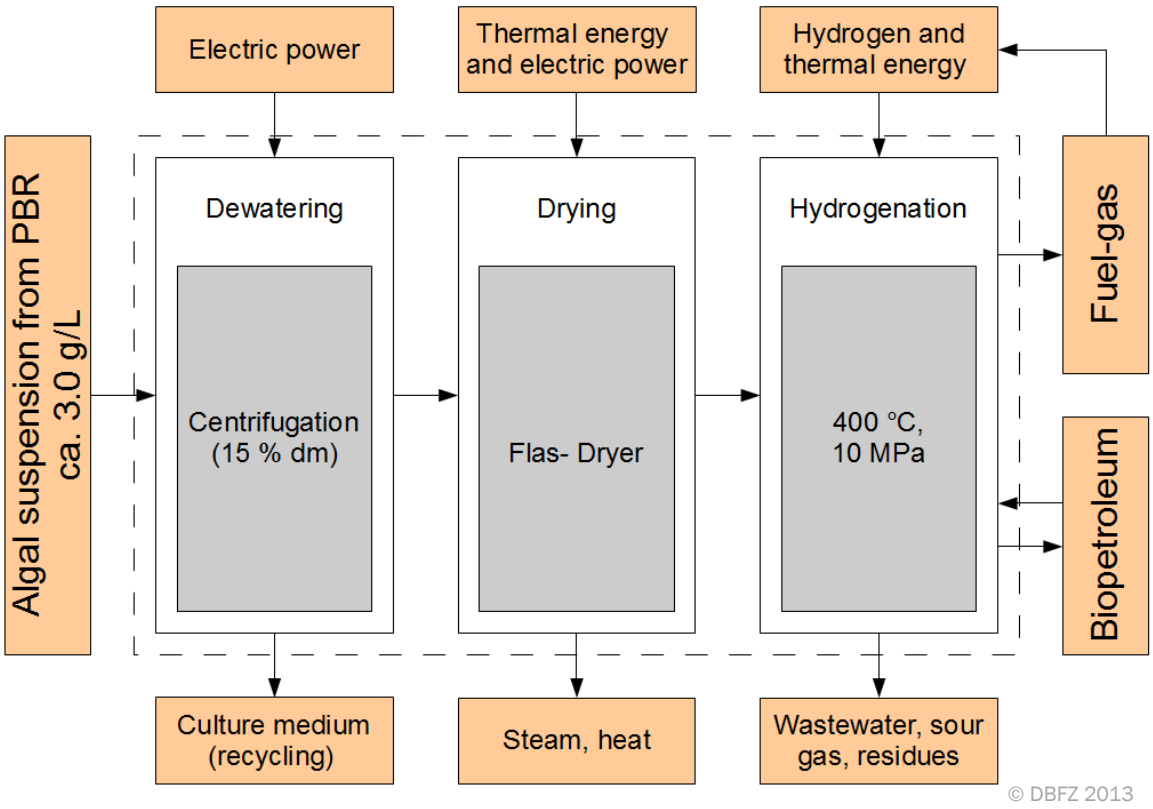


Figure 10.7 Schematic of direct hydrogenation recovery path

The process of hydrogenation of micro-algae has been little studied to date, so a number of ideal assumptions had to be made with regard to energy, and in particular to the mass balance study. The results obtained therefore serve primarily as a means of orientation, though they should be quite suitable for an initial estimation of feasibility.

To calculate the energy demand of the hydrogenation stage it is assumed that the perceptible heat of the products can be used for feed pre-heating and only a small portion (20 %) has to be fed in as "peak heat", for example due to the combustion of the gaseous products. The reaction heat released in the exothermic hydrogenation reactions was calculated, but was not included in the enthalpy balance. This keeps the energy assessment on the safe side. The hydrogenating hydrogen is obtained from natural gas by steam reforming.

The material use balance analysis of the process chain was carried out by way of example for the micro-algae *Scenedesmus obliquus*. It is based on ideal reactions of the organic matter, with separate reaction mechanisms being postulated for the protein, carbohydrate and lipid material classes:

- Polypeptides

Polypeptides are polycondensation products of the amino acids. The polymer structure is broken down into the functional groups (peptide grouping). The nitrogen already pre-bound with hydrogen is hydrogenated to form ammonia, while the oxygen is split off in the form of carbon oxides. The hydrocarbon residues are hydrogenated; the chain lengths result from the amino acid profile of the algal mass (appendix Algae data sheets).

- Carbohydrates

It is assumed that the carbohydrates are composed entirely of glucose building blocks interconnected via oxygen bridges. Under the reaction conditions of hydrogenation, the macro-molecules decay into their monomers. The oxygen bound into the OH groups is split off as water; the loop and bridge oxygen is probably released as CO/CO_2. What remains are n-alkanes with chain lengths of 5 and 6 C-atoms.

- Lipids:

Only part of the lipid fraction contained in the alga *Scenedesmus obliquus* consists of triglycerols or free fatty acids (appendix Algae data sheets). Since no information is available concerning the nature of the remaining constituents, the fatty acid structures were assumed in idealised form for the total lipid content. In view of the relatively low lipid content in the Scenedesmus, this assumption should not place in question the informative value of the results.

The ester groups of the triglycerols are cracked and split off, as are the carboxyl groups of the free fatty acids. The oxygen bound into the functional groups is released partially as CO_2 and partially as water.¬ The ratio of oxygen hydrogenation and decarboxylation depends on the reaction conditions, and is specified here as 1:1. The remaining hydrocarbon residues are hydrogenated to form long-chained alkanes. The chain length distribution results from the fatty acid profile and from the aforementioned hydrogenation/decarboxylation ratio.

In addition to these primary crack reactions, which result in removal of the hetero-atoms, the hydrocarbons are also split off – to a greater or lesser extent, depending on the reaction temperature and time. This entails a secondary formation of short-chained alkanes. For the material and enthalpy balances two cases are considered:

- Case 1 (little cracking): The entire thermal energy demand is covered by external sources, such as by burning natural gas. Hydrogen recovery by steam reforming is likewise based on natural gas.
- Case 2 (intensive cracking): The cracked gases produced under sharper conditions fully cover the thermal energy demand. The hydrogenating hydrogen is obtained from natural gas, as in case 1.

Balance of materials

Based on the assumptions regarding the reaction sequence, the product distributions presented in Figure 10.8 are obtained. The chart on the left is based on a low cracking intensity, while the chart on the right shows the results for major influence of thermal cracking.

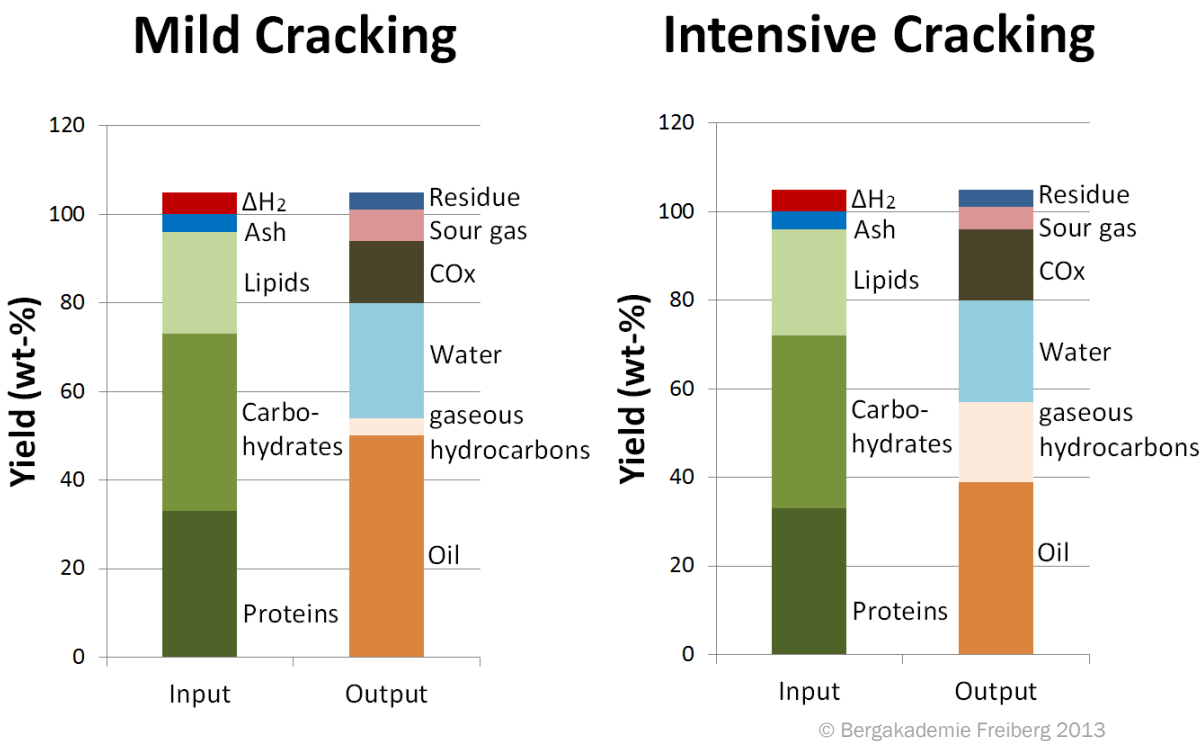


Figure 10.8 Product distribution in hydrogenation of algal biomass

Case 1 with a yield of approximately 51 % (referred to the application of hydrogenation) delivers maximum liquid products. The gaseous products (sour gas, hydrocarbons) can be co-incinerated in heating and power plants. The need for separate gas purification might be eliminated as a result. Case 2 is characterised by large quantities of gas, linked to lower oil yields (39 %). This quantity of gas does, in principle, cover the total thermal energy demand. However, the necessary hydrogenating hydrogen must be obtained from natural gas. For incineration, though, it does necessitate a gas purification stage which severely impairs the efficiency of the process.

Table 10.7 shows the elemental balance with regard to the liquid product for both cases.

Table 10.7. Elemental balance of direct hydrogenation

Case 1:	Little cracking		
Element	Percentage of algal biomass, absolute, in kg	Percentage of liquid product, absolute, in kg	Difference in kg
C	483.0	388.6	94.4
H	65.0	72.9	-7.9
O	336.3	0.0	336.3
N	64.9	0.0	64.9
S	6.7	0.0	6.7
K and P	44.1	0.0	44.1
Total	1000.0	461.6	538.4

Case 2:	Intensive cracking		
Element	Percentage of algal biomass, absolute, in kg	Percentage of liquid product, absolute, in kg	Difference in kg
C	483.0	293.7	189.3
H	65.0	55.7	9.3
O	336.3	0.0	336.3
N	64.9	0.0	64.9
S	6.7	0.0	6.7
K and P	44.1	0.0	44.1
Total	1000.0	349.4	650.6

The idealised reaction sequences in the calculation result in complete conversion and complete removal of the hetero-atoms from the hydrogenation product. For vegetable oils this is quite possible. For the algal biomass it must be assumed that, on the one hand, residues of oxygen and nitrogen remain in the hydrogenation product and, on the other, a solid, coke-like hydrogenation residue is produced due to incomplete conversion and as a result of build-up reactions.

The carbon efficiency is 80.5 % for low-intensity cracking and 60.8 % for high-intensity cracking.

Energy balance

Table 10.8 shows the results of the energy balance.

Table 10.8. Energy balance of direct hydrogenation

Case 1:	Little cracking		
Step	Electrical energy demand kWh	Thermal energy demand MJ	Total energy demand MJ
Culture	0	0	
Dewatering	333	0	1199
Drying	918	6120	9425
Heat-up	0	360	360
Compaction	94	0	338
Hydrogenation	0	0	0
Reforming	0	89521)	8952
Total	1345	15432	20274
Energy input			43274 MJ
Algal biomass	(chemically bound) Energy consumption		23000 MJ 20274 MJ
Energy yield (liquid product)			22132 MJ
Thermal efficiency			51 %
Usage rate			96 %
Energy factor			1,0

1) Also includes the feed demand (methane) for steam reforming

Case 2:	Intensive cracking		
Step	Electrical energy demand kWh	Thermal energy demand MJ	Total energy demand MJ
Culture	0	0	
Dewatering	333	0	1199
Drying	918	6120	9425
Heat-up	0	360	360
Compaction	105	0	378
Hydrogenation	0	0	0
Reforming H2 Heat	0	6144 1953	6144 1953
Total	1356	145772)	19459
Energy input			34027 MJ
Algal biomass	(chemically bound) Energy consumption		23000 MJ 11027 MJ
Energy yield (liquid product)			16797 MJ
Thermal efficiency			49 %
Usage rate			73 %
Energy factor			1,5

2) Heat demand is covered by product gas

The low-intensity cracking variant (case 1) is more favourable in energy terms. Less hydrogen is needed to saturate the hydrocarbon residues. This results in an increase in energy efficiency for the overall chain from 49 % to 51 %. A high proportion (approximately 96 %) of the chemically bound energy (calorific value) introduced with the algal biomass is to be found in the liquid product. However, the ratio of energy yield to external energy consumption is just 1.0 in this case, as opposed to 1.5 for intensive cracking (case 2).

10.3 Assessment and comparison of product lines

Table 10.9 summarises the assessment of the product lines.

The following comments should be noted when evaluating this summary:

1. The assessment is carried out for the process chain downstream of the PBR. This means all consumption for the production of the algal biomass from conditioning of the flue gas, cultivation in the PBR, the required nutrients etc., are not taken into account.

2. Different products are made on each product line analysed. No product line to product line comparison of the data obtained is therefore possible!

3. Further technological steps may be necessary in order to progress from the product analysed in this comparison to the usable product. For example, a gas treatment stage must be included on the way from biogas to biomethane (or bio-natural gas). This also applies to other product paths!

4. Various criteria were identified for the comparison (see section 0). Their significance is as follows:

   a. Yield

      The yield indicates the mass of product which can be obtained relative to the input mass of dry alga. This is the basic criterion of the mass balance.

   b. Carbon efficiency

      This criterion, describing the mass of carbon obtained in the products in relation to the carbon of the algal biomass, specifies the conversion rate of this element. This is becoming increasingly important at present for the implementation of carbon cycles.

   c. Thermal efficiency

      The thermal efficiency is the ratio of energy output to energy input. taking into account the chemically bound energy of the micro-algae and the energy required for conversion.

   d. Usage rate

      In order to depict the specifics of the conversion process more fully, the usage rate was included as an assessment criterion. It places the energy of the products in relation to the chemical energy of the algae. This indicates how much energy from the micro-alga is contained in the product.

   e. Energy factor

      This factor does not take into account the chemical energy content of the algae. The energy factor places the energy recovered with the products in relation to the process energy required for the conversion.

Table 10.9. Summary of assessment of product lines

Criterion	Process chain Base case	Biogas	HTC	HTL	Low-intensity hydrogenation	High-intensity hydrogenation
Product(s)	Biodiesel	Biogas	Biocoal (HTC)	Bio-oil (HTL) Biocoal (HTL)	Hydrogenated oil	Hydrogenated oil
Use	Motor fuel	CHP heating gas Biomethane	Incineration Regular fuel	Motor fuel Incineration	Motor fuel	Motor fuel
Yield (m/m alga)	5 %	71 %	70 %	36 %	51 %	39 %
Carbon efficiency	7.3 %	64.8 %	89.7 %	52.7 %	80.5 %	60,8 %
Thermal efficiency	5 %	55 %	69 %	44 %	51 %	49 %
Usage rate	8 %	60 %	85 %	52 %	96 %	73 %
Energy factor	0.16	6.4	3.7	3.0	1.0	1,5
Development status of technology in Biomass	Industrial scale	Industrial scale	Demonstration	Demonstration	Laboratory and pilot plant	
Development status of technology in Micro-algae	Laboratory scale	Laboratory scale	Laboratory scale	Laboratory scale	Research and development	

The following advantages and disadvantages can be set forth in summary for the individual technology chains:

10.3.1 Base case: Production of biodiesel

With the process chain considered as the base case, FAMEs with biodiesel quality can be obtained. However, the yield is directly linked to the fatty acid content of the biomass, which in autotrophically cultivated micro-algae is very low. If the focus is on producing motor fuel components, this method is unsuitable.

Advantages:

- The steps in the process chain are tried and tested, and are fundamentally available.
- Relatively simple technology, which can also be implemented on a small scale.
- The liquid product has the same properties as the biodiesel obtained from vegetable oils.
- Experience in processing vegetable oils exists.

Disadvantages:

- Process chain for micro-algae (incl. cell decomposition and extraction) not yet implemented on a technical scale.
- Autotrophically cultivated micro-algae, such as the species *Chlorella vulgaris* and *Scenesemus obliquus* considered, contain only small amounts of lipids, and their fatty acids content capable of being transesterified/esterified is extremely low.
- The path is therefore only economically viable if use of the extraction and transesterification residues, which occur in large quantities, is also considered (e.g. substrate for biogas production or production of animal foodstuffs).
- The algal biomass has to be dried.
- Biodiesel can only be mixed with conventional diesel fuels to a limited extent.

10.3.2 Biogas path:

This biological process is characterised primarily by the low energy input into the process, underpinned by a high energy factor. Biogas use is well established in Germany. It can be linked in to existing plants. The usage rate and carbon efficiency values also indicate, however, that uses for the residual products also need to be sought.

Advantages:

- Use of the moist biomass (complete cell or also residual product fractions).
- Little energy input required (-> high energy factor)
- Biogas use well established in Germany.

- Co-digestion possible in existing plants.
- Combination with alga production (use of CO_2, grain recycling).
- Recycling of the nutrients (nitrogen, phosphor) possible.

Disadvantages:

- Decomposition of the cell necessary depending on alga species.
- Adaptation of the biogas process to high nitrogen content required.

10.3.3 HTC path

Hydrothermal carbonisation, with a usage rate of 85 % and a carbon efficiency of just under 90 %, offers the possibility to convert virtually the entire algal biomass into an energy source material. The thermal efficiency of 69 % indicates that this method can be designed comparatively efficiently. The product, the biocoal, opens up numerous potential applications alongside the classic use as a replacement for fossil fuel coals. Mention should be made in this context of the establishment of biocoal pellets for small-scale furnaces and of material use, for example as activated carbon. There is a need for considerable further research and development in relation to these new forms of use.

Advantages:

- High carbon content in the product.
- Moist biomass can be used.
- Dewatering of the coal much easier than in the case of the algal suspension.
- Relatively mild reaction conditions (180 °C to 250 °C).
- Recycling of the nutrients (nitrogen, phosphor) possible.
- Direct use of the dewatered coal in combustion.
- Development of a regular fuel (pelletising) possible for enhanced value creation.
- At present several HTC pilot plants in operation or under construction.

Disadvantages:

- Micro-algae development still in the R&D phase.
- Product development (pellets, nanostructures) still in the development phase.

10.3.4 HTL path

Hydrothermal liquefaction is suitable for the production of an interesting range of products. Despite its lower characteristic material and energy balance data as compared to HTC and hydrogenation, it offers interesting potential for algal biomass refining.

Advantages:

- The complete dewatered biomass can be used.
- No need for drying. This is a significant saving in terms of process energy.
- No cell decomposition is required.
- The products are bio-oil and biocoal, in ratios dependent on the process conditions.
- The resultant low-calorie gas can be utilised (not included in the balance).
- Can be operated in mixtures with other biogenic and non-biogenic carbon sources.
- The resultant aqueous phase contains nitrogen, which can be used where appropriate in the culture medium of the PBR.

Disadvantages:

- The bio-oil and biocoal products have to undergo further processing.
- Complex and costly plant engineering (high pressure and high temperatures).
- The technology is at the pilot scale stage for biomasses containing lignocellulose; economic viability is not yet proven.
- The technology is being tested on a laboratory scale for micro-algae.
- The product spectrum is specific to alga species.

10.3.5 Direct hydrogenation

Hydrogenation can be used to convert micro-algae into hydrocarbon-rich oils relatively independently of their material composition and with a high yield. The yield and product quality are controllable by way of the process conditions. The process does, however, require very complex and costly plant engineering.

Advantages:

- Good quality of liquid hydrogenation products (high calorific value, low hetero-atom content, boiling behaviour).
- Possible to use the complete algal mass.
- The cell decomposition and extraction steps of the process are not needed.
- Combustion gases and sour gases can be co-incinerated at heating/power plants.
  → Energy recovery (not credited in the calculations!).
  → Use of the power/heat plant's flue gas cleaning systems, so no separate gas purification required.
- High rate of energy and carbon transfer from the raw material (micro-alga) into the liquid product.

Disadvantages:

- Complex and costly plant engineering (pressure reactors, distillation, hydrogen supply).
- Drying of the feedstock required. This severely impairs the energy balance.
- By-products (water, combustion and sour gases) have to be disposed of.
- Usage path still in R&D stage.

11 Summary

The summary assessment of the derived product lines (section 10.3) and evaluation of the literature search reveals the following research requirements:

Biogas:

Co-digestion is a possible means of utilising micro-algae in the short term. However, this does require adaptation of the bacterial cultures of the biogas reactor. This adaptation should be specific to the micro-alga species used.

Integration into an overall process must be investigated, and more specifically the integration of CO_2 and grain from the biogas plant. Research results on this are sparse.

HTC:

The behaviour of micro-algae in the HTC reactor must be investigated as a fundamental requirement. The experiments at the DBFZ have demonstrated promising results, but because only a small number have been carried out they are not representative. A wider variation of reaction parameters and use of different alga species is necessary.

Use of the eluate is an interesting issue, as it enables savings to be made in fertiliser consumption. This requires a more detailed analysis of the aqueous phase of the HTC process.

Supercritical CO_2 – Extraction:

The literature search reveals no indication that SFE with moist algal biomass (15-25 % dm) has been investigated. Since the thermal drying stage is not needed when using the dewatered algal biomass, this analysis is advantageous.

HTL:

Hydrothermal liquefaction is suitable for the production of an interesting range of products. It is specific to alga species and dependent on the process parameters (pressure, temperature, catalyst). This field has not been adequately studied on a small technical scale.

The need for further research emerges from the possibility of liquefying other carbon carriers, such as brown coal or biological residual products, with algae.

The use of all products, especially the eluate, must be investigated.

Hydrogenation:

Although hydrogenating processes are established in the petrochemicals and mineral oil processing sectors, there is a need for research into the hydrogenation of algal biomass with regard to basic questions such as:

- The influence of key process parameters (pressure, temperature, time) on product distribution and quality.
- Use of a mashing agent to assist hydrogen transport from the gas phase to the algal mass and to improve pumping capability
  (use of different carrier oils, variation of quantity).
- Co-processing together with vegetable oils.
- Studies on the influence of the micro-alga species.
- Testing and optimisation of catalysts.
- Use of moist biomass.

The finishing touch leads to points of affinity with hydrothermal liquefaction, which was investigated in part under hydrogen atmosphere and with the aid of catalysts.

In these focus areas the research partners see the basis for one or more additional research funding applications.

Biomass		Temp. (°C)	Pressure (MPa)	Catalyst	Reaction time (min)	Atmosphere	Solvent	Yield (% (m/m)) Oil	Water Soluble	Solid	Gas	Com.	Source
Micro-alga	*Nannochloropsis sp.*	350	0.07-3.5	Without Pd/C Pt/C Ru/C Ni/SiO2-Al2O3 CoMo/γ-Al2O3 Zeolite	60	He, H2	Water	35-47 45-57	No data No data				(Duan and Savage 2011)
Micro-alga	*Dunaliella tertiolecta*	250-340	10	Na2CO3 (0-5 % (m/m))	5 and 60	N2	No data	37	50	Negligible	10		(Minowa et al. 1995)
Micro-alga	*Chlorella vulgaris*	300-350	No data	Alkali (KOH, Na2CO3)	60	No data	Water	9 to 14	No data	4 to 5	6 to 20		(Ross et al. 2010)
	Spirulina			Organic acids (methanoic acid, ethanoic acid)				16-20	No data	4 to 5	16-30		
Micro-alga	*Dunaliella salina*	200	2	Without REHY Ni/REHY	60	H2	Ethanol	35 52 72	No data				(Yang et al. 2011)

Biomass		Temp. (°C)	Pressure (MPa)	Catalyst	Reaction time (min)	Atmosphere	Solvent	Yield (% (m/m)) Oil	Water Soluble	Solid	Gas	Com.	Source
Micro-alga	*Nannochloropsis sp.*	200-500	35	Without	60	He	Water	27-43	No data				(Brown et al. 2010)
Micro-alga	*Spirulina*	300-425	5	Without Fe(CO)5-S		H2, N2, CO	Water, tetralin, toluene, 1-methyl naphthalene	52-79 63-83	No data		5 to 9 4 to 12		(T. Suzuki T. Matsui C. Ueda N. Ikenaga 2006)
Micro-alga	*Spirulina platensis*	200-380	2 (?)	Without	0-120	N2	No data	18-40	30-57	5 to 21	5 to 28		(Jena et al. 2011a)
Micro-alga/ Coal	*Chlorella, Chlorella/Coal, Spirulina, Spirulina/Coal, Littorale, Littorale/Coal*	300-400	5	Without	60	H2	1-methyl naphthalene	5 to 59	No data	Max. 30 (gesch.)	≈10-18		(Ikenaga et al. 2001)
				Fe(CO)5-S, Mo(CO)6-S, Ru3(CO)12				22-82	No data	Max. 55 (gesch.)	≈2-18		
Macro-alga	*Enteromorpha prolifera*	220-320	No data	Na2CO3 (0-5 % (m/m))	30	N2	Water	9 to 20	34-45	17-20	21-30		(Zhou et al. 2010)
Cattle dung		270-350	0-0.7	NaOH	0-40	Air, N2, CO, H2	(water)	8 to 49	No data	2 to 25	5 to 47		(Yin et al. 2010)

Biomass		Temp. (°C)	Pressure (MPa)	Catalyst	Reaction time (min)	Atmosphere	Solvent	Yield (% (m/m)) Oil	Water Soluble	Solid	Gas	Com.	Source
Jack pine sawdust		280-380	2 (?)	Without FeSO4*7H2O, Ca(OH)2, Ba(OH)2	15-60	N2	Water	27-39 20-45	6 to 26 5 to 25	20-40 No data	25-51 (gas+water) 20-70 (gas+water)		(Xu and Lad 2008)
Cellulose		200-350	4.3-16.5	Na2CO3	0-60	N2	Water	0-44	12 to 42	10 to 88	0-19		(Fang et al. 2004)
Waste	Food industry/ Sewage treatment plant waste	300-350	p < 22.1	K2CO3, ZrO2	5 to 10	No data	Water	0-13	79-97	0-32 (org. phase)	0-0.5	Reaction mixture cycled	(Hammerschmidt et al. 2011)
Wood (sawdust)		200-350	2 (?)	Without	15	N2	Water, methanol, ethanol, water/methanol, water/ethanol	23-66	6 to 26	3 to 70	≈5		(Cheng et al. 2010)
Macro-alga	*Laminaria Saccharina*	250-370	No data	Without KOH	15-120	No data	Water	4 to 19	23-68	11 to 31	8 to 18		(Anastasakis and Ross 2011)
Peat		150-380	25.1	Without	30	N2	Without adding solvent	No data		53-99	No data		(Mursito et al. 2010)

Biomass	Temp. (°C)	Pressure (MPa)	Catalyst	Reaction time (min)	Atmosphere	Solvent	Yield (% (m/m)) Oil	Water Soluble	Solid	Gas	Com.	Source
Waste sludge from paper factory + newspaper	250-380	2 (?)	Without	20	N2	Water	17-28	30-45	18-47	6 to 18		(Zhang et al. 2011)
							25-34					
			KOH, methanoic acid, FeS					31-42	13-22	9 to 14		
Poplar leaves	300-450	7.5-18.5	Without	20	No data	Water	8 - 10	25-28	37-45	10 to 16		(Wu et al. 2008)
							10 - 16					
			T203, Z409					19-28	32-37	17-25		
Wood	280	No data	Without	15	N2	Water	6 – 9	8-41	39-45	8 - 45		(Karagöz et al. 2006)
			K2CO3				18-36	35-51	4 - 36	10 - 11		
Sword grass	235-260	13.8, 23.5	Without			Water						(Kumar and Gupta 2009)
			K2CO3									
Eucalyptus	150-350	2	Without	0	N2	Water, waste water, NaOH solution	1 to 38	8 to 71	4 to 77	1 to 31		(Sugano, et al., 2008)
Micro-alga *Spirulina*	280-320	4.8-14	Without	No data	No data	Ethanol	35-45	No data	17-22	No data		(Huang et al. 2011)
							42-47					
			FeS, FeSO4, NaOH, Na2CO3						10 to 21			

Biomass	Temp. (°C)	Pressure (MPa)	Catalyst	Reaction time (min)	Atmosphere	Solvent	Yield (% (m/m)) Oil	Water Soluble	Solid	Gas	Com.	Source
Sewage sludge	260-400	7-13	Without Na2CO3, NaOH, K2CO3, KOH, iron-containing catalysts	No data	No data	Ethanol, ethanol-water mixture	28-57 39-47	No data	32-52 32-43	No data		(Hui et al. 2010)

Biomass	Temp. (°C)	Catalyst	Reaction time	Solvent (extraction)	Yield (% (m/m))		Comments	Source
Micro-alga ***Chlorella prototthecoides***	30-90	Concentrated H2SO4	4 h	n-hexane	Lipids FAME	Up to 55 80 of lipids 44 of alga	Heterotrophic	(Miao and Wu 2006)
Micro-alga ***Chlorella prototthecoides***	30-45	Enzyme (Lipase of Candida sp.)	12 h	n-hexane	Lipids FAME	44 to 49 98 of lipids Up to 48 of alga	Heterotrophic	(Li et al. 2007)
Micro-alga ***Schizochytrium limacinum*** ***(Lipid content: 51 %)***	90	H2SO4	40 min	Methanol/ Chloroform/ Methanol/ Chloroform or hexane	Biodiesel Biodiesel	98 of lipids 50 of alga Up to 116 of lipids Up to 67 of alga	2-stage 1-stage (simultaneous extraction and transesterification)	(Johnson and Wen 2009)
Micro-alga **(heterotrophic)**	90 90	HCl	60 min 15 to 120 min	Methanol/chloroform (10:1)	FAME FAME	Up to 34 of alga Up to 40 of alga	2-stage 1-stage (simultaneous extraction and transesterification)	(Lewis et al. 2000)

Micro-alga ***Nannochloropsis sp.***	65	Mg-Zr (solid)	4 h	Methanol/ Dichloromethane (2:1)	FAME	22 % of alga (27.2 MJ/kg)	2-stage	(Li et al. 2011)
					FAME	28 % of alga (31.5 MJ/kg)	1-stage (simultaneous extraction and transesterification)	
Soya bean oil	23-25	CaO powder coating	12 h		FAME	2 % of oil	Monocrystalline, heterogeneous catalysts	(Venkat Reddy et al. 2006)
		Nanocrystalline CaO	24 h		FAME	>99 % of oil		
Biomass	**Temp. (°C)**	**Catalyst**	**Reaction time**	**Solvent (extraction)**	**Yield (% (m/m))**		**Comments**	**Source**
Nagchamba oil	30-65	H2SO4 for esterification	Up to 50 min		3 to 10 fold increase in reaction speeds in both stages, particularly at low temperatures		1st stage: Esterification of free fatty acids	(Gole and Gogate 2012)
		KOH for transesterification					2nd stage: Transesterification of the triglycerols	
Palm oil	40	H2SO4 (98 %)	300		FAME	93 % of oil	Without ultrasound	(Deshmane et al. 2009)
			150		FAME	95 % of oil	With ultrasound	
Rape-seed oil	40-60	NaOH or KOH	1 to 5 min		FAME	90 to 94 % of oil	Microwaves	(Azcan and Danisman 2008)

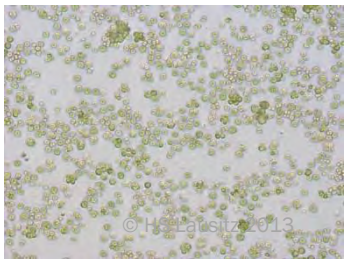
Biomass	Temp. (°C)	Pressure (bar)	Catalyst	Reaction time	Atmosphere	Mash oil	Yield (% (m/m))	Comments	Source
Hydrocracking									
Micro-alga oil Botryococcus Braunii	400	200	CoMo		H2	Without	Product oil: 80 Composition: 66 % petrol (RON 82) 16 % diesel, 14 % kerosene		(HILLEN et al. 1982)
Hydrogenation									
Fast pyrolysis oil from lignocellulose	250	100	Pd/C	4 h	H2	Without	Oil: 35 (residual O: 18,5 %)	Mild deoxygenation	(Wildschut et al. 2009)
	350	200	Ru/TiO2				Oil: 65 (residual O: 10 %)	Deep deoxygenation	
Vegetable oil Rape-seed oil Jatropha oil	320	60	NiMo/Al2O3	1 h	H2	Without	n-alkanes (diesel): 85 (residual O: 0, complete conversion)	Continuous on solid catalyst	(Kuchling et al. 2010)
Micro-alga Chlorella pyrenoidosa	400-430	70-140	NiWo CoMo	210 min	H2	Tetralin or "white oil"	Oil: 46,7 (residual O: 5 %) By-products: Water: 10 COx: Up to 30	In autoclave	(Chin 1979)

Biomass	Temp. (°C)	Pressure (bar)	Catalyst	Reaction time	Atmosphere	Yield (% (m/m)) Liquid product	Com.	Source
Wood (beech) Miscanthus	500	No data	Al-MCM-41	15 min	N2	32 to 51	Fixed bed reactor	(Antonakou et al. 2006
Micro-alga *Chlorella protothecoides*	500	No data	Without	2-3 s	N2	Pyrolysis oil 57 (41 MJ/kg) Coke 11 Pyrolysis oil 17 (30 MJ/kg) Coke 54	Heterotro-phic Autotrophic	(Miao et al. 2004)
Micro-alga	500 500 400	No data	Without	1 sec. 10-20 sec "Very long"	Inert	Oil: 75 Coke: 12 Gas: 13 50 20 30 30 35 35		(Brennan and Owende 2010)
Micro-alga *Chl. protothecoides* *Microcystis auruginosa*	500	No data	Without	2-3 seconds	N2	Pyrolysis oil 17.5 (30 MJ/kg) Pyrolysis oil 23.7 (29 MJ/kg)		(Miao et al. 2004)
Micro-alga *Chlorella protothecoides*	250-500	No data	Without	No data	No data	Pyrolysis oil 55.3 at 500 °C (32.5 – 39.7 MJ/kg)		(Demirbas et al. 2006)

Biomass	Temp. (°C)	Pressure (bar)	Catalyst	Reaction time	Atmosphere	Yield (% (m/m)) Liquid product	Com.	Source
Micro-alga oil *Nannochloropsis sp.* *Chaetoceros muelleri* *Naviculus saprophilla* *Manoraphidium minutum*	400 500	Low	HZSM-5	No data	No data	Hydrocarbons 76 to 86 (splitting off of CO2 and H2O)	Lipids by extraction with butanol or chloroform/ methanol	(Milne et al. 1990)
Micro-alga oil *Botryococcus braunii*	450-500	1	Zeolite	75 s	Inert	Petrol 62 (RON 95) (Oil conversion rate 85 %)	Fixed bed reactor	(Kitazato et al. 1989)

Algae data sheets

Chlorella vulgaris FHL 132 (Versuch 4a/b)



Taxonomische Einordnung

Abteilung:	*Chlorophyta*
Klasse:	*Chlorophyceae*
Ordnung:	*Chlorococcales*
Familie:	*Oocystaceae*
Gattung:	*Chlorella*
Art:	*Chlorella vulgaris*

Herkunft

Stammsammlung	Sammlung von Algenkulturen der Uni Göttingen (SAG)
Lagerbedingung^	½ Tamiya, RT

Morphologie

Zellgröße:	Ø 4 - 13 µm
Zellform:	kugelig, oval
Begeißelung:	keine
Zellaggregate/-verbände:	vereinzelt oder in Kolonien
Besonderheiten:	-
Farbe der Suspension:	grasgrün
Vermehrung:	asexuell durch Autosporen

Wachstumsbedingungen

½ Tamiya, 24 – 25° C; pH 6,52 – 7,78; ca. 100 µE/(m²*s); 2 % CO_2, Magnetrührer

Wachstumsparameter

Dauer der lag-Phase:	nicht erkennbar
Wachstumsdauer (0,3-3,0 g/L):	ca. 16 d
Biomasseproduktivität:	0,145 – 0,148 g/L*d
Verdopplungszeit:	4,7- 4,8 d

Physiologische Parameter

keine Sedimentation innerhalb von 48 h (auch nicht bei pH-Werten von 10-12)
nach Zugabe von 5 * 10^{-4} mol/l $Al_2(SO_4)_3$ bereits nach 30 min deutliche Sedimentation (nach 24 h bei allen getesteten Konz. von 1,5 – 8.8 * 10^{-4} mol/l $Al_2(SO_4)_3$
Zentrifugation für 5 min bei 8000 rpm bringt klare Trennung Alge - Medium

Analytik (Werte jeweils als MW ± SD)

Gesamtlipid (% TS):	20,7 ± 0,9
Lipidproduktivität (mg Lipid x g TS^{-1} x d^{-1}):	12,9 ± 0,6
Gesamt-Fettsäuregehalte (%TS):	6,6 ± 0,19
Gesamt-Fettsäureproduktivität (mg Fettsäure x g TS^{-1} x d^{-1}):	4,13
Gesamtprotein* (%TM):	38,1 ± 1,2
Proteinproduktivität* (mg Protein x g TS^{-1} x d^{-1}):	23,9 ± 1,2

* : als summierte Gehalte an Glycin, Alanin, Valin, Leucin, Isoleucin, Phenylalanin, Prolin, Serin, Threonin, Cystein, Methionin, Tyrosin, Asparaginsäure, Glutaminsäure, Lysin, Arginin, Histidin

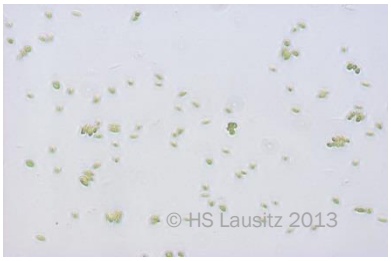
Aminosäure- und Fettsäuregehalte (%TS):

Aminosäuren	MW ± SD	Fettsäuren	MW ± SD
Alanin	3,13 ± 0,03	Palmitinsäure (C16:0)	1,21 ± 0,04
Asparginsäure	4,01 ± 0,06	Palmitolinsäure (C16:1)	0,034 ± 0,001
Arginin	2,88± 0,09	Stearinsäure (C18:0)	0,11 ± 0,02
Cystein	0,18 ± 0,01	Ölsäure (C18:1w9)	0,334 ± 0,01
Glutaminsäure	4,60 ± 0,08	Linolsäure (C18:2)	2,91 ± 0,06
Glycin	2,39 ± 0,03	α - Linolensäure (C18:3α)	0,294 ± 0,01
Histidin	1,26 ± 0,03		
Isoleucin	1,59 ± 0,02		
Leucin	3,67 ± 0,05		
Lysin	2,65 ± 0,03		
Methionin	0,28±0,02		
Phenylalanin	2,07 ± 0,04		
Prolin	1,88 ± 0,02		
Serin	1,77 ± 0,02		
Threonin	1,95 ± 0,02		
Tyrosin	1,43 ± 0,03		
Valin	2,45 ± 0,03		

Scenedesmus obliquus (Versuch 3a/3b)

Taxonomische Einordnung

Abteilung:	*Chlorophyta*
Klasse:	*Chlorophyceae*
Ordnung:	*Chlorococcales*
Familie:	*Scenedesmaceae*
Gattung:	*Scenedesmus*
Art:	*Scenedesmus obliquus*



Herkunft

Stammsammlung	Sammlung von Algenkulturen der Uni Göttingen (SAG)
Lagerbedingung	ESP Ag (=Basalmedium mit Pepton), RT

Morphologie

Zellgröße:	Ø 12 - 15 µm
Zellform:	kugelig, oval, ellipsoidisch
Begeißelung:	keine
Zellaggregate/-verbände:	vereinzelt oder in Kolonien

Besonderheiten: -
Farbe der Suspension: grasgrün
Vermehrung: asexuell durch Autosporen

Wachstumsbedingungen
½ Tamiya, 24 – 25° C; pH 6,38 – 8,08; ca. 100 µE/(m^2*s); 2 % CO_2, Magnetrührer

Wachstumsparameter
Dauer der lag-Phase: nicht erkennbar
Wachstumsdauer (0,3-3,0 g/L): ca. 24 d
Biomasseproduktivität: 0,119 – 0,129 g/L*d
Verdopplungszeit: 5,4-5,8 d

Physiologische Parameter
deutliche Sedimentation nach 48 h (Sedimentation nach 24 h bei einem pH-Wert von 10)
Zentrifugation für 5 min bei 8000 rpm bringt klare Trennung Alge - Medium

Analytik (Werte jeweils als MW ± SD)
Gesamtlipid (% TS): 22,7 ± 0,9
Lipidproduktivität (mg Lipid x g TS^{-1} x d^{-1}): 9,4 ± 0,4
Gesamt-Fettsäuregehalte (%TS): 4,9 ± 0,36
Gesamt-Fettsäureproduktivität (mg Fettsäure x g TS^{-1} x d^{-1}): 2,04
Gesamtprotein* (%TM): 33,6 ± 0,75
Proteinproduktivität* (mg Protein x g TS^{-1} x d^{-1}): 14 ± 0,31
* : als summierte Gehalte an Glycin, Alanin, Valin, Leucin, Isoleucin, Phenylalanin, Prolin, Serin, Threonin, Cystein, Methionin, Tyrosin, Asparaginsäure, Glutaminsäure, Lysin, Arginin, Histidin

Aminosäure- und Fettsäuregehalte (%TS):

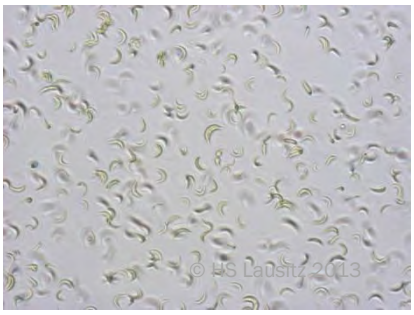
Aminosäuren	MW ± SD	Fettsäuren	MW ± SD
Alanin	3,13 ± 0,07	Palmitinsäure (C16:0)	0,917 ± 0,12
Asparginsäure	3,36 ± 0,09	Palmitolinsäure (C16:1)	0,019 ± 0,0003
Arginin	1,76 ± 0,04	Stearinsäure (C18:0)	0,075 ± 0,02
Cystein	0,33 ± 0,002	Ölsäure (C18:1w9)	0,425 ± 0,01
Glutaminsäure	3,84 ± 0,17	Linolsäure (C18:2)	0,091 ± 0,06
Glycin	2,27 ± 0,05	α - Linolensäure (C18:3α)	0,287 ± 0,01
Histidin	0,84 ± 0,03		
Isoleucin	1,47 ± 0,03		
Leucin	3,38 ± 0,06		
Lysin	2,15 ± 0,04		
Methionin	0,37 ± 0,04		
Phenylalanin	2,04 ± 0,04		

Prolin	1,77 ± 0,05		
Serin	1,64 ± 0,05		
Threonin	1,98 ± 0,04		
Tyrosin	1,11 ± 0,05		
Valin	2,14 ± 0,06		

Selenastrum rinoi MACC-67 (Versuch 9a/9b

Taxonomische Einordnung

Abteilung:	Chlorophyta
Klasse:	Chlorophyceae
Ordnung:	Sphaeropleales
Familie:	Selenastraceae
Gattung:	*Selenastrum*
Art:	*Selenastrum rinoi*



Herkunft

Stammsammlung	SAG Göttingen
Lagerbedingung	BG 11, RT

Morphologie

Zellgröße:	10-12 µm lang, 2-3 µm breit
Zellform:	sichelförmig
Begeißelung:	keine
Zellaggregate/-verbände:	vereinzelt oder in Kolonien
Besonderheiten:	-
Farbe der Suspension:	grasgrün
Vermehrung:	asexuell durch Autosporen

Wachstumsbedingungen

BG 11, 25 – 28° C; pH 7,95 – 8,95; ca. 100 µE/(m²*s); 2 % CO_2, Magnetrührer

Wachstumsparameter

Dauer der lag-Phase:	nicht erkennbar
Wachstumsdauer (0,3-3,0 g/L):	ca. 12 d
Biomasseproduktivität:	0,235-0,279 g/L*d
Verdopplungszeit:	2,48-2,95 d

Physiologische Parameter

Keine Sedimentation innerhalb von 48 h (auch nicht bei pH-Werten von 10-12)
Zentrifugation für 10 min bei 8000 rpm bringt klare Trennung Alge - Medium

Analytik (Werte jeweils als MW ± SD)

Gesamtlipid (% TS): 22,4 ± 2,6
Lipidproduktivität (mg Lipid x g TS^{-1} x d^{-1}): 18,7 ± 2,2
Gesamt-Fettsäuregehalte (%TS): 4,9 ± 0,02
Gesamt-Fettsäureproduktivität (mg Fettsäure x g TS^{-1} x d^{-1}): 4,08
Gesamtprotein* (%TM): 42,1 ± 1,7
Proteinproduktivität* (mg Protein x g TS^{-1} x d^{-1}): 35,1 ± 1,4

* : als summierte Gehalte an Glycin, Alanin, Valin, Leucin, Isoleucin, Phenylalanin, Prolin, Serin, Threonin, Cystein, Methionin, Tyrosin, Asparaginsäure, Glutaminsäure, Lysin, Arginin, Histidin

Aminosäure- und Fettsäuregehalte (%TS):

Aminosäuren	MW ± SD	Fettsäuren	MW ± SD
Alanin	4,18 ± 0,22	Palmitinsäure (C16:0)	0,961 ± 0,034
Asparginsäure	3,80 ± 0,20	Palmitolinsäure (C16:1)	0,15 ± 0,0049
Arginin	3,89 ± 0,35	Stearinsäure (C18:0)	0,065 ± 0,012
Cystein	0,17 ± 0,01	Ölsäure (C18:1w9)	0,458 ± 0,01
Glutaminsäure	4,63 ± 0,24	Linolsäure (C18:2)	0,257 ± 0,005
Glycin	2,81 ± 0,11	α - Linolensäure (C18:3α)	0,619 ± 0,008
Histidin	1,09 ± 0,03		
Isoleucin	1,74 ± 0,10		
Leucin	4,22 ± 0,21		
Lysin	2,82 ± 0,13		
Methionin	0,34 ± 0,03		
Phenylalanin	2,25 ± 0,13		
Prolin	2,26 ± 0,10		
Serin	1,79 ± 0,10		
Threonin	2,02 ± 0,11		
Tyrosin	1,61 ± 0,09		
Valin	2,47 ± 0,13		

Base data of assessment

See also section 0 "Figure 10.1 Schematic of assessment system

"

Tabelle A3.1. Energy demand of selected consumers

Consumer	Value	Unit	Source
Centrifuge	3.60	MJ/m³	
Flash dryer, thermal	4.32	MJ/kg H_2O	
Flash dryer, electrical	0.65	MJ/kg H_2O	

Bases for assessment of the biogas path

Tabelle A3.2. Material data of the biogas path

Micro-alga components	Value	Unit	Source
Proteins	33.6	% dm	According to (Hochschule Lausitz (FH) 2011)
Lipids	22.7	% dm	According to (Hochschule Lausitz (FH) 2011)
Ash	20.0	% dm	Assumption
Carbohydrates	23.7	% dm	from difference

Tabelle A3.3. Technical parameters of the biogas path

Parameter	Value	Unit	Source
Biogas plant internal power demand	94.7	kJ/kg(substrate)	(Weiland et al. 2010)
Heat capacity of water	4.2	kJ/kgK	
Heat capacity of algal biomass	2.0	kJ/kgK	
Calorific value of methane	39.8	MJ/m³	

Literature references

AVA-CO_2 Hydrothermale Karbonisierung / Erneuerbare Energie - Energiebilanz. Online verfügbar unter http://www.ava-co2.com/web/pages/de/technologie/energiebilanz.php, zuletzt geprüft am 24.04.2012.

DIN EN 228:2008-11, Norm - Beuth.de. Online verfügbar unter http://www.beuth.de/de/norm/din-en-228/109897746, zuletzt geprüft am 19.04.2012.

DIN EN 590:2010-05, Norm - Beuth.de. Online verfügbar unter http://www.beuth.de/de/norm/din-en-590/125423850?SearchID=400507049, zuletzt geprüft am 19.04.2012.

german.alibaba.com. Online verfügbar unter http://german.alibaba.com.

Zentrifugal-Separatoren von GEA Westfalia Separator. Online verfügbar unter http://www.westfalia-separator.com/de/produkte/separatoren.html, zuletzt geprüft am 26.04.2012.

Ahmad, A.L; Mat Yasin, N.H; Derek, C.J.C; Lim, J.K (2011): Optimization of microalgae coagulation process using chitosan. In: Chemical Engineering Journal 173 (3), S. 879–882.

Amin, Sarmidi (2009): Review on biofuel oil and gas production processes from microalgae. In: Energy Conversion and Management 50 (7), S. 1834–1840.

Anastasakis, K.; Ross, A.B (2011): Hydrothermal liquefaction of the brown macro-alga Laminaria Saccharina: Effect of reaction conditions on product distribution and composition. In: Bioresource Technology 102 (7), S. 4876–4883.

Andrich, Gianpaolo; Nesti, Ugo; Venturi, Francesca; Zinnai, Angela; Fiorentini, Roberto (2005): Supercritical fluid extraction of bioactive lipids from the microalgaNannochloropsissp. In: European Journal of Lipid Science and Technology 107 (6), S. 381–386.

Antal, Michael Jerry; Mok, William S.L; Richards, Geoffrey N. (1990): Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from d-fructose and sucrose. In: Carbohydrate Research 199 (1), S. 91–109.

Antonakou, Eleni; Lappas, Angelos; Nilsen, Merete H.; Bouzga, Aud; Stöcker, Michael (2006): Evaluation of various types of Al-MCM-41 materials as catalysts in biomass pyrolysis for the production of bio-fuels and chemicals. In: Fuel 85 (14-15), S. 2202–2212, zuletzt geprüft am 26.04.2012.

ARAL (1995): ARAL Fachreihe Forschung und Technik. Dieselkraftstoffe.

ARAL (2000): ARAL Fachreihe Forschung und Technik. Ottokraftstoffe.

Arlt, A. (2003): Systemanalytischer Vergleich zur Herstellung von Ersatzbrennstoffen aus biogenen Abfällen. Forschungszentrum Karlsruhe GmbH. Karlsruhe. Online verfügbar unter http://www.itas.fzk.de/deu/lit/2003/arlt03a.pdf, zuletzt aktualisiert am 08.01.2004, zuletzt geprüft am 26.04.2012.

Azcan, Nezihe; Danisman, Aysegul (2008): Microwave assisted transesterification of rapeseed oil. In: Fuel 87 (10-11), S. 1781–1788, zuletzt geprüft am 26.04.2012.

Balat, M. (2008): Mechanisms of Thermochemical Biomass Conversion Processes. Part 3: Reactions of Liquefaction. In: Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 30 (7), S. 649–659.

Barbier, J.; Charon, N.; Dupassieux, N.; Loppinet-Serani, A.; Mahé, L.; Ponthus, J. et al. (2011): Hydrothermal conversion of glucose in a batch reactor. A detailed study of an experimental key-parameter: The heating time. In: The Journal of Supercritical Fluids 58 (1), S. 114–120.

Becker, E. W. (1994): Microalgae Biotechnology and Microbiology.

Becker, T. (1983): Downstream processing of proteins. In: Biotechnology Advances 1 (2), S. 247–261.

Becker, W. (2004): Microalgae in human and animal nutrition. In: Amos Richmond (Hg.): Handbook of microalgal culture. Biotechnology and applied phycology. Oxford, OX, UK ;, Ames, Iowa, USA: Blackwell Science.

Behrendt, F.; Neubauer, Y.; Oevermann, M.; Wilmes, B.; Zobel, N. (2008): Direct Liquefaction of Biomass. In: Chem. Eng. Technol 31 (5), S. 667–677.

Belay, A.; Kato, T.; Ota, Y. (1996): *Spirulina* (Arthrospira): potential application as an animal feed supplement. In: Journal of Applied Phycology 8, S. 303–311.

Benemann, John R.; Oswald, William J.: Systems and Economic Analysis of Microalgae Ponds for Conversion of CO_2 to Biomass.

Berenguer, J.; Pedro, M. A. de; Vázquez, D. (1982): Induction of cell lysis in Escherichia coli - cooperative effect of Nocardicin A and Mecillinam. In: Antimicrobial agents and chemotherapy 21 (2), S. 195–200.

Berges, J. A.; Fisher, A. E.; Harrison, P. J. (1993): A comparison of Lowry, Bradford and Smith protein assays using different protein standards and protein isolated from the marine diatom Thalassiosira pseudonana. In: Marine Biology 115, S. 187–193.

Bergius, Friedrich (1932): Chemical reactions under high pressure. Nobel Lecture, May 21, 1932, 21.05.1932, zuletzt geprüft am 24.04.2012.

Bilanovic, D.; Shelef, G. (1988): Flocculation of Microalgae with Cationic Polymers –Effects of Medium Salinity. In: Biomass 17, S. 65–76. Online verfügbar unter http://www.sciencedirect.com/science/article/pii/0144456588900716.

Biller, P.; Ross, A.B (2011): Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. In: Bioresource Technology 102 (1), S. 215–225.

Bligh, E.G.; Dyer, W.J. (1959): A rapid method for total lipid extrction and purification. In: Canadian Journal of Biochemical Physiology 37, S. 911–917.

Bonora, A.; Mares, D. (1982): A simple colorimetric method for detecting cell viability in cultures of eukaryotic microorganisms. In: Current Microbiology 7, S. 217–222.

Borowitzka, M. A. (1997): Microalgae for aquaculture: Opportunities and constraints. In: Journal of Applied Phycology 9, S. 393–401.

Bradford, M. M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the prinziple of protein-dye binding. In: Analytical Biochemistry 72, S. 248–254.

Brebu, M.; Vasile, C. (2010): Thermal degradation of lignin - a review. In: Cellulose Chemistry and Technology 44 (9), S. 353–363.

Brennan, Liam; Owende, Philip (2010): Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. In: Renewable and Sustainable Energy Reviews 14 (2), S. 557–577.

Brentner, L. B. (2011): Life cycle analysis of microalgal biodiesel and future prospects for microalgae biotechnology. 5. Interantionaler Algenkongress. European society of Microalgae Biotechnology. Berlin, 01.12.2011. Online verfügbar unter www.algaecongress.com.

Brown, M. R.; Mular, M.; Miller, I.; Farmer, C.; Trenerry, C. (1999): The vitamin content of microalgae used in aquaculture. In: Journal of Applied Phycology 11, S. 247–255.

Brown, Robert C. (Hg.) (2011): Thermochemical processing of biomass. Conversion into fuels, chemicals and power. Chichester: Wiley (Wiley series in renewable resources).

Brown, Tylisha M.; Duan, Peigao; Savage, Phillip E. (2010): Hydrothermal Liquefaction and Gasification of Nannochloropsis sp. In: Energy Fuels 24 (6), S. 3639–3646.

Bruton, T.; u.a. (2009): A Review of the Potential of Marine Algae as a Source of Biofuel in Ireland. Dublin. Online verfügbar unter www.sei.ie.

Burlew, John S. (1976): Algal Culture from labatory to pilot plant: Burlew, John S.

Cao, Xiaoyan; Ro, Kyoung S.; Chappell, Mark; Li, Yuan; Mao, Jingdong (2011): Chemical Structures of Swine-Manure Chars Produced under Different Carbonization Conditions Investigated by Advanced Solid-State 13 C Nuclear Magnetic Resonance (NMR) Spectroscopy †. In: Energy Fuels 25 (1), S. 388–397.

Carlson, A.S; van Beilen, J.B; Möller, R.; Clayton, D. (2007): MICRO- AND MACRO-ALGAE: UTILITY FOR INDUSTRIAL APPLICATIONS: CPL Press. Online verfügbar unter www.cplbookshop.com.

Cartens, M.; Molina Grima, E.; Robles Medina, A.; Giménez Giménez, A.; Ibánez González, M. J. (1996): Eicosapentaenoic acid (20:5n-3) from the marine microalga Phaeodactylum tricornutum. In: JAOCS 73 (8), S. 1026–1033.

Chang, C.; Ma, X.; Cen, P. (2006): Kinetics of levulinic acid formation from glucose decomposition at high temperature. In: Chinese J. Chem. Eng. 14 (5), S. 708–712.

Char, J-M; Wang, J-K; Chow, T-J; Chien, Q-C (2011): Biodiesel production from microalgae through supercritical carbon dioxide extraction. In: Journal of the Japan Institute of Energy 90, S. 369–373.

Chen, Paris Hanglay; Oswald, William J. (1998): Thermochemical treatment for algae fermentation. In: Environment International 24 (8), S. 889–897.

Cheng, Shuna; D'cruz, Ian; Wang, Mingcun; Leitch, Mathew; Xu, Chunbao (2010): Highly Efficient Liquefaction of Woody Biomass in Hot-Compressed Alcohol−Water Co-solvents †. In: Energy Fuels 24 (9), S. 4659–4667.

Chin, Lih-Yen (1979): Fuels by Algae Hydrogenation. Dissertation. Pennsylvania State University, Pennsylvania. Department of Chemical Engineering, zuletzt geprüft am 30.04.2012.

Chisti, Yusuf (2007): Biodiesel from microalgae. In: Biotechnology Advances 25 (3), S. 294–306.

Chisti, Yusuf; Moo-Young, Murray (1986): Disruption of microbial cells for intracellular products. In: Enzyme and Microbial Technology 8 (4), S. 194–204.

Choubert, Georges; Heinrich, Olivier (1993): Carotenoid pigments of the green alga *Haematococcus* pluvialis: assay on rainbow trout, Oncorhynchus mykiss, pigmentation in comparison with synthetic astaxanthin and canthaxanthin. In: Aquaculture 112 (2-3), S. 217–226.

Christen, Daniel S. (2010): Praxiswissen der chemischen Verfahrenstechnik. Handbuch für Chemiker und Verfahrensingenieure. 2. Aufl. Berin: Springer.

Collet, Pierre; Hélias, Arnaud; Lardon, Laurent; Ras, Monique; Goy, Romy-Alice; Steyer, Jean-Philippe (2011): Life-cycle assessment of microalgae culture coupled to biogas production. In: Bioresource Technology 102 (1), S. 207–214.

Damann, Roland (1998): AQUATECTOR® Microfloat®-TECHNOLOGIE IN DER MODERNEN ABWASSERBEHANDLUNG GRUNDLAGEN UND ANWENDUNGSBEREICHE. In: Weka Fachverlage, 1998.

DBFZ (2011): internes Material.

DELANOUE, J.; DEPAUW, N. (1988): The potential of microalgal biotechnology: A review of production and uses of microalgae. In: Biotechnology Advances 6 (4), S. 725–770.

Demirbas, A. (2000): Mechanisms of liquefication and pyrolysis reactions of biomass. In: Energy Conversion & Management 41, S. 633–646.

Demirbas, Ayhan (2009): Biorefineries: Current activities and future developments. In: Energy Conversion and Management 50 (11), S. 2782–2801.

Demirbas, Ayhan (2010): Use of algae as biofuel sources. In: Energy Conversion and Management 51 (12), S. 2738–2749.

Demirbas, Ayhan (2011): Biodiesel from oilgae, biofixation of carbon dioxide by microalgae: A solution to pollution problems. In: Applied Energy 88 (10), S. 3541–3547.

Demirbas, Ayhan; Demirbas, M. Fatih (2010): Algae Energy. Algae as a New Source of Biodiesel. London: Springer-Verlag London (Green Energy and Technology).

Demirbas, Ayhan; Ozturk, Temel; Demirbas, M. Fatih (2006): Recovery of Energy and Chemicals from Carbonaceous Materials. In: Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 28 (16), S. 1473–1482.

Denery, J.R.; Dragull, K.; Tang, C.S.; Li, Q.X. (2004): Pressurized fluid extraction of carotenoids from *Haematococcus* pluvialis and *Dunaliella salina* and kavalactones from Piper methysticum. In: Analytica Chimica Acta, 501, 2004, S. 175–181. 501, S. 175–181.

Deshmane, Vishwanath G.; Gogate, Parag R.; Pandit, Aniruddha B. (2009): Ultrasound-Assisted Synthesis of Biodiesel from Palm Fatty Acid Distillate. In: Ind. Eng. Chem. Res. 48 (17), S. 7923–7927, zuletzt geprüft am 26.04.2012.

Dodd, Joseph C. (1979): Algae production and harvesting from animal wastewaters. In: Agricultural Wastes 1 (1), S. 23–37. Online verfügbar unter http://www.sciencedirect.com/science/article/pii/0141460779900040.

Dote, Y.; Sawayama, S.; Inoue, S.; Minowa, T.; Yokoyama, S. (1994): Recovery of liquid fuel from hydrcarbon-rich microalgae by thermochemical liquefaction. In: Fuel 73 (12), S. 1855–1857.

Doucha, J.; Lívanský, K. (2008): Influence of processing parameters on disintegration of Chlorella cells in various types of homogenizers. In: Appl Microbiol Biotechnol 81 (3), S. 431–440.

DSTAN (2008): Defence Standard 91-91 Issue 6. Online verfügbar unter http://www.seta-analytics.com/documents/DEF_STAN_91-91_R6.pdf, zuletzt aktualisiert am 18.08.2008, zuletzt geprüft am 26.04.2012.

Duan, Peigao; Savage, Phillip E. (2011): Hydrothermal Liquefaction of a Microalga with Heterogeneous Catalysts. In: Ind. Eng. Chem. Res 50 (1), S. 52–61.

Dufour, J.; Moreno, J.; Rodriguez, R.: Life cycle assessment of biodiesel production from microalgae oil: effect of algae species and cultivation system. DOI: 10.1007/978-94-007-1899-9-42

Ebermann, Robert; Elmadfa, Ibrahim (2008): Lehrbuch Lebensmittelchemie und Ernährung. Vienna: Springer-Verlag/Wien.

Ecke, M. (2003): 2,5 Milliarden Jahre Erfahrung der Natur – Mikroalgen als alternative Roh – und Wirkstoffquelle. In: F. Begemann und S. Schröder (Hg.): Schriften zu Genetischen Ressourcen. Produktvielfalt durch Ressourcenvielfalt - Potenziale genetischer Ressourcen -. Tagungsband eines

Symposiums vom 24.-25. September 2003 im Gustav-Stresemann-Institut in Bonn. Zentralstelle für Agrardokumentation und -information Informationszentrum Biologische Vielfalt (IBV). Bonn: Zentralstelle für Agrardokumentation und -information (ZADI) (23), S. 140–148.

Ehimen, E.A.; Connaughton, S.; Sun, Z.; Carrington, G.C. (2009): Energy recovery from lipid extracted, transesterified and glycol codigested microalgae biomass. In: GCB Bioenergy, Bd. 1, S. 371–381.

Ehimen, E.A; Sun, Z.F; Carrington, C.G; Birch, E.J; Eaton-Rye, J.J (2011): Anaerobic digestion of microalgae residues resulting from the biodiesel production process. In: Applied Energy 88 (10), S. 3454–3463.

Elliot, Douglas C. (2011): Hydrothermal Processing. In: Robert C. Brown (Hg.): Thermochemical processing of biomass. Conversion into fuels, chemicals and power. Chichester: Wiley (Wiley series in renewable resources), S. 200–231.

Elliott, D.C.; Sealook, L.J. (1999): Chemical processing in high-pressure aqueous environmemts: Low temperature gasification. In: Trans IChemE 74, S. 563–566.

Elliott, Douglas C. (2007): Historical Developments in Hydroprocessing Bio-oils. In: Energy Fuels 21 (3), S. 1792–1815.

Endisch, M.; Balfanz, U.; Olschar, M.; Kuchling, T. (2008): Hydierung von Pflanzenölen zu hochwertiegen Dieselkomponenten. In: DGMK (Hg.): Energetische Nutzung von Biomassen. DGMK Tagungsbericht 2009-2. DGMK Fachbereichstagung. Velen, 14-16.04.2008. DGMK, S. 261–270.

Eppink, M. Wijffels R.; Barbosa, M. (2011): Biorefinery of Microalgae: Production of high valuable products, bulk chemicals and biofuels. 5. Internationaler Algenkongress. Berlin, 01.12.2011.

Erlitz, Marcel (2010): Optimierung der Lipidextraktion aus Algen. Bachelor Thesis. Senftenberg.

Fachagentur Nachwachsende Rohstoffe (2005): Leitfaden Biogas. Gülzow.

Fajardo, A.R.; Cerdan, L.E.; Medina, A.R.; Fernandez, F.G.A.; Moreno, P.A.G.; Grima, E.M. (2007): Lipid extraction from the microalga Phaeodactylum tricornutum. In: European Journal of Lipid Science and Technology 109, S. 120–126.

Fang, Z.; Minowa, T.; Smith, R. L.; Ogi, T.; Koziński, J. A. (2004): Liquefaction and Gasification of Cellulose with Na 2 CO 3 and Ni in Subcritical Water at 350 °C. In: Ind. Eng. Chem. Res 43 (10), S. 2454–2463.

Fang, Zhen; Sato, Takafumi; Smith, Richard L.; Inomata, Hiroshi; Arai, Kunio; Kozinski, Janusz A. (2008): Reaction chemistry and phase behavior of lignin in high-temperature and supercritical water. In: Bioresource Technology 99 (9), S. 3424–3430.

Feinberg, Daniel A. (1984): Fuel Options from Microalgae with Representative Chemical Compositions. Colorado.

Food and Agriculture Organization of the United Nations (FAO) (2009): ALGAE-BASED BIOFUELS. A Review of Challenges and Opportunities for Developing Countries. Online verfügbar unter www.fao.org/bioenergy/aquaticbiofuels.

Frenz, J.; Largeau, C.; Casadevall, E. (1989): Hydrocarbon Recovery and Biocompatibility of Solvents for Extraction from Cultures of Botryococcus braunii. In: Biotechnology and Bioengineering 34, S. 755–762.

Funke, Axel; Ziegler, Felix (2011): Heat of reaction measurements for hydrothermal carbonization of biomass. In: Bioresource Technology 102 (16), S. 7595–7598, zuletzt geprüft am 24.04.2012.

G. Shelef; A. Sukenik; M. Green (1984): Microalgae Harvesting and Processing. A Literature Review. Haifa.

Giménez Giménez, A.; Ibánez González, M. J.; Robles Medina, A.; Molina Grima, E.; Garcia Salas, S.; Esteban Cerdán, L. (1998): Downstream processing and purification of eicosapentaenoic (20:5n-3) and arachidonic acids (20:4n-6) from the microalga Porphyridium cruentum. In: Bioseparation 7, S. 89–99.

Gole, Vitthal L.; Gogate, Parag R. (2012): Intensification of Synthesis of Biodiesel from Nonedible Oils Using Sonochemical Reactors. In: Ind. Eng. Chem. Res., S. 120410150545008, zuletzt geprüft am 26.04.2012.

Golueke, C. G.; Oswald, W. J.; Gotaas, H. B. (1957): Anaerobic digestion of microalgae. In: Applied Microbiology 5, 1957, S. 47–55.

Goto, Motonobu; Obuchi, Ryusaku; Hirose, Tsutomu; Sakaki, Tsuyoshi; Shibata, Masao (2004): Hydrothermal conversion of municipal organic waste into resources. In: Bioresource Technology 93 (3), S. 279–284.

Gouveia, L.; Batista, A. P.; Sousa, I.; Raymundo, A.; Bandarra, N. M. (2008): Microalgae in novel food products. In: Food Chemistry Research Development.

Grange, P.; Laurent, E.; Maggi, R.; Centeno, A.; Delmon, B. (1996): Hydrotreatment of pyrolysis oils from biomass: reactivity of the various categories of oxygenated compounds and preliminary techno-economical study. In: Catalysis Today 29, S. 297–301, zuletzt geprüft am 26.04.2012.

Green, Don W.; Perry, Robert H. (2008): Perry's Chemical Engineers' Handbook: McGraw-Hill.

Greenwell, H. C.; Laurens, L. M. L.; Shields, R. J.; Lovitt, R. W.; Flynn, K. J. (2010): Placing microalgae on the biofuels priority list: a review of the technological challenges. In: Journal of The Royal Society Interface 7 (46), S. 703–726.

Grierson, Scott; Strezov, Vladimir; Bray, Sargent; Mummacari, Raffaella; Danh, Luu Thai; Foster, Neil (2012): Assessment of Bio-oil Extraction from Tetraselmis chui Microalgae Comparing Supercritical CO 2, Solvent Extraction, and Thermal Processing. In: Energy Fuels 26 (1), S. 248–255.

Guckert, James B.; Cooksey, Keith E.; Jackson, Larry L. (1988): Lipid solvent systems are not equivalent for analysis of lipid classes in the microeukaryotic green alga, Chlorella. In: Journal of Microbiological

Methods 8, S. 139–149. Online verfügbar unter http://ac.els-cdn.com/0167701288900152/1-s2.0-0167701288900152-main.pdf?_tid=eecaf0aca0ba8f418c43891a203169b6&acdnat=1335274203_fe8c91983f42c59ddc92cbff8331c095, zuletzt geprüft am 24.04.2012.

Guil-Guerrero, J. L.; Belabi, E-H; Rebollosos-Fuentes, M. M. (2001): Eicosapentaenoic and arachidonic acids purification from the red microalga Porphyridium cruentum. In: Bioseparation 9, S. 299–306.

Halim, R.; Danquah, M. k. and Webley, P. A. (2012): Extraction of oil from microalgae for biodiesel production: a review. Biotechnology advances. 2012, S. 709-732.

Halim, R.; Gladman, B.; Danquah, M. K.; Webley, P. A. (2011): Oil extraction from microalgae for biodiesel production. In: Bioresource Technology

Halle, I.; Janczyk, P.; Freyer, G.; Souffrant, W. B. (2009): Effect of microalga *Chlorella vulgaris* on laying hen performance. In: Archiva Zootechnica 12 (2), S. 5–13.

Hammerschmidt, Alexander; Boukis, Nikolaos; Hauer, Elena; Galla, Ulrich; Dinjus, Eckhard; Hitzmann, Bernd et al. (2011): Catalytic conversion of waste biomass by hydrothermal treatment. In: Fuel 90 (2), S. 555–562.

Hans Binder Maschinenbau GmbH (Hg.) (2011): BINDER Trocknungsanlagen – Verfahrenstechnik. Online verfügbar unter http://www.binder-trockner.de/, zuletzt geprüft am 26.04.2012.

Harun, Razif; Davidson, Michael; Doyle, Mark; Gopiraj, Rajprathab; Danquah, Michael; Forde, Gareth (2011): Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. In: Biomass and Bioenergy 35 (1), S. 741–747. Online verfügbar unter http://www.sciencedirect.com/science/article/pii/S0961953410003594.

Harun, Razif; Singh, Manjinder; Forde, Gareth M.; Danquah, Michael K. (2010): Bioprocess engineering of microalgae to produce a variety of consumer products. In: Renewable and Sustainable Energy Reviews 14 (3), S. 1037–1047.

He, B. J.; Zhang, Y.; Yin, Y.; Riskowski, G. L.; Funk, T. L. (1999): THERMAL CHEMICAL CONVERSION OF SWINE MANURE TO PRODUCE FUEL, REDUCE WASTE AND ODOR. ASAE/CASE Annual International Meeting. Toronto, 18.07.1999.

Hedenskog, G.; Ebbinghaus, L. (1972): Reduction of the nucleic acid content of single-cell protein concentrates. In: Biotechnology and bioengineering 14, S. 447–457.

Hedenskog, G.; Enebo, L. (1969): Investigation of some methods for increasing the digestibility in vitro of microalgae. In: Biotechnology and bioengineering 11, S. 37–51.

Heger, Monica (2009): A New Processing Scheme for Algae Biofuels. OriginOil claims that its technology extracts oil from algae cheaply and efficiently. Online verfügbar unter http://www.technologyreview.com/energy/22572/page1/, zuletzt aktualisiert am 01.05.2009, zuletzt geprüft am 02.02.2012.

Heilmann, Steven M.; Davis, H. Ted; Jader, Lindsey R.; Lefebvre, Paul A.; Sadowsky, Michael J.; Schendel, Frederick J. et al. (2010): Hydrothermal carbonization of microalgae. In: Biomass and Bioenergy 34 (6), S. 875–882.

Heilmann, Steven M.; Jader, Lindsey R.; Harned, Laurie A.; Sadowsky, Michael J.; Schendel, Frederick J.; Lefebvre, Paul A. et al. (2011): Hydrothermal carbonization of microalgae II. Fatty acid, char, and algal nutrient products. In: Applied Energy 88 (10), S. 3286–3290, zuletzt geprüft am 24.04.2012.

Hemaiswarya, S.; Raja, R.; Ravi Kumar, R.; Ganesan, V.; Anbazhagan, C. (2011): Microalgae: a sustainable feed source for aquaculture. In: World J Microbiol Biotechnol 27 (8), S. 1737–1746.

Hempel, Niels; Petrick, Ingolf; Behrendt, Frank (2012): Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. In: J Appl Phycol.

Henderson, Rita; Parsons, Simon A.; Jefferson, Bruce (2008): The impact of algal properties and pre-oxidation on solid-liquid separation of algae. In: Water Research 42 (8-9), S. 1827–1845. Online verfügbar unter http://www.sciencedirect.com/science/article/pii/S004313540700704X.

Heng, Liang; Jun, Nan; Wen-jie, He; Guibai, Li (2009): Algae removal by ultrasonic irradiation–coagulation. In: Desalination 239 (1-3), S. 191–197.

Herrero, M.; Cifuentes, A.; Ibañez, E. (2006): Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgaeA review. In: Food Chemistry 98 (1), S. 136–148.

Hielscher Ultrasonics GmbH (Hg.): UIP500hd - Industrielle Beschallung im kleinen Maßstab. Online verfügbar unter http://www.hielscher.com/ultraschall/i500_p.htm, zuletzt geprüft am 02.02.2012.

HILLEN, L.W.; POLLARD, G.; WAKE, L.V.; WHITE, N. (1982): Hydrocracking of the Oils of Botryococcus braunii to Transport Fuels. In: Biotechnology and bioengineering 24, S. 193–205.

Hintz, H. F.; Heitman jr., H.; Weir, W. C.; Torell, D. T.; Meyer, J. H. (1966): Nutritive value of algae grown on sewage. In: Journal of Animal science 25, S. 675–681.

Hochschule Lausitz (FH) (2011): Datenblätter zu den kultivierten Algenspezies *C. vulgaris*, *S. obliquus* und S. rinoi.

Hölker, Udo (2008): Deutschlandweit in die Fermenter geschaut. In: BauernZeitung (6. Woche 2008), S. 28–30.

Hosikian, Aris; Lim, Su; Halim, Ronald; Danquah, Michael K. (2010): Chlorophyll Extraction from Microalgae: A Review on the Process Engineering Aspects. In: International Journal of Chemical Engineering 2010, S. 1–11.

Huang, Huajun; Yuan, Xingzhong; Zeng, Guangming; Wang, Jingyu; Li, Hui; Zhou, Chunfei et al. (2011): Thermochemical liquefaction characteristics of microalgae in sub- and supercritical ethanol. In: Fuel Processing Technology 92 (1), S. 147–153.

Hui, L.; Xingzhong, Y.; Guangming, Z.; Danlian, H.; Huajun, H.; Jingyi, T. et al. (2010): The formation of bio-oil from sludge by deoxy-liquefaction in supercritical ethanol. In: Bioresource Technology 101 (8), S. 2860–2866.

Huihua Mao, H.; Moo-Yount, M. (1990): Disruption of baker's yeast by a new bead mill. In: Biotechnolgy Techniques 4 (5), S. 335–340.

Igl, N.; Schulmeyr, J. (2006): Neue Perspektiven der Hochdruckextraktion mit CO2. In: Chemie Ingenieur Technik 78 (9), S. 1241.

Ikenaga, Na-oki; Ueda, Chiyo; Matsui, Takao; Ohtsuki, Munetaka; Suzuki, Toshimitsu (2001): Co-liquefaction of Micro Algae with Coal Using Coal Liquefaction Catalysts. In: Energy Fuels 15 (2), S. 350–355.

Itoh, Shinji; Suzuki, Akira; Nakamura, Tadashi; Yokoyama, Shin-ya (1994): Production of heavy oil from sewage sludge by direct thermochemical liquefaction. In: Desalination 98 (1-3), S. 127–133.

Jacobs, Ullrich (2009): SYSTEMVERGLEICH KLÄRSCHLAMMTROCKNUNG.

Janczyk, P.; Langhammer, M.; Renne, U.; Guiard, V.; Souffrant, W. B. (2006): Effect of feed supplementation with *Chlorella vulgaris* powder on mice reproduction. In: Archiva Zootechnica 9, S. 123–134.

Janczyk, Pawel (2006): Evaluation of nutritional value and activity of green microalgae *Chlorella vulgaris* in rats and mice. Untersuchungen zur Verwertung und zur Wirkung von grünen Mikroalgen *Chlorella vulgaris* bei Ratten und Mäusen. FU Berlin, FB Veterinärmedizin. Online verfügbar unter http://www.diss.fu-berlin.de/diss/receive/FUDISS_thesis_000000002523, zuletzt aktualisiert am 19.02.2010, zuletzt geprüft am 13.03.2012.

Jena, Umakanta; Das, K.C; Kastner, J.R (2011a): Effect of operating conditions of thermochemical liquefaction on biocrude production from *Spirulina* platensis. In: Bioresource Technology 102 (10), S. 6221–6229.

Jena, Umakanta; Vaidyanathan, Nisha; Chinnasamy, Senthil; Das, K.C (2011b): Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass. In: Bioresource Technology 102 (3), S. 3380–3387, zuletzt geprüft am 24.04.2012.

Johnson, Michael B.; Wen, Zhiyou (2009): Production of Biodiesel Fuel from the Microalga Schizochytrium limacinum by Direct Transesterification of Algal Biomass. In: Energy Fuels 23 (10), S. 5179–5183, zuletzt geprüft am 26.04.2012.

Kaltschmitt, Martin (Hg.) (2001): Energie aus Biomasse. Grundlagen, Techniken und Verfahren; mit 124 Tabellen. Berlin ;, Heidelberg [u.a.]: Springer.

Kaltschmitt, Martin (Hg.) (2009): Energie aus Biomasse. Grundlagen, Techniken und Verfahren. 2. Aufl. Dordrecht ; Heidelberg, London, New York, NY: Springer.

Kamio, Eiji; Sato, Hisayoshi; Takahashi, Susumu; Noda, Hidehiko; Fukuhara, Chouji; Okamura, Takanari (2008): Liquefaction kinetics of cellulose treated by hot compressed water under variable temperature conditions. In: J Mater Sci 43 (7), S. 2179–2188.

Kampen, I.: Mechanischer Zellaufschluss. Praktikumsskript.

Kampen, I. (2005): Einfluss der Zellaufschlussmethode auf die Expanded Bed Chromatographie. Dissertation. Technische Universität Carolo-Wilhelmina zu Braunschweig, Braunschweig. Fakultät für Maschinenbau.

Karagöz, S.; Bhaskar, T.; Muto, A.; Sakata, Y. (2006): Hydrothermal upgrading of biomass: Effect of KCO concentration and biomass/water ratio on products distribution. In: Bioresource Technology 97 (1), S. 90–98.

Kelly, William J.; Muske, Kenneth R. (2004): Optimal operation of high-pressure homogenization for intracellular product recovery. In: Bioprocess Biosyst Eng 27 (1), S. 25–37.

Kim, Dong-Geol; La, Hyun-Joon; Ahn, Chi-Yong; Park, Yong-Ha; Oh, Hee-Mock (2011): Harvest of Scenedesmus sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. In: Bioresource Technology 102 (3), S. 3163–3168.

Kitazato, H.; Asaoka, S.; Iwamoto, H. (1989): Catalytic cracking of hydrocarbons from microalgae. In: Sekiyu Gakkaishi 32, S. 28–34.

Klass, D. L. (2004): Biomass for renewable energy and fuels. In: Encyclopedia of Energy 1, S. 193–212.

Knežević, D.; van Swaaij, W. P. M.; Kersten, S. R. A. (2009): Hydrothermal Conversion of Biomass: I, Glucose Conversion in Hot Compressed Water. In: Ind. Eng. Chem. Res 48 (10), S. 4731–4743.

Koch, Heinz Josef: Landbauliche Verwertung von HTC-Produkten aus Bioabfal. ifz Göttingen. Online verfügbar unter http://www.ifz-goettingen.de/site/de/318/htc-biokohle.html, zuletzt geprüft am 24.04.2012.

Kong, L.; Li, G.; Zhang, B.; He, W.; Wang, H. (2008): Hydrogen Production from Biomass Wastes by Hydrothermal Gasification. In: Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 30 (13), S. 1166–1178.

Kranich, W. L.; Eralp, A. E. (1984): Conversion of sewage sludge to oil by hydroliquefaction. Project Summary. Hg. v. United States Environmental Protection Agency.

Kruse, Andrea (2011): Behandlung von Biomasse mit überkritischem Wasser. In: Chemie Ingenieur Technik, S. 1381–1389.

Krzack, S. (2008): Grundlagen der Vergasung. In: Jörg Schmalfeld (Hg.): Die Veredlung und Umwandlung von Kohle. Technologien und Projekte 1970 bis 2000 in Deutschland. Hamburg: DGMK.

Kuchling, Thomas; Nitz, Beate; Endisch, Matthias; Roscher, Jan (2010): Die Hydrierung von Pflanzenölen zur Herstellung von ochwertigen Dieselkraftstoffkomonenten. In: Zeitschrift für Freunde und Förderer der TU Bergakademie Freiberg 17, S. 75–79, zuletzt geprüft am 26.04.2012.

Kügler, Ingo; Öhlinger, Andreas; Walter, Birgit (2004): DEZENTRALE KLÄRSCHLAMMVERBRENNUNG. Wien. Online verfügbar unter http://www.umweltbundesamt.at/fileadmin/site/publikationen/BE260.pdf, zuletzt aktualisiert am 10.11.2004, zuletzt geprüft am 26.04.2012.

Kumar, Sandeep; Gupta, Ram B. (2009): Biocrude Production from Switchgrass Using Subcritical Water. In: Energy Fuels 23 (10), S. 5151–5159.

Laxhuber, T. (2009): Trocknungstechnik - Professionelle Trocknung von Gärsubstrat.

Lee, J-Y; Yoo, C.; Jun, S-Y; Ahn, C-Y; Oh, H-M (2010): Comparison of several methods for effective lipid extraction from microalgae. In: Bioresource Technology 101, S. 575–577.

Lee, S. J.; Yoon, B.-D.; Oh, H.-M. (1998): Rapid method for the determination of lipid from the green alga Botryococcus braunii; In: Biotechnology Techniques .

Leibnitz, E.; Könnecke, H.G.; Schröter, M.: Zur Kenntnis der Druckinkohlung von Braunkohlen in Gegenwart von Wasser. IV, zuletzt geprüft am 24.04.2012.

Lewis, Tom; Nichols, Peter D.; McMeekin, Tom A. (2000): Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. In: Journal of Microbiological Methods 43, S. 107–116, zuletzt geprüft am 26.04.2012.

LfL (2009): Einsatz von Gärresten aus der Biogasproduktion als Düngemittel.

Li, Hui; Yuan, Xingzhong; Zeng, Guangming; Huang, Danlian; Huang, Huajun; Tong, Jingyi et al. (2010): The formation of bio-oil from sludge by deoxy-liquefaction in supercritical ethanol. In: Bioresource Technology 101 (8), S. 2860–2866.

Li, Xiufeng; Xu, Han; Wu, Qingyu (2007): Large-scale biodiesel production from microalgaChlorella protothecoides through heterotrophic cultivation in bioreactors. In: Biotechnol. Bioeng. 98 (4), S. 764–771, zuletzt geprüft am 26.04.2012.

Li, Yuesong; Lian, Shuang; Tong, Dongmei; Song, Ruili; Yang, Wenyan; Fan, Yong et al. (2011): One-step production of biodiesel from Nannochloropsis sp. on solid base Mg–Zr catalyst. In: Applied Energy 88 (10), S. 3313–3317, zuletzt geprüft am 26.04.2012.

Liau, Bing-Chung; Shen, Chun-Ting; Liang, Fong-Ping; Hong, Siang-En; Hsu, Shih-Lan; Jong, Ting-Ting; Chang, Chieh-Ming J. (2010): Supercritical fluids extraction and anti-solvent purification of carotenoids from microalgae and associated bioactivity. In: The Journal of Supercritical Fluids 55 (1), S. 169–175.

Libra, Judy A.; Ro, Kyoung S.; Kammann, Claudia; Funke, Axel; Berge, Nicole D.; Neubauer, York et al. (2011): Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. In: Biofuels 2 (1), S. 71–106.

Lindfors, Lars (2010): Überblick des NSE Biofuels - Prozesses zur Herstellung von Synthesegasen aus Reststoffen der Forstwirtschaft. In: A. Sonnleitner (Hg.): 4. BtL-Kongress. Bericht über den 4. internationalen BtL-Kongress der Fachagentur Nachwachsende Rohstoffe am 1. und 2. Dezember 2010 in Berlin. BIOENERGY 2020+ GmbH.

Liu, Yang (2010): Green algae as a substrate for biogas production - cultivation and biogas potentials. In: Journal of Biotechnology 150 (1).

Lognone, V. (2003): Algen in der Tierernährung (3), S. 1–4.

Lorenz, R.Todd; Cysewski, Gerald R. (2000): Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. In: Trends in Biotechnology 18 (4), S. 160–167.

Lowry, O. H.; Rsebrough, N. J.; Far, A. L.; Randall, R. J.: Protein measurement with the folin phenol reagent. 1951, The Journal of Biological Chemistry, 193, 265-275

Lu, X.; Yuan, L.; Liu, X.: Kinetics of Fructose Decomposition Catalyzed by Organic Acids in High Temperature Liquid Water.

Lundquist, T.J.; Woertz, I.C.; Quinn, N.T.W.; Bennemann, J.R. (2010): A realistic technology and engineering assesment of algae biofuel production. Berkeley.

Lynam, Joan G.; Coronella, Charles J.; Yan, Wei; Reza, Mohammad T.; Vasquez, Victor R. (2011): Acetic acid and lithium chloride effects on hydrothermal carbonization of lignocellulosic biomass. In: Bioresource Technology 102 (10), S. 6192–6199, zuletzt geprüft am 24.04.2012.

Mann, G.: Nutzungsoptionen phototropher Mikroalgen im Bereich der Biogaserzeugung.

Mann, G.; Schlegel, M.; Schumann, R.; Sakalauskas, A. (2009): Biogas-conditioning with microalgae. In: Agronomy Research 7 (1), S. 33–38.

Mata, Teresa M.; Martins, António A.; Caetano, Nidia S. (2010): Microalgae for biodiesel production and other applications: A review. In: Renewable and Sustainable Energy Reviews 14 (1), S. 217–232.

Matsui, T.; Hishihara, A.; Ohtsuki, M.; Ikenaga, N.; Suzuki, T. (1997): Liquefaction of micro-algae with iron catalyst Liquefaction iron catalyst. In: Fuel 76 (11), S. 1043–1048, zuletzt geprüft am 05.05.2012.

McKendry, P. (2002): Energy production from biomass (part 1): overview of biomass. In: Bioresource Technology 83, S. 37–46.

Medina, A. Robles; Grima, E. Molina; Giménez, A. Giménez; González, M.J. Ibáñez (1998): DOWNSTREAM PROCESSING OF ALGAL POLYUNSATURATED FATTY ACIDS. In: Biotechnology Advances 16 (3), S. 517–580.

Meier, D. (2007): Verfahren zur Biomasseverflüssigung - Möglichkeiten und Grenzen. BFH Insitut für Holzchemie und chemische Technologie des Holzes, Hamburg. Magdeburg, 29.11.2007.

Meier, R.L. (1955): Biological cycles in the transformation of solar energy into useful fuels. Madison (WI): University of Wisconsin Press (Solar energy research), S. 179–183.

Mendes, R. (2003): Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. In: Inorganica Chimica Acta 356, S. 328–334.

Mendes, Rui L.; Fernandes, Helena L.; Coelho, JoséP; Reis, Elsa C.; Cabral, Joaquim M.S; Novais, Júlio M.; Palavra, António F. (1995): Supercritical CO_2 extraction of carotenoids and other lipids from *Chlorella vulgaris*. In: Food Chemistry 53 (1), S. 99–103.

Mercer, Paula; Armenta, Roberto E. (2011): Developments in oil extraction from microalgae. In: Eur. J. Lipid Sci. Technol 113 (5), S. 539–547.

Miao, Xiaoling; Wu, Qingyu (2006): Biodiesel production from heterotrophic microalgal oil. In: Bioresource Technology 97 (6), S. 841–846, zuletzt geprüft am 26.04.2012.

Miao, Xiaoling; Wu, Qingyu; Yang, Changyan (2004): Fast pyrolysis of microalgae to produce renewable fuels. In: Journal of Analytical and Applied Pyrolysis 71 (2), S. 855–863.

Middelberg, Anton P.J (1995): Process-scale disruption of microorganisms. In: Biotechnology Advances 13 (3), S. 491–551.

Milne, Thomas A.; Evans, Robert J.; Nagle, Nicholas (1990): Catalytic Conversion of Microalgae and Vegetable Oils to Premium Gasoline, with Shape-Selective Zeolites. In: Biomass 21, S. 219–232, zuletzt geprüft am 26.04.2012.

Minarick, Mitchell; Zhang, Yuanhui; Schideman, Lance; Wang, Zhichao; Yu, Guo; Funk, Ted; Barker, Doug (2011): Product and Economic Analysis of Direct Liquefaction of Swine Manure. In: Bioenerg. Res 4 (4), S. 324–333.

mineralit GmbH (2011): Produktinformation mineralit- Universal- Trommeltrockner.

Minowa, T.; u.a. (1995): Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction. In: Fuel 74 (12), S. 1735–1738.

Minowa, Tomoaki; Zhen, Fang; Ogi, Tomoko (1998): Cellulose decomposition in hot-compressed water with alkali or nickel catalyst. In: The Journal of Supercritical Fluids 13 (1-3), S. 253–259.

Molina Grima, E.; Acien Fernandes, F.G.; Robles Medina, A. (2004): Downstreaming processing of cell mass and products. In: Amos Richmond (Hg.): Handbook of microalgal culture. Biotechnology and applied phycology. Oxford, OX, UK ;, Ames, Iowa, USA: Blackwell Science.

Molina Grima, E.; Belarbi, E.-H; Acien Fernandez, F. G.; Robles Medina, A.; Chisti, Yusuf (2003): Recovery of microalgal biomass and metabolites: process options and economics. In: Biotechnology

Advances 20 (7-8), S. 491–515. Online verfügbar unter http://www.sciencedirect.com/science/article/B6T4X-47C3Y5B-1/2/4e6eaae6aa41e9b29df5ba5db43748e2.

Molina Grima, E.; Robles Medina, A.; Giménez Giménez, A.; Sánchez Perez, J. A.; Garcia Camacho, F.; Garcia Sánchez, J. L. (1994): Comparison between extraction of lipids and fatty acids from microalgal biomass. In: JAOCS 71 (9), S. 956–959.

Muller-Feuga, A. (2004): Microalgae for aquaculture: the current global situation and future trends. In: Amos Richmond (Hg.): Handbook of microalgal culture. Biotechnology and applied phycology. Oxford, OX, UK ;, Ames, Iowa, USA: Blackwell Science, S. 352–364.

Mursito, Anggoro Tri; Hirajima, Tsuyoshi; Sasaki, Keiko (2010): Upgrading and dewatering of raw tropical peat by hydrothermal treatment. In: Fuel 89 (3), S. 635–641.

Mussgnug, J. H.; Klassen, V.; Schlüter, A.; Kruse, O. (2010): Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. In: Journal of Biotechnology 150 (1), S. 51–56. Online verfügbar unter http://www.sciencedirect.com/science/article/pii/S016816561000369X.

Nagle, N.; Lemke, P. (1990): Production of methyl ester fuel from mircoalgae; In: Applied biochemistry and biotechnology.

NEUERO Farm- und Fördertechnik GmbH (2011): Datenblatt BAndtrockner. Online verfügbar unter http://www.neuero-farm.de/uploads/tx_products4neuero/nbt_datenblatt.pdf, zuletzt aktualisiert am 09.11.2011, zuletzt geprüft am 26.04.2012.

Ni, Meng; Leung, Dennis Y.C; Leung, Michael K.H; Sumathy, K. (2006): An overview of hydrogen production from biomass. In: Fuel Processing Technology 87 (5), S. 461–472.

Oh, Hee-Mock; Lee, Seog June; Park, Myung-Hwan; Kim, Hee-Sik; Kim, Hyoung-Chin; Yoon, Jung-Hoon et al. (2001): Harvesting of *Chlorella vulgaris* using a bioflocculant from Paenibacillus sp. AM49. In: Biotechnology Letters 23 (15), S. 1229–1234.

Oilgae (2011): Algal Oil Extraction. Online verfügbar unter http://www.oilgae.com/algae/oil/extract/extract.html, zuletzt aktualisiert am 22.09.2011.

Okuda, K.; Man, X.; Umetsu, M.; Takami, S.; Adschiri, T.: Complete disassembly of lignin in phenol-water solvent. In: Hydrothermal Reactions and Techniques.

OriginOil, Inc (Hg.) (2012): Single-Step Extraction. Online verfügbar unter http://www.originoil.com/technology/single-step-extraction.html, zuletzt geprüft am 02.02.2012.

Pasquet, Virginie; Chérouvrier, Jean-René; Farhat, Firas; Thiéry, Valérie; Piot, Jean-Marie; Bérard, Jean-Baptiste et al. (2011): Study on the microalgal pigments extraction process: Performance of microwave assisted extraction. In: Process Biochemistry 46 (1), S. 59–67.

Pernet, F.; Tremblay, R. (2003): Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters. In: Lipids, 38, 2003, S. 1191–5. 38, S. 1191–1195.

Perretti, G.; Bravi, E.; Montanari, L.; Fantozzi, P.: Extraction of PUFAs rich oils from algae with supercritical carbon dioxide.

Peterson, Andrew A.; Vogel, Frédéric; Lachance, Russell P.; Fröling, Morgan; Antal, Jr.Michael J.; Tester, Jefferson W. (2008): Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies. In: Energy Environ. Sci 1 (1), S. 32.

Prabakaran, P.; Ravindran, A. D. (2011): A comparative study on effective cell disruption methods for lipid extraction from microalgae. In: Letters in Applied Microbiology 53, S. 150–154.

Pulz, Otto (2009): Mikroalgen als Energieträger der Zukunft. In: Thomas Bley (Hg.): Biotechnologische Energieumwandlung. Gegenwärtige Situation, Chancen und künftiger Forschungsbedarf. Berlin ;, Heidelberg: Springer, S. 87–96.

Qureshi, M. A.; Garlich, J. D.; Kidd, M. T. (1996): Dietary *Spirulina* platensis enhances humoral and cell-mediated immune functions in chickens. In: Immunopharmacology and immunotoxicology 18 (3), S. 465–476.

Raach-Moujahed, A.; Hassani, S.; Zairi, S.; Bouallegue, M.; Darej, C.; Haddad, B.; Damergi, C. (2011): Effect of dehydrated *Spirulina* platensis on performances and meat quality of broilers. In: ROVAS 1 (8), S. 505–509.

Ramke, Hans-Günter; et al (2010): Machbarkeitsstudie zur Energiegewinnung aus organischen Siedlungsabfällen durch Hydrothermale Carbonisierung, 2010, zuletzt geprüft am 24.04.2012.

Randall, L. G. (1982): The Present Status of Dense (Supercritical) Gas Extraction and Dense Gas Chromatography: Impetus for DGC/MS Development. In: Separation Science and Technology 17 (1), S. 1–118.

Ras, Monique; Lardon, Laurent; Bruno, Sialve; Bernet, Nicolas; Steyer, Jean-Philippe (2011): Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. In: Bioresource Technology 102 (1), S. 200–206. Online verfügbar unter http://www.sciencedirect.com/science/article/pii/S096085241001151X.

Reitan, Kjell Inge; Rainuzzo, Jose R.; Øie, Gunvor; Olsen, Yngvar (1997): A review of the nutritional effects of algae in marine fish larvae. In: Aquaculture 155 (1-4), S. 207–221.

Roberts, M.; Williams, J.; Halberstadt, P.; Sanders, D.; Adams, T. (2004): Animal Waste to Marketable Products. Hg. v. Natural Gas Technologies Conference.

Robles Medina, A.; Giménez Giménez, A.; Garcia Camacho, F.; Sánchez Perez, J. A.; Molina Grima, E.; Contreras Gómez, A. (1995): Concentration and purification of stearidonic, eicosapentaenoic, and docosahexaenoic acids from cod liver oil and the marine microalga Isochrysis galbana. In: JAOCS 72 (5), S. 576–583.

Rosello Sastre, R.; Posten, C. (2010): Die vielfältige Anwendung von Mikroalgen als nachwachsende Rohstoffe. In: Chemie Ingenieur Technik 82 (11), S. 1925–1939.

Ross, A.B; Biller, P.; Kubacki, M.L; Li, H.; Lea-Langton, A.; Jones, J.M (2010): Hydrothermal processing of microalgae using alkali and organic acids. In: Fuel 89 (9), S. 2234–2243.

Russell, J.; Miller, R. K.; Molton, P. M. (1983): Formation of aromatic compounds from condensation reactions of cellulose degradation products. In: Biomass 3 (1), S. 43–57.

Salecker, Hano (2009): Energiegewinnung aus Mikroalgen - Konzepte und deren Bewertung -.

Salerno, Michael; Nurdogan, Yakup; Lundquist, Tryg J. (2009): Biogas Production from Algae Biomass Harvested at Wastewater Treatment Ponds. An ASABE Conference Presentation. Bioenergy Engineering Conference, 11.10.2009. Online verfügbar unter http://digitalcommons.calpoly.edu/cgi/viewcontent.cgi?article=1181&context=cenv_fac.

Salim, Sina; Bosma, Rouke; Vermuë, Marian H.; Wijffels, René H. (2011): Harvesting of microalgae by bio-flocculation. In: J Appl Phycol 23 (5), S. 849–855.

Samson, Réjean; Leduy, Anh (1982): Biogas production from anaerobic digestion of *Spirulina* maxima algal biomass. In: Biotechnology and bioengineering 24 (8), S. 1919–1924.

Sander, K.; Murthy, G. S. (2009): Enzymatic degradation of microalgal cell walls. ASABE, 2009.

Sargent, J.R; McEvoy, L.A; Bell, J.G (1997): Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. In: Aquaculture 155 (1-4), S. 117–127.

Sasaki, Mitsuru; Fang, Zhen; Fukushima, Yoshiko; Adschiri, Tadafumi; Arai, Kunio (2000): Dissolution and Hydrolysis of Cellulose in Subcritical and Supercritical Water. In: Ind. Eng. Chem. Res 39 (8), S. 2883–2890.

Sawayama, S.; Minowa, T.; Yokoyama, S-Y (1999): Possibility of renewable energy production and CO2 mitigation by thermochemical liquefaction of microalgae. In: Biomass and Bioenergy 17, S. 33–39.

Saxena, P. N.; Ahmad, M. R.; Shyam, R.; Amla, D. V. (1983): Cultivation of *Spirulina* in sewage for poultry feed. In: Experientia 39, S. 1077–1083.

Sazdanoff, Nick (2006): Modeling and Simulation of the Algae to Biodiesel Fuel Cycle. Honors Undergraduate Thesis.

Schamphelaire, Liesje de; Verstraete, Willy (2009): Revival of the Biological Sunlight-to-Biogas Energy Conversion System. In: Biotechnology & Bioengineering 103, 2009 (2), S. 296–304.

Schmack, Doris; Schneider, Gregor; Nusko, Robert (2008): Effizienzsteigerung der Biogasnutzung durch Solarenergie: Schmack Biogas AG.

Schnecker, John (2011): World Water Works AHTO System for Algae Harvesting :Algae Industry Magazine. Online verfügbar unter http://www.algaeindustrymagazine.com/world-water-works-ahto-system-for-algae-harvesting/.

Serp, Philippe; Figueiredo, José Luís (2009): Carbon materials for catalysis. Hoboken, N.J: John Wiley & Sons.

Sheehan, John; Dunahay, Terri; Benemann, John; Roessler, Paul (1998): A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae.

Shen, Y.; Pei, Z.; Yuan, W.; Mao, E. (2009): Effect of nitrogen and extraction method on algae lipid yield. In: Int. J. Agric. & Biol. Eng. 2 (1), S. 51–57.

Sim, T.-S; Goh, A.; Becker, E.W (1988): Comparison of Centrifugation, Dissolved Air Flotation and Drum Filtration Techniques for Harvesting Sewage-grown Algae. In: Biomass 16, S. 51–66.

Simon, R. D. (1974): The use of an ultrasonic bath to disrupt cells suspended in volumes of less than 100 µliters. In: Analytical Biochemistry 60 (1), S. 51–58.

SMITH, P.; KROHN, R.; HERMANSON, G.; MALLIA, A.; GARTNER, F.; PROVENZANO, M. et al. (1985): Measurement of protein using bicinchoninic acid. In: Analytical Biochemistry 150 (1), S. 76–85.

Sommer, T.R; D'Souza, F.M.L; Morrissy, N.M (1992): Pigmentation of adult rainbow trout, Oncorhynchus mykiss, using the green alga *Haematococcus* pluvialis. In: Aquaculture 106 (1), S. 63–74.

Sommer, T.R; Potts, W.T; Morrissy, N.M (1991): Utilization of microalgal astaxanthin by rainbow trout (Oncorhynchus mykiss). In: Aquaculture 94 (1), S. 79–88.

Spolaore, Pauline; Joannis-Cassan, Claire; Duran, Elie; Isambert, Arsène (2006): Commercial applications of microalgae. In: Journal of Bioscience and Bioengineering 101 (2), S. 87–96.

Stahl, E.; Quirin, K. W.; Gerard, D. (1987): Verdichtete Gase zur Extraktion und Raffination. Berlin ;, New York: Springer.

Stark, Oliver K.; Damann, Roland; Stein, Andreas (2008): Abtrennung von Biomasse mittels Mikroflotation. In: KA 55 (5).

Steiger, Wolfgang (2012): Algenernte mit verbesserter Energieeffizienz. In: 5. Bundesalgenstammtisch. Algenbiotechnologie für Ernährung und Bioökonomie. München-Pullach, 26.-27.03.2012. DECHEMA, S. 12.

Stephens, Evan; Ross, Ian L.; King, Zachary; Mussgnug, Jan H.; Kruse, Olaf; Posten, Clemens et al. (2010): An economic and technical evaluation of microalgal biofuels. In: Nat Biotechnol 28 (2), S. 126–128.

Stuhlberger, Johannes (2012): Biotreibstoffe: aus der Forschung in die Umsetzung. In: 5. Bundesalgenstammtisch. Algenbiotechnologie für Ernährung und Bioökonomie. München-Pullach, 26.-27.03.2012. DECHEMA.

Suckow, M.; Szepietowska-Nelz, A.; Petrick, I. (2011): Methods for establishing of fatty acid methyl ester, 23.06.2011, zuletzt geprüft am 26.04.2012.

Sugano, Motoyuki; Takagi, Hirokazu; Hirano, Katsumi; Mashimo, Kiyoshi (2008): Hydrothermal liquefaction of plantation biomass with two kinds of wastewater from paper industry. In: J Mater Sci 43 (7), S. 2476–2486.

T. Suzuki T. Matsui C. Ueda N. Ikenaga (2006): LIQUEFACTION OF MICRO ALGAE WITH IRON CATALYST. Online verfügbar unter http://www.anl.gov/PCS/acsfuel/preprint%20archive/Files/42_1_SAN%20FRANCISCO_04-97_0074.pdf.

Tchorbanov, B.; Bozhkova, M. (1988): Enzymatic hydrolysis of cell proteins in green algae Chlorella and Scenedesmus after extraction with organic solvents. In: Enzyme Microb. Technol 10, S. 233–238.

Tempest, R. K. (1950): The Supersonic Flow of Compressible Fluid through Axially Symmetric Tubes of Uniform and Varying Section. In: Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences 200 (1063), S. 511–523.

Titirici, Maria-Magdalena; Thomas, Arne; Antonietti, Markus (2007): Back in the black: hydrothermal carbonization of plant material as an efficient chemical process to treat the CO_2 problem? In: New J. Chem. 31 (6), S. 787, zuletzt geprüft am 24.04.2012.

Toor, Saqib Sohail; Rosendahl, Lasse; Rudolf, Andreas (2011): Hydrothermal liquefaction of biomass: A review of subcritical water technologies. In: Energy 36 (5), S. 2328–2342.

Toyomizu, M. (2001): Effects of dietary *Spirulina* on meat colour in muscle of broiler chickens. In: British poultry science 42 (2), S. 192–202.

Tran, N.H; Bartlett, J.R; Kannangara, G.S.K; Milev, A.S; Volk, H.; Wilson, M.A (2010): Catalytic upgrading of biorefinery oil from micro-algae. In: Fuel 89 (2), S. 265–274.

Tsukahara, K.; Sawayama, S. (2005): Liquid fuel production using microalgae. In: Journal of the Japan Petroleum Institute 48 (5), S. 251–259.

TU Dresden - AG Abwasserbehandlung (2005): Abwasserbehandlung - Entwicklung eines Verfahrens zur biologischen Trennung von Biogas aus der anaeroben Klärschlammbehandlung und aus nachwachsenden Rohstoffen am Beispiel von Algen und Methan - Teil 2. In: Technische Universität Dresden - Professur Siedlungswasserwirtschaft. Online verfügbar unter http://tu-dresden.de/die_tu_dresden/fakultaeten/fakultaet_forst_geo_und_hydrowissenschaften/fachrichtung_wasserwesen/isiw/sww/abwasserbehandlung/afoi/Algen2005.

Ugwu, C.U; Aoyagi, H.; Uchiyama, H. (2008): Photobioreactors for mass cultivation of algae. In: Bioresource Technology 99 (10), S. 4021–4028.

Valcárcel, M.; Tena, M. T. (1997): Applications of supercritical fluid extraction in food analysis. In: Fresenius J. Anal. Chem. 358, S. 561–573.

Valderrama, José O.; Perrut, Michel; Majewski, Wieslaw (2003): Extraction of Astaxantine and Phycocyanine from Microalgae with Supercritical Carbon Dioxide. In: J. Chem. Eng. Data 48 (4), S. 827–830.

van Harmelen, Toon; Oonk, Hans (2006): Microalgae biofixation process: TNO.

Vardon, Derek R.; Sharma, B.K; Scott, John; Yu, Guo; Wang, Zhichao; Schideman, Lance et al. (2011): Chemical properties of biocrude oil from the hydrothermal liquefaction of *Spirulina* algae, swine manure, and digested anaerobic sludge. In: Bioresource Technology 102 (17), S. 8295–8303.

VDI- Richtlinie 4630: Vergärung organischer Stoffe - Substratcharakterisierung, Probennahme, Stoffdatenerhebung, Gärversuch.

Venkat Reddy, Chinta Reddy; Oshel, Reed; Verkade, John G. (2006): Room-Temperature Conversion of Soybean Oil and Poultry Fat to Biodiesel Catalyzed by Nanocrystalline Calcium Oxides. In: Energy Fuels 20 (3), S. 1310–1314, zuletzt geprüft am 26.04.2012.

Vito, F. de (2006): Application of Pulsed Electric Field (PEF) Techniques in Food Processing. Dissertation, Salerno.

Wagner, Wolfgang; Kruse, Alfred (1998): Properties of water and steam. The industrial standard IAPWS-IF97 for the thermodynamic properties and supplementary equations for other properties : tables based on these equations = Zustandsgrößen von Wasser und Wasserdampf : der Industrie-Standard IAPWS-IF97 für die thermodynamischen Zustandsgrößen und ergänzende Gleichungen für andere Eigenschaften : Tafeln auf der Grundlage dieser Gleichungen. Begleit CD-ROM. Berlin [etc.]: Springer.

Wauquier, Jean-Pierre; Trambouze, Pierre; Favennec, Jean-Pierre (1995): Petroleum refining. Paris: Éditions Technip.

Weiland, Peter; Gemmeke, Burga; Rieger, Christa; Plogsties, Vincent; Kissel, Rainer; Bachmaier, Hans et al. (2010): Biogas- Messprogramm II. Gülzow: Fachagentur Nachwachsende Rohstoffe e. V. (FNR).

Weißbach, F. (2009): Die Bewertung von nachwachsenden Rohstoffen für die Biogasgewinnung. Teil 1: Das Gasbildungspotential der fermentierbaren Nährstoffe. In: Pflanzenbauwissenschaften 13, S. 72–85.

Weissman, J. C.; Goebel, R. P. (1987): Design and Analysis of Microalgal Open Pond Systems for the Purpose of Producing Fuels. Golden, Colorado.

WHITE, D. H.; WOLF*, D. (1995): DIRECT BIOMASS LIQUEFACTION BY AN EXTRUDER-FEEDER SYSTEM. In: Chemical Engineering Communications 135 (1), S. 1–19.

Wildschut, Jelle; Mahfud, Farchad H.; Venderbosch, Robbie H.; Heeres, Hero J. (2009): Hydrotreatment of Fast Pyrolysis Oil Using Heterogeneous Noble-Metal Catalysts. In: Ind. Eng. Chem. Res. 48 (23), S. 10324–10334, zuletzt geprüft am 26.04.2012.

Wiltshire, K.H.; Boersma, M.; Moller, A.; Buhtz, H. (2000): Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae). In: Aquatic Ecology 34, S. 119–126.

Wirth, B.; Mumme, J. (2011): Anaerobic Treatment of HTC Waste Water. In:

Wu, Libin; Guo, Shipeng; Wang, Chao; Yang, Zhengyu (2008): Direct Deoxy-Liquefaction of Poplar Leaves to Biopetroleum with Two Kinds of Catalysts. In: Ind. Eng. Chem. Res 47 (23), S. 9248–9255.

Xiang, Qian; Lee, Yong Y.; Torget, Robert W. (2004): Kinetics of Glucose Decomposition During Dilute-Acid Hydrolysis of Lignocellulosic Biomass. In: Applied Biochemestry and Biotechnology, 113-116, S. 1127–1138.

Xu, Chunbao; Lad, Nimisha (2008): Production of Heavy Oils with High Caloric Values by Direct Liquefaction of Woody Biomass in Sub/Near-critical Water. In: Energy Fuels 22 (1), S. 635–642.

Xu, Ling; Weathers, Pamela J.; Xiong, Xue-Rong; Liu, Chun-Zhao (2009): Microalgal bioreactors: Challenges and opportunities. In: Eng. Life Sci. 9 (3), S. 178–189.

Yamaguchi, K. (1997): Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. In: Journal of Applied Phycology 8, S. 487–502.

Yang, Chao; Jia, Lishan; Chen, Changping; Liu, Guangfa; Fang, Weiping (2011): Bio-oil from hydro-liquefaction of *Dunaliella salina* over Ni/REHY catalyst. In: Bioresource Technology 102 (6), S. 4580–4584.

Yen, Hong-Wei; Brune, David E. (2007): Anaerobic co-digestion of algal sludge and waste paper to produce methane. In: Bioresource Technology 98 (1), S. 130–134. Online verfügbar unter http://www.sciencedirect.com/science/article/pii/S0960852405005365.

Yin, Sudong; Dolan, Ryan; Harris, Matt; Tan, Zhongchao (2010): Subcritical hydrothermal liquefaction of cattle manure to bio-oil: Effects of conversion parameters on bio-oil yield and characterization of bio-oil. In: Bioresource Technology 101 (10), S. 3657–3664.

Yu, G.; Zhang, Y.; Schideman, L.; Funk, T. L.; Wang, Z. (2011): Hydrothermal liquefaction of low lipid content microalgae into bio-crude oil. In: ASABE 54 (1), S. 239–246.

Zhang, Linghong; Champagne, Pascale; Xu, Chunbao (2011): Bio-crude production from secondary pulp/paper-mill sludge and waste newspaper via co-liquefaction in hot-compressed water. In: Energy 36 (4), S. 2142–2150.

Zheng, H.; Yin, J.; Gao, Z.; Huang, H.; Ji, X.; Dou, C. (2011): Disruption of Chlorella vulagris cells for the release of biodiesel-producting lipids - a comparison of grinding, ultrasonication, bead milling, enzymatic lysis and microwaves. In: Appl Microbiol Biotechnol 164, S. 1215–1224.

Zhou, Dong; Zhang, Liang; Zhang, Shicheng; Fu, Hongbo; Chen, Jianmin (2010): Hydrothermal Liquefaction of Macroalgae Enteromorpha prolifera to Bio-oil. In: Energy Fuels 24 (7), S. 4054–4061.