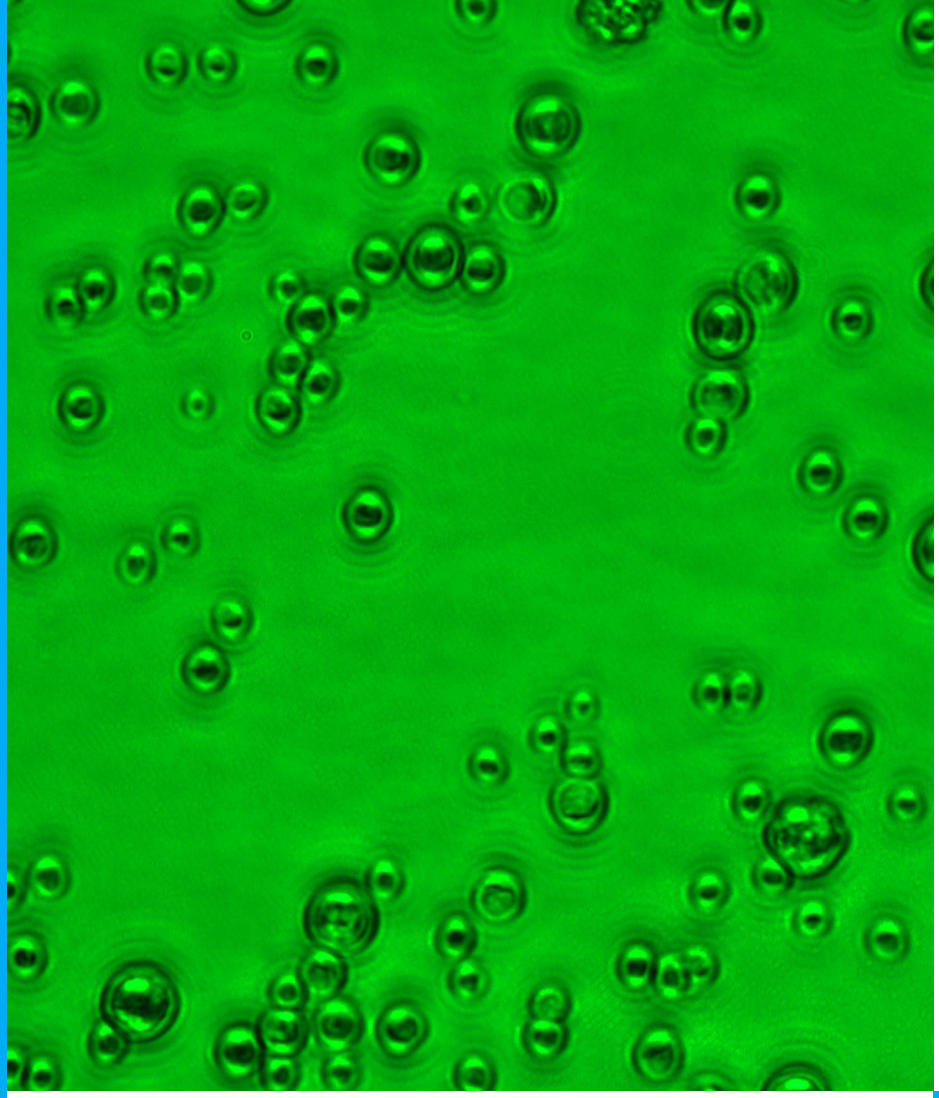

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Sustainable algal biomass products by cultivation in waste water flows

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Mona Arnold (Ed.)

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Sustainable algal biomass products by cultivation in waste water flows

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Abstract

Algae are predicted to play an important role in tomorrow's bioeconomy. The technical goal of the project was to develop enhanced algal cultivation processes utilising waste flows and to increase the overall material and energy efficiency of algal processing for biodiesel and biogas production.

The project produced new knowledge on the boundary conditions for cost efficient algal cultivation and productivity. The use of algae as a tertiary treatment of municipal waste water, such as the utilisation of waste water flows from biowaste handling, was assessed with positive results. Process concepts based both on CO_2 uptake and (waste) organic carbon were assessed. In a Nordic climate the utilisation of spill heat is requisite and with restricted available daylight in the winter time, an alga's ability to shift from autotrophic to heterotrophic growth provides a potential strategy for algal cultivation in Nordic circumstances.

The fractionation of algal residuals for biopolymers is a new research area with potential long term impact in the bioeconomy sector.

Cost efficient Integrated production concepts still need to be developed, as premises to successful business models.

It is apparent that an economically viable algae-to-biodiesel commercialization will initially depend on government subsidies and the future price of oil, in addition to optimized biomass yields. However, algae to biofuels is globally a topical sector with a high interest from several stakeholders. The markets are likewise global. From the biofuel point of view, air traffic is particularly interesting, as this sector will probably need to rely on liquid fuel still during the next decade.

Keywords microalgae, biofuels, waste, biorefinery

Preface

This publication gives the overview of the results from the Tekes Biorefine programme project Algae from Waste for Combined Biodiesel and Biogas Production – ALDIGA coordinated by VTT and executed 02/2010–12/2012.

The project was carried out as an extensive collaboration between five Finnish research organisation and eight international laboratories. The research partners in addition to VTT were: University of Helsinki (Prof. M. Romantschuk), Finnish Environment Institute (Senior Scientist Dr K. Spilling), HAMK University of Applied Sciences (Principal lecturer Dr M. Kymäläinen), LAMK Lahti University of Applied Sciences (Dean Dr S. Kostia).

The international co-operators were Waterloo University (CA), Aalborg University (DK), DTU (DK), Lawrence Berkeley Natl. Lab (US), San Diego Center for Algae Biotechnology, UCSD (US), Ludwig Maximilian University (DE), University of London, Imperial College (UK) and Hamburg University of Applied Sciences (DE).

The steering group consisted of representatives for the companies participating in the project: Neste Oil Oyj (chair), Kemira Oyj, Gasum Oy, Ekokem Oy, Wärtsilä Finland Oy, Bioste Oy, Biovakka Suomi Oy, PHJ Oy, Kujalan komposti Oy, Clewer Ltd, Sybimar Oy, Envor Group Oy, LHJ Group, the main funding organisation, Tekes, and the responsible leader of the coordinating organisation, professor Merja Penttilä at VTT.

The following chapters give an overview of the results obtained in the project. Due to the large number of research question and tasks involved in the project the overviews are focussed on the results, whereas detailed information on research methods can be found in publications i.e. articles and theses, that have been produced by the participating organisations during and after the project.

Espoo 12.12.2013

Mona Arnold, VTT

Project manager

Contents

Terms and abbreviations

AD	anaerobic digestion
AMPTS	automatic methane potential test system
autotrophy	being capable of synthesizing its own food from inorganic substances, using light or chemical energy (e.g. CO_2)
axenic	free of other organisms
BOD_7	Biological Oxygen Demand after 7 days, i.e. the quantity of oxygen consumed over 7 days
cetane number	cetane number or CN is a measure of a fuel's ignition delay, the time period between the start of injection and the first identifiable pressure increase during combustion of the fuel
CSTR	completely stirred tank reactor
FTIR-PAS	Fourier transform infrared photoacoustic spectroscopy
FVW	fruit and vegetable waste
HRAP	high rate algal pond
mixotrophy	a mixotroph is an organism that can use a mix of different sources of energy and carbon. In mixotrophic culture both CO_2 and organic carbons are supplied and both are assimilated. Both respiratory and photosynthetic metabolism occur in the same population.
OLR	organic loading rate
PBR	photobioreactor
qPCR	quantitative real time polymerase chain reaction. A laboratory technique based on the polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule.
reject water	liquid generated in dewatering of digested sewage sludge

SCE	supercritical fluid extraction
SEM	scanning electron microscopy
tertiary wastewater treatment	advanced cleaning of wastewater during which nutrients (such as phosphorous and nitrogen) and most suspended solids are removed
VFA	volatile fatty acid
VS	volatile solids

1. Introduction

Mona Arnold, VTT

By the year 2035, global energy demand is predicted to grow by 35% from 2010 (International Energy Agency 2012). Biobased fuels are considered as sustainable alternatives to fossil fuels but in order to be able to compete against more traditional fuels, these alternative fuels must be technically acceptable, economically competitive, environmentally acceptable and easily available.

The importance of algae has increased with the search for renewable energy sources. Algae can thrive and produce valuable products such as lipids (oils), carbohydrates, proteins, and various feedstocks that can be converted into other biofuels and useful materials. The oil produced is suitable for biodiesel with very small modifications. The fatty acid composition is also suitable for production of functional foods and feed, and as raw material for bioplastics and the biochemical industry.

Algae-based biofuel production has a number of potential advantages:

- Biofuels and byproducts can be synthesized from a large variety of algae.
- Algae have a rapid growth rate, in comparison with plants.
- Algae can be cultivated in brackish coastal water and seawater.
- Some land areas that are unsuitable for agricultural can be used to cultivate algae.
- Algae can take up high concentrations of nitrogen, silicon, phosphate, and sulfate nutrients from municipal, agricultural or industrial waste streams.
- Algae can sequester carbon dioxide (CO_2) from industrial sources.

1.1 Cultivation and processing

The overall process of producing high-quality biofuels from microalgae includes two major steps: cultivation of the algal biomass and processing the biomass into a final product (Fig. 1).

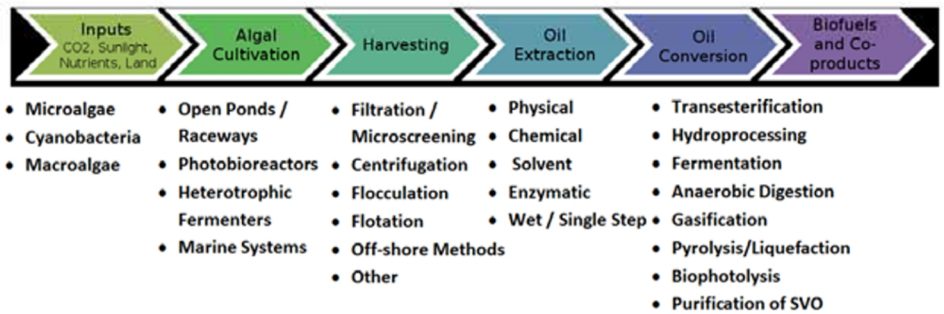

Figure 1. Simplified Schematic Diagram of Major Stages Involved in Producing Algal-derived Liquid Biofuel (IEA 2011).

1.2 Microalgal cultivation

As photosynthetic organisms, microalgae require light and CO_2, appropriate temperature (generally 15–35°C) and pH (1.5–10, but generally 6–7.5), and a supply of nutrients (Havlik et al. 2013). Some algae may also consume organic carbon when it is available.

Light and CO_2 are the two most important growth factors for phototrophic microalgae (Havlik et al. 2013). Light provides the energy for microalgal biomass production, whereas CO_2 provides the carbon for that biomass. Provision of light is largely dependent on the reactor design, which may be an open system, such as a pond, or a closed photobioreactor (PBR). In either case, light may be obtained directly from sunlight, but PBRs may also be fed with artificial light. CO_2 is generally provided by sparging air or CO_2 enriched gas to the production system, but may also be added as bicarbonate solution.

Nitrogen and phosphorous, plus other nutrients are essential for growth, but their depletion can result in accumulation of storage molecules such as lipids, starch, or pigments. When these are the desired end-product (as in lipids for biodiesel), nutrient levels must be carefully monitored.

When microalgae utilise organic carbon sources for growth, light is no longer limiting and higher biomass concentrations and higher growth rates can be achieved than by phototrophic growth. Growth on organic carbon sources may be completely heterotrophic, but with some species may also be mixotrophic if light is supplied. Biomass production under mixotrophic conditions is generally similar to that in heterotrophic conditions, or slightly higher (Wang et al. 2013). Mixotrophy has been shown to increase the lipid content and yield of lipid on organic substrate in some species (Wan et al. 2011, Wang et al. 2013). Indeed cultivation processes based on both phototrophic, heterotrophic, and mixotrophic growth are being commercialized (Eckerberry 2012).

1.3 Sustainability and utilisation of waste streams

Culturing and processing of algae still faces several technical problems that need to be overcome in order to increase the sustainability of the overall process. For example, reviews of the technological challenges and life-cycle assessments of algal biofuel production have highlighted the supply of nutrients (especially nitrogen and phosphorous) as important cost factors. Furthermore, even if the microalgae are grown on non-arable land, if their cultivation for biofuel production requires fertilizers to provide N and P their cultivation will be in direct competition with food growers for these fertilizers (Peccia et al. 2013).

The capture of carbon dioxide during autotrophic algal growth provides an opportunity to establish "carbon neutral" energy production, whereby CO_2 released from use of fossil fuel during the cultivation and subsequent burning of the algal fuel would be linked to carbon capture during cultivation. However, to be truly carbon neutral, algal fuels would have to be produced without external inputs of energy. This has not been realized, so an important first sustainability aim for algal biofuel production is to reduce the emissions that are associated with producing the fuel to a minimum. The process would be more economical if combined with sequestration of CO_2 from flue gas emissions, with wastewater treatment processes, and/or with the extraction of high value compounds for application in other industries.

Cultivation of algae requires large amounts water as algae generally grow in very dilute suspension (0.2–0.3%). This means that harvesting and drying is also still a significant cost in algal biofuel production.

At higher latitudes, the use of spill heat or strains suitable for colder climates and both high and low light provision is essential to achieve productive processes (Shukla et al. 2013). Stimulation of algal biomass production, and stabilization of conditions during cold seasons should be cost-efficiently achieved by utilizing spill heat and CO_2 emissions from other bioprocesses such as composting or digestion. Waste streams could provide nutrients at lower costs if the nutrients are in a form available to the algae. Alternatively, it may be possible to find cheap organic substrates that meet the nutritional requirements. The key to using side streams and waste streams is to maintain both lipid yield and growth rates at or near the levels found on purer nutrients (fertilisers, glucose) while decreasing the media costs. In Finland for instance, municipalities and the paper and pulp industry are the major sources of nitrogen and phosphorus inputs to waters.

However, it is important to remember that although waste streams provide interesting sources of nutrients, including carbon and nitrogen, these also carry costs in the form of inhibitors, polymers which need hydrolysing, variability of sugars, variability in the concentration of other nutrients, incorrect ratios of nutrients, and biological contaminants (e.g. bacteria). The waste stream may also be too low or too high in pH. However, there are also some algae that grow at pH 1.5–3.0, utilise multiple carbon sources, sequester nutrients (e.g. phosphate) and degrade complex molecules (e.g. dyes), making their use in treating and valorising waste streams of continued interest.

1.4 CO_2 absorption or utilising carbon containing waste streams?

Heterotrophic cultivation of algae has the advantages of being independent of a light source and utilising conventional fermentation technologies in compact reactors, which require less space than photobioreactors or ponds. The contained systems allow the use of GMO species to increase product yields. Utilisation of carbon containing waste streams in heterotrophic cultivations can also overcome one of the main bottlenecks in algal cultivation, namely obtaining sufficiently high biomass density to reduce de-watering problems.

Generally, high cell densities require hetero- (or mixo-) trophic growth, which are dependent on the concentration of organic carbon provided.

- "Photoautotrophic culture presents severely limiting biomass production due to cellular self-shading that hinders light availability towards the end of growth. The low biomass concentration obtained in the photoautotrophic culture increases the biomass harvesting cost." (Cheirsilp & Torpee 2012.)
- Heterotrophic culture can provide high biomass concentrations and more effective strategies to induce the accumulation of high amounts of lipid.
- Mixotrophic culture of microalgae also provides high biomass concentrations, but simultaneous provision of light can be used to reduce the CO_2 footprint of the heterotrophic growth. This will need to be balanced with the cost of providing light and the increased risks of contamination. Mixotrophic growth is still poorly understood. It is important to recognise that not all algae can grow heterotrophically in the presence of light, while others may not photosynthesise in the presence of organic carbon. The ability of an alga to survive and grow in an organic carbon containing waste stream cannot be described as mixotrophic growth unless the organic carbon is also consumed (by the alga, not by cohabiting bacteria). More insight is needed on mixotrophic growth in order to develop systems that can truly valorise the benefits of such cultivation regimes.

1.5 Algae for biofuel production

Algae are potential producers of large quantities of lipids: the oil yield is up to 6 times higher than in oil palm, which is ranked second (Table 1). A realistic current value of microalgal biomass production lies between 15 and 25 tonne/ha/year. With an assumption that there would be 30% lipid content in the microalgal cells (without optimizing the growth condition), this is equivalent to a lipid production of 4.5–7.5 tonne/ha/year (Lam and Lee 2012).

Table 1. Potential oil yields from oil rich plants or microalgae (Darzins et al. 2010).

	Litres per hectar per year
Oil palm	5 940
Jatropha	1 890
Rapeseed	1 400
Sunflower	955
Camelina	560
Soybean	450
Microalgae	3 800–50 000*

*projected maximal yield (Hu et al. 2008)

Microalgae can be utilized in energy and fuel production in several ways, not only as a lipid source for biodiesel (Fig. 2).

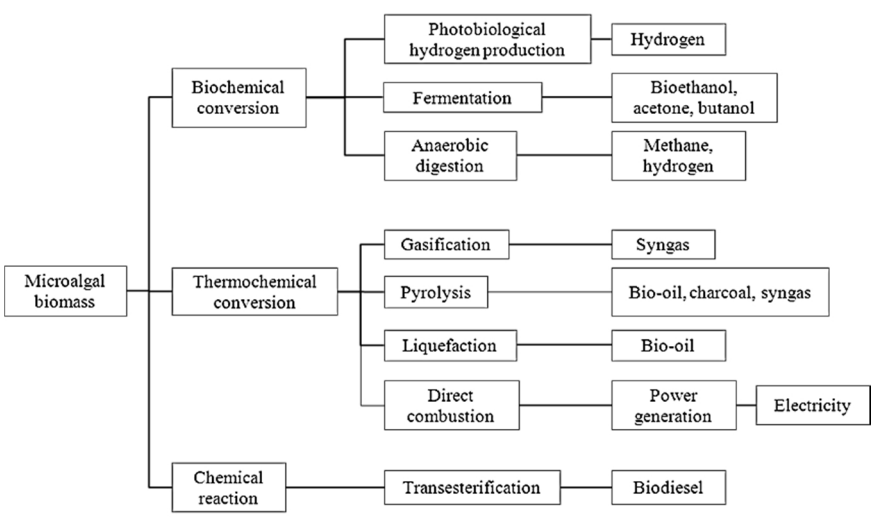

Figure 2. Alternatives of microalgae based energy (Hytönen 2012, edited from Brennan and Owende 2010, Tsukahara and Sawayama 2005, Wang et al. 2008).

Carbohydrate- and protein-rich biomass residuals from biodiesel production are suitable for biogas and possibly hydrogen gas production. Further many algal strains have been recognized as potential biogas substrate without lipid extraction, alone or even more efficiently, as co-substrate in the digestion process. Algal biomass can also be digested to ethanol, utilized as a substrate for electricity production in microbial fuel cells (MFCs), or processed under high temperature, in the absence of oxygen to produce bio-oil and bio-syngas. Dewatered biomass can be incinerated.

1.6 Residual valorisation

Currently there are no commercial algal plants operating on a consistent schedule for the purpose of producing biofuel. Commercial production of algae is limited to small scale, high value products. None-the-less, the value of the non-lipid algal components should not be ignored. Depending on the algal species, the amount of the residual matter after extraction of lipids is significant (Biller and Ross 2010). In fact, up-grading of the residue is one of the key issues to determine the cost-effectiveness of the algal concept. The US Department of Energy listed the following options for recovering economic value of de-fatted biomass (DoE 2010):

1. Maximum energy recovery from the lipid extracted biomass with use of residuals as soil amendments (via anaerobic digestion)
2. Recovery of protein from the de-fatted biomass for use in food and feed
   - Industrial enzymes (requires selected or engineered algal strains)
3. Recovery and utilization of non-fuel lipids
   - Separation of non-fuel lipids to be utilized as chemical feedstocks for surfactants, bioplastics and speciality products (urethanes, epoxides, lubricants etc.)
4. Recovery and utilization of carbohydrates from de-fatted biomass and the glycerol from the transesterification of lipids to biodiesel
   - Conversion through anaerobic fermentation to H_2 and solvents (acetone, ethanol, butanol), organic acids (formic, acetic, propionic etc)
   - Glycerol to 1,3-propanediol and formulation to e.g. polymers, adhesives polyesters
5. Recovery/extraction of fuel lipids only, with use of the residual biomass as soil fertilizer and conditioner.

The polysaccharide and protein rich residue may also be a source for valuable biopolymers, which can be exploited in various industrial material applications.

2. Goal

The project's goal was to design and validate integrated concepts of utilising waste streams for algal biomass production. The sustainable processes developed should involve efficient utilisation of all side streams generated in addition to biodiesel and biogas. This also included material valorisation of residual algal biomass.

3. Algal cultivation for lipid production in waste water

Main writers: Anne Ojala, Marika Tikka, Kalle Valkonen, University of Helsinki; Silja Kostia, Pekka Järvelin, Lahti University of Applied Sciences

3.1 Selection of algal species for feasibility testing

Of the estimated 10 million algal species on the earth, some 40 000 separate species have been identified. Most strains are photoautotrophs, but some genera (e.g. Chlamydomonas) also grow heterotrophically in the dark (Hu et al. 2008).

An algal strain suitable for energy applications should have a high growth rate and a high capacity for biomass and lipid production, as well as a suitable fatty acid composition. A good strain should also be easy to cultivate and harvest, and lipids from the cells should be easily extractable. Three algal strains were chosen, after pre-cultivation in organic medium and preliminary testing of lipid content. Two strains (*Chlorella pyrenoidosa* UTEX 1230 and *Euglena gracilis* CCAP 1224/5Z) originated from culture collections.

One specific target of algal strain selection was the selection of strains capable of mixotrophic growth, since most of the waste waters of interest contain organic carbon and there is evidence that mixotrophy may enhance lipid production (Wan et al. 2011). This was the main reason for also using a strain of *Selenastrum*, isolated by the University of Helsinki and originating from a typical Finnish lake (Lake Iso-Ruuhijärvi) with a high concentration of coloured organic, humic compounds and thus from conditions favouring mixotrophy. Algae from northern humic lakes are also presumably well adapted to low light conditions and can grow at low temperatures, which are characteristics useful in commercial applications.

3.2 Algal growth in various waste waters

To develop a concept for algal energy, including the utilization of waste resources in production of biodiesel and biogas from algae, it was important to identify available and suitable waste streams, as well as to choose the algal strains for testing.

Suitable waste streams were identified both from literature and by interviewing stakeholder companies in the project. The companies also provided data on their own waste water streams for evaluation of their possible use as algal cultivation media. Based on that information, the waste streams with the most potential were tested in small scale laboratory assays and at pilot scale. The main focus was on waste streams rich in organic carbon, nitrogen and phosphorus (Kautto 2011). Carbon and nutrient rich waste streams are most suitable for heterotrophic or mixotrophic species, while autotrophic species require only nutrient rich waste streams.

The companies interviewed were interested in manure and agricultural waste waters, municipal waste waters, reject water from sewage treatment, cooling water from biogas plants, distillery effluents, and leachate from landfills. Except for the landfill leachate these waste streams were in principle all suitable for algal growth. Landfill leachate was, however, low in carbon and nutrients and generally containing toxins (Päijät-Hämeen Jätehuolto Oy). The other waste streams appeared more suitable. Easy availability and company stakeholder interest were the pragmatic criteria applied to choose the waste streams for laboratory and pilot scale testing. The chosen waste streams were also seen to be suitable for algal cultivation in the literature (Kautto 2011). Thus reject water from a biogas plant, fish farm water, fluid pressed from municipal organic waste before digestion, (hereafter called press water) and two waste waters from a local composting plant in Lahti were assessed (Table 2). The reject water was high in COD and BOD and rich in nitrogen and phosphorus. Water from fish cultivation was collected from two different farms. Nutrient concentrations, COD, and BOD in these waters were low. The biowaste press water was very rich in nutrients and organic matter, but usually very acidic. The press water analytical data was provided by Irene Bohn at Helsingborg University (I. Bohn, Avfall Sverige, personal communication, 2010). The waste streams from the composting plant in Lahti were leachate from the first stage of the composting process (‘precomposting water’) and composting wetting liquid (‘process water’). Characterization of these waste waters is presented in Table 2.

Table 2. Characterization of waste waters used in algal cultivation. Fish farm waste water was not sterilized (*) or sterilized (**). BOD_7 = the concentration of oxygen consumed over 7 days.

		Reject	Press	Process	Precomp	Fish farm*	Fish farm**
Total N	mg L^{-1}	3 860	10 595	486	2 540	31.4	21.9
NH_4^+	mg N L^{-1}	3 800	237.6	333.2	1 650	0.8	
NO_3^-	mg N L^{-1}	112	333	72	40	33	0
Total P	mg L^{-1}	540	357.5	6.4		1.7	
PO_4	mg P L^{-1}	168	308	3.4	348	1.6	6.2
BOD_7	mg O L^{-1}	9 300	13 556	124	46 000	3.3	91.8
COD	mg L^{-1}	17 850	40 640	643	80 000	28	175
pH		8.2	4.3	9.1	4.3	6.8	6.5

The waste waters included in this study would typically require pretreatment (filtering and sterilization) before they could be used in algal cultivation. Reject water and liquid fractions from the composting process needed to be diluted to lower the high concentration of nutrients. Sterilised and non-sterilized fish farm waters were used without dilution. In laboratory scale experiments, *E. gracilis* and *C. pyrenoidosa* were cultivated in each of the selected waste waters. Reject water and non-sterilized fish farm water were not used for the cultivation of *Selenastrum* sp (Table 3).

Waste fractions originating from biowaste processing (compost leachate, process water and press water) were found to be the most promising for algal biomass production. The nitrogen and phosphorous concentration of the fish farm waters were lower and also contained less organic carbon (BOD) than the other waste fractions. This probably limited algal growth. Algal growth in the reject water was also poor, probably due to the high concentration of ammonium (>100 mg NH_4 -N L^{-1}), which was converted to toxic ammonia when the pH increased during cultivation. The pH increase was a result of algal photosynthesis, that utilizes dissolved carbon dioxide from the solution and thus raises the pH. The very low concentration of magnesium in the reject water may also have hampered algal growth (Park et al. 2010).

The composting leachate, in contrast, was not only rich in macro nutrients, but also contained plenty of all micronutrients. Pre-composting water also contained considerable amounts of dissolved zinc (0.5 mg L^{-1}) and some nickel and chromium (0.25 mg L^{-1}). If the water is recycled within the cultivation process, the concentrations of heavy metals would need to be monitored and controlled to ensure that the concentrations would not exceed the toxic thresholds of the cultivated algae.

Table 3. Strains and waste water sources tested (+) at lab scale.

	Reject water	Press	Process	Precomp	Fish farm non sterilized	Fish farm, sterilized
Dilutions %	5/10	*20/50/100*			*20/50/100*	*20/50/100*
Selenastrum sp	-	+	+	+	-	-
E. gracilis	+	+	+	+	+	+
C. pyrenoidosa	+	+	+	+	+	+

3.3 Bacterial contamination of algal cultivations

Waste streams rich in organic carbon may also contain abundant heterotrophic microorganisms. In our preliminary cultivation of algae in these wastewaters we found that sterilization of the waste streams was necessary to avoid adverse contamination originating from the waste stream. At industrial scale, sterilization may not be feasible and thus we assessed how reduction of contaminants prior to cultivation affected subsequent algal growth. Quantitative PCR (qPCR) with appropriate primers was used to estimate the number of bacterial and algal cells during the cultivation of *Selenastrum* sp. in small scale laboratory experiments designed to test whether the growth of contaminating bacteria during the cultivation was a reason for poor growth of algae in some conditions. To validate the qPCR results, which is a relatively new method for determination of algal growth, microscopy was also used to determine algal and bacterial cell numbers.

Process water, press water and pre-composting water were used to assess bacterial growth. All waste waters were sterilized and used at two dilutions. Algae were grown in non-sterile environments. Initially, the number of bacterial cells was below the detection limit, but bacterial populations developed during the cultivation. In only one out of six experiments did strong bacterial growth correlate with poor algal growth. In the other cultivations, algal and bacterial growth curves were similar, implying that the populations could co-exist and did not out-compete each other. Thus bacterial contamination would probably not inhibit algal growth if bacterial numbers can be reduced, e.g. by sterilization of the waste stream, at the beginning of the process. Since complete sterilization is unlikely to be feasible at an industrial scale, other approaches to limiting bacterial growth will be needed.

3.4 Biomass production in waste water

The laboratory experiments indicated that *E. gracilis* and *Selenastrum* sp. had a higher capacity to produce biomass than *C. pyrenoidosa*. *C. pyrenoidosa* had usually higher maximum cell numbers than the two other strains but the cells were small and thus biomass production remained low. On the other hand, *C. pyrenoidosa* was stable in variable culture conditions while the growth of other strains was

more sensitive. For example, although in a 10% dilution of process water all species grew well, in a 50% dilution growth of *E. gracilis* and *Selenastrum* was poor. In reject water, survival of *E. gracilis* cells was very weak.

Our measurements also revealed that *Selenastrum* was capable of consuming nutrients very rapidly at the beginning of the cultivation when growth was vigorous. Thus, algae have potential for waste water purification and the processes of biomass production and water purification can be combined if the waste water is otherwise suitable for algae cultivation.

3.5 Lipid production and characterization in waste water grown algae

The fatty acid composition of raw material greatly influences biodiesel characteristics such as cetane number, cold-flow properties, viscosity and oxidative stability. Especially in cold climates, the fuel's cold-flow properties should be noted. Saturated and mono-unsaturated fatty acids with 14–18 carbon atoms are most suitable for biodiesel production. Saturated fatty acids with long carbon chain have unsatisfactory cold-flow properties, but inversely cetane number increases with increasing chain length and decreasing saturation (Stansell et al. 2012). Fatty acids with several double bonds have poor oxidative stability and the proportion of these fatty acids should be low (Stansell et al. 2012).

In our research *E. gracilis* and *Selenastrum* sp. had a higher capability to produce lipids suitable for biodiesel production than *C. pyrenoidosa* (Table 4). All species demonstrated the highest lipid content when grown in organic press water (diluted 10%). The total fatty acid content in *E. gracilis* was 14.5% of DW, whereas in *Selenastrum* and *C. pyrenoidosa* the corresponding values were only 7.1% and only 4.1%, respectively. A substantial proportion of fatty acids in *Selenastrum* sp. in different waste water cultivations were saturated or monounsaturated with a carbon chain length 16–18. The most abundant fatty acid in all *Selenastrum* cultivations was oleic acid (18:1n9*c*) (23.6–36.8% of total FA:s). The amount of palmitic acid (16:0) was also high (19.4–22.1%). *C. pyrenoidosa* had a high proportion of palmitic acid (20.9–32.2%), but the amount of oleic acid was low (2.6–8.0%). Instead, *C. pyrenoidosa* had a significant amount of rumenic acid (18:2n6*c*) (16.5–23.5%). In press water, in *E. gracilis* over half of the total fatty acids were myristic acid (14:0), but in other waste waters the proportion of this fatty acid in *E. gracilis* was highly variable (5.6–35.5%). The proportion of alphalinolenic acid (18:3n3) which is the precursor for other polyunsaturated fatty acids (PUFAs), was generally higher in *Selenastrum* and *C. pyrenoidosa* strains than in *E. gracilis* (9.9–33.5, 16.9–36.5 and 3.3–20.3) but *E. gracilis* had a distinct profile, with relatively high amounts of polyunsaturated fatty acids with more than three double bonds.

According to our results *Selenastrum* sp. had a more suitable fatty acid profile for biodiesel than *E. gracilis* or *C. pyrenoidosa*, since it had a higher proportion of monounsaturated fatty acids and lower proportion of polyunsaturated fatty acids (Table 4). The unsaturated fatty acids with several double bonds present in

E. gracilis would not be optimal for biodiesel production, but are excellent in functional food products, as also recognized by the Japanese company Euglena Co. Ltd. (http://www.euglena.jp/english/.) Synthesis of polyunsaturated fatty acids with more than 18 atoms of carbon is limited in humans and thus these must be obtained from food.

Nitrogen limitation is known to enhance lipid production in algae. Since most of the waste waters assessed here contained abundant nitrogen (Table 2), high concentrations of lipid in the biomass were not expected. However, even though the nitrogen content of fish farm water was low (Table 2), lipid accumulation in fish farm water was not higher than in other waste waters. Other stress factors may have contributed to differences in lipid production obtained in different conditions, but the complexity of waste waters hampered identification of specific factors. In general, the fatty acid profiles were more dependent on the species than on the culture medium, but growth conditions had some effect on the relative amounts of fatty acids in the different species.

Table 4. Total fatty acid content (% DW) and proportion of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of *E. gracilis, C. pyrenoidosa* and *Selenastrum* sp. cultivated in different waste waters and waste water concentrations. The total FAs was determined as the sum of the 37 known fatty acids, which were used in the calibration standard. SFAs, MUFAs and PUFAs present as > 1% of total FAs are included. (**sterilized fish farm effluent).

E. gracilis	**total FAs**	**SFAs**	**MUFAs**	**PUFAs**
precomposting water 2.5%	6.7 ± 1.5	61.7 ± 5.3	3.4 ± 0.4	31.8 ± 3.0
precomposting water 5%	3.5 ± 0.2	48.5 ± 6.3	5.5 ± 0.5	43.9 ± 6.6
process water 25%	7.6 ± 0.1	68.7 ± 5.5	13.6 ± 7.4	17.0 ± 1.8
fish farm water** 100%	3.8 ± 0.6	35.3 ± 3.4	5.8 ± 1.0	54.2 ± 3.8
press water 10%	14.5 ± 0.6	78.4 ± 9.2	1.8 ± 0.5	13.0 ± 2.7
C. pyrenoidosa				
precomposting water 2.5%	3.8 ± 0.7	38.1 ± 3.1	8.7 ± 0.1	53.3 ± 3.0
precomposting water 5%	3.9 ± 0.4	31.2 ± 2.3	12.6 ± 2.4	50.3 ± 4.3
process water 25%	3.3 ± 1.0	41.4 ± 8.3	11.9 ± 0.4	43.1 ± 10.0
process water 50%	2.7 ± 0.9	46.1 ± 3.7	18.2 ± 8.8	34.7 ± 12.0
Fish farm water** 100%	2.3 ± 1.0	30.7 ± 0.5	11.6 ± 1.0	53.5 ± 3.1
press water 10%	4.1 ± 0.7	45.1 ± 1.7	5.8 ± 0.8	47.7 ± 1.7
***Selenastrum* sp.**				
precomposting water 2.5%	5.5 ± 0.5	36.7 ± 4.3	38.1 ± 8.9	23.8 ± 5.3
precomposting water 5%	4.7 ± 0.7	42.3 ± 5.5	40.0 ± 2.5	13.6 ± 0.8
Fish farm water** 100%	6.7 ± 0.5	31.8 ± 2.5	25.5 ± 1.2	38.8 ± 1.7
press water 10%	7.1 ± 0.8	34.8 ± 1.7	41.0 ± 0.2	19.8 ± 0.6

We also tested the suitability of supercritical fluid extraction (SFE; 80°C, 500 atm, 1 h) for extraction of lipids from the *Selenastrum* strain and *C. pyrenoidosa*. Solvent extraction of lipids has several disadvantages, for example toxicity of solvents and limited selectivity for some neutral lipid fractions. Supercritical fluid extraction with CO_2 is a promising alternative because it is non-toxic and has minimal co-extraction of polar- and non-acyl-glycerol neutral lipids and non-lipid components (Grierson et al. 2012, Halim et al. 2012, Soh & Zimmerman 2011). High energy requirements and installation costs have limited scaling-up of this method (Halim et al. 2012). Our preliminary results indicated that the cell matrix influences the extraction capacity with supercritical CO_2. Lipid extraction with supercritical CO_2 was more efficient and easier from *Selenastrum* cells than from *C. pyrenoidosa,* which required higher temperature and longer extraction time. That is probably due the strong cell wall structure of *Chlorella* species and many studies have demonstrated enhancement of lipid extraction from *Chlorella* with either supercritical CO_2 or solvent extraction methods when cell disruption was used before extraction (Mendes et al. 2003).

3.6 Light and temperature requirement

The light intensity required for efficient photosynthesis of the tested microalgae was quite low, i.e. ca 100 µmol m^{-2} s^{-1}, implying that in Southern Finland there is enough natural light for algal growth from February to October.

The algae were photosynthetically active at 10°C, but the optimum temperature for photosynthesis of *C. pyrenoidosa*, *E. gracilis* and *Selenastrum* sp. was higher, i.e. 15, 25 and 20°C, respectively. In the winter, when light would be the growth limiting factor in autotrophic cultivations, the ability to shift from autotrophic to heterotrophic growth could be utilised (Section 4). Either mixotrophy or heterotrophy could be implemented to use waste carbohydrates as the organic carbon source.

3.7 Pilot scale cultivation

The viability of using the most promising waste water, i.e. composting leachate, was verified in pilot scale cultivations. Four flat panel photo bioreactors were purpose built in two sizes (Fig. 3). Three had an effective volume of 170 l whereas the volume of a thinner reactor was 80 l.

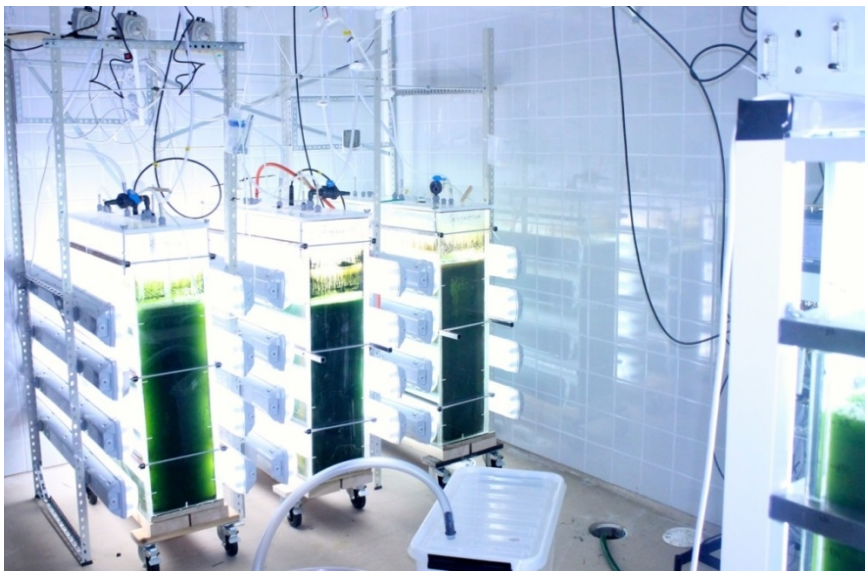

Figure 3. Algae cultivated in photobioreactors in a temperature-controlled room in the Department of Environmental Sciences, University of Helsinki.

The photobioreactors were robust in design. Filtered compressed air was pumped through a perforated pipe to the reactor to provide mixing and to strip out excess oxygen, resulting from photosynthesis. Bioreactors were illuminated with daylight fluorescent tubes and installed with temperature control, as well as pH control based on controlled CO_2 input. This was a simple, one-way control which opened the CO_2 valve when the pH rose above a set value. In a full-scale operation this type of control can be applied to adjust the flue gas flow to the cultivation process.

Composting leachate was diluted to 2.5% with tap water for reactor cultivation of *Selanastrum* (Fig. 4). In a 17 day experiment, the algae gained a biomass of 1.2 g DW L^{-1}, which was similar to that obtained in a control cultivation, in which a defined inorganic medium was used as a substrate (data not shown). The nutrient reduction was > 99% for ammonium, 83% for total nitrogen, 73% for phosphorus from phosphate and 70% for total phosphorus. The experiments so far have shown that composting leachates are suitable for algal cultivation also at this larger scale. When reject water (2.5%) was used as the source for major nutrients, supplemented with micronutrients (e.g. Mg), *Selenastrum* sp. produced 2.7 g DW L^{-1} biomass in a 35 day long experiment. Nutrients were reduced 91% for ammonium, 85% for total nitrogen, and ≥ 98% for phosphorus and phosphorus from phosphate. Therefore reject water also showed potential for mass cultivation of algae, although mineral supplements were needed.

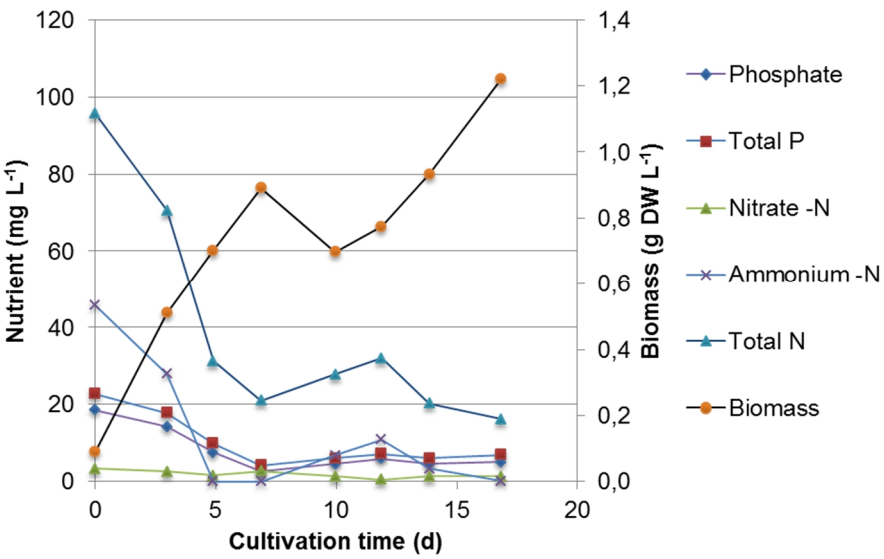

Figure 4. Growth (g DW L^{-1}) of *Selenastrum* strain when cultivated in compost leachate (2.5%) in 80 l bioreactor.

3.8 Case study – Kujalan Komposti Oy

The concept of integrating microalgal cultivation into a biowaste management center was assessed using Kujalan Komposti Oy's process as a case study. The study included a scenario in which the algae would utilize spill heat and CO_2 from the composting plant and the residual microalgal biomass after lipid extraction would be utilized as a biogas co-substrate Fig. 5). The area allocated for algal cultivation was 2.2 ha (Fig. 6). The feasibility study was done using results from laboratory scale tests (algal growth rates and removal of nutrients originating from organic waste streams). Due to restricted resources, the role of illumination, CO_2, nutrients and heat variation were not examined.

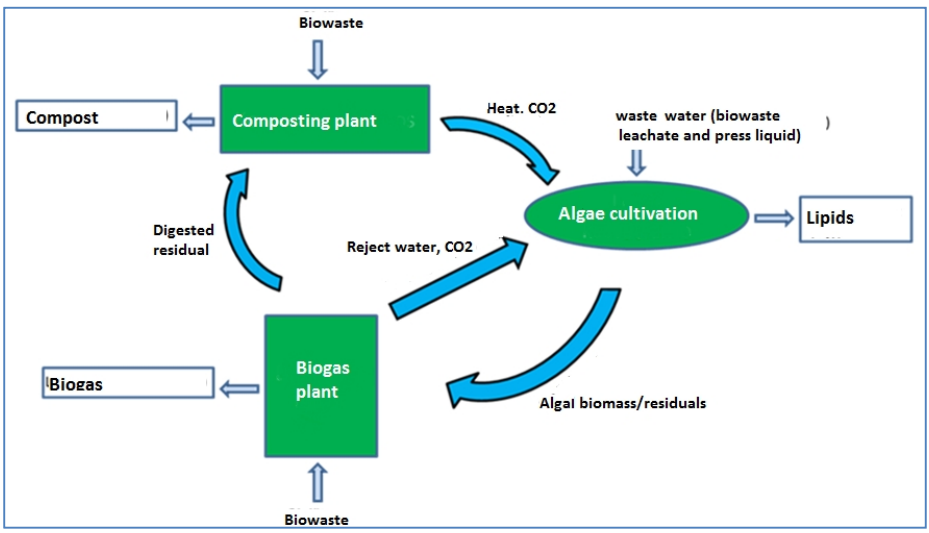

Figure 5. Algal cultivation integrated into biowaste processing.

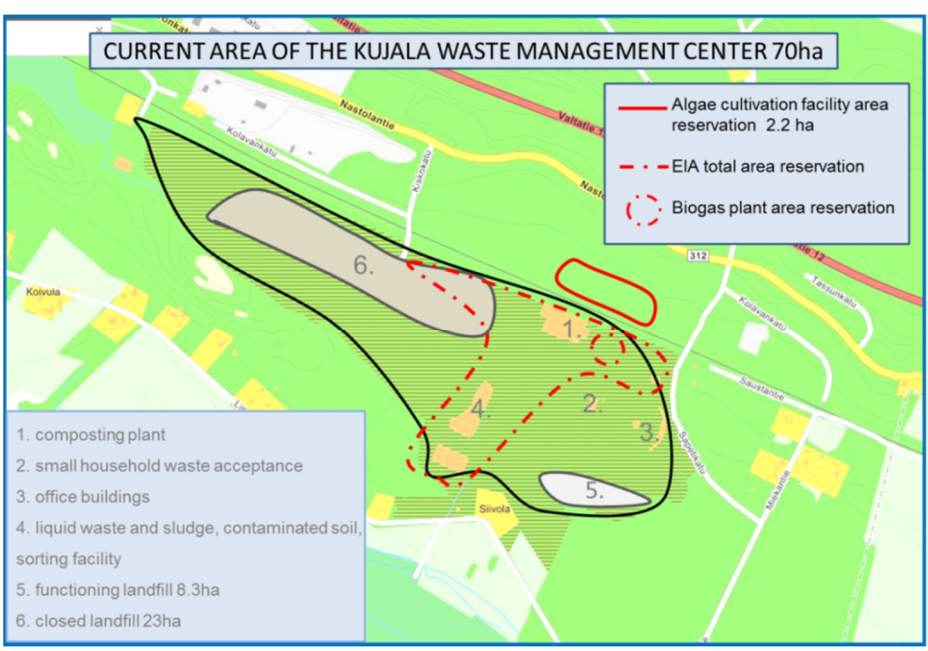

Figure 6. Schematic representation of Kujala waste management centre and area reservations included in the study (Järvelin 2011).

The case study assessed the reserved area's (Fig. 6) potential for three scenarios: a worst-case (low harvesting density of 0.6 g/l per 6 d, an average of the lowest values found in the literature), a moderate case (3.0 g/l per 6 d, (literature and project result average) and an optimal case (5.0 g/l per 6 d, an average of the maximum values found in the literature). The scenarios modelled the biomass productivity and determined the area and water requirements in the three situations. This gave a range and understanding of the minimum and maximum capacity of the cultivation facility area.

The results showed that with the algal production rates presented, the reserved area in Kujala (2.2 ha) was far too small for the targeted biomass production 60 000 t/a, (Kujalan Komposti Oy 2011). In addition the corresponding water consumption would be 14 700–44 100 m^3 per year of raw waste water, meaning significant pumping and treatment costs (Järvelin 2011).

Key findings:

- A Finnish isolate of *Selenastrum* sp. had good tolerance in industrial waste streams (e.g. from a composting plant) and a lipid profile which could be suitable for biodiesel production, making it an interesting choice for algal biomass production in Finland.
- Several waste sources are available in Finland, which are suitable for cultivation of algae. However, individual waste sources need to be assessed to ensure they provide adequate nutrition and some may need to be diluted.
- Algal processes which utilise waste streams containing organic matter will be subject to contamination. Either cheap methods of reducing contaminants (e.g. pasteurisation) will need to be developed or co-cultivation with a heterotroph with desirable properties, which can compete with the natural contaminants, should be developed.
- The lipid content of the algal species grown in waste water was lower than expected, even when the nitrogen content of the water was low (e.g. fish farm water).
- Algal growth and nutrient consumption was similar at pilot scale, to that observed at lab scale.
- Although waste water from a composting plant could support the production of a large amount of algae, the space and water required to grow the algae would be prohibitive at this time.

4. The productivity of algae in mixotrophic conditions

Marilyn Wiebe and Yanming Wang, VTT

The focus of the productivity analysis was to determine production (biomass and lipid, CO_2 and/or O_2) rates and yields, as well as relevant consumption rates (CO_2 and/or O_2 and glucose), in order to gain an understanding of the potential of mixotrophic growth for providing high biomass algal cultures as well as providing data to the techno-economic study. The chosen strains (*Chlorella protothecoides* and *Euglena gracilis*) were known to grow heterotrophically, which is important when assessing the potential of mixotrophic growth, and corresponded with two of the strains assessed for their ability to grown in waste waters (Section 3).

Algae were grown in 2 L glass-walled stirred tank reactors (1–2 L, Sartorius Biostat B), and light was supplied from 1, 2 or 3 fluorescent lights positioned vertically around the reactor, each supplying ~400 µmol photos m^{-1} s^{-1} at the inner surface of the reactor. Some experiments also utilised a Sartorius 3 L photobioreactor (Sartorius PBR-2S) (Fig. 7). For heterotrophic growth, the vessel was completely covered with thick aluminium foil.

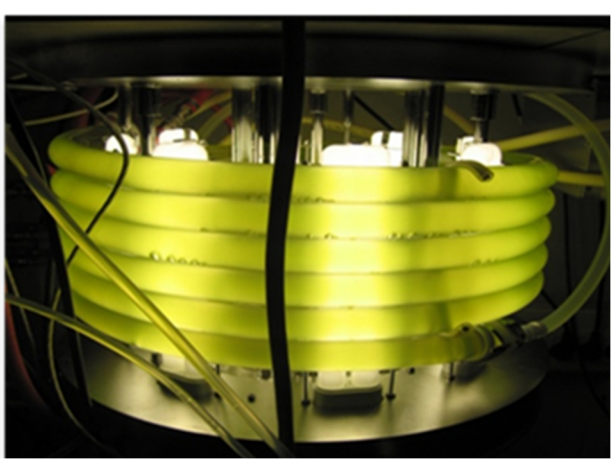

Figure 7. Sartorius PBR S2 photobioreactor (VTT).

4.1 Phototrophic growth

Phototrophic cultures were established with both *Chlorella protothecoides* and *Euglena gracilis*. In addition, some cultivations were carried out with the obligate phototroph *Scenedesmus obliquus*, which had contaminated a *Chlorella pyrenoides* culture. *S. obliquus* has previously been used as a source of algal biomass to assess the potential of converting residual algal biomass to ethanol or microbial biodiesel, after initial lipid extraction (Microbes and algae for biodiesel production – Microfuel, Tekes 40258/07). All three strains were able to grow in the stirred tank bioreactor in chemically defined medium and accumulated lipids in their biomass in some conditions.

Although algae are able to grow exponentially, as do heterotrophic microorganisms, light and CO_2 provision rapidly become limiting. As a result, in phototrophic conditions throughout most of the growth phase algae grow at a linear, rather than an exponential rate (Fig. 8). Since the exponential phase is relatively short, algal growth rates are typically reported for the linear phase (g L^{-1} day^{-1}) rather than as specific growth rates (g g^{-1} day^{-1}). As light and/or nutrients become more limiting, the prolonged period of decelerating growth may result in a shift to a slower linear rate of growth than is initially sustained. Growth rates reported here refer to the first period of linear growth.

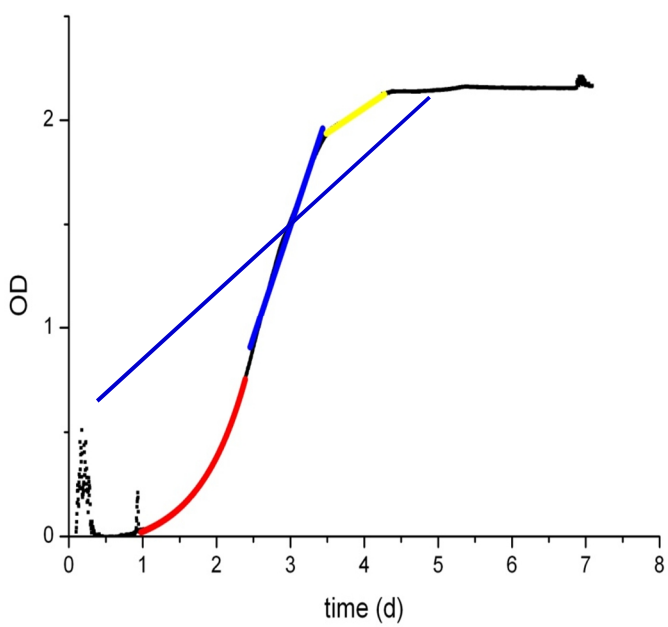

Figure 8. In phototrophic algal cultures, initial exponential growth (red), is followed by a period of prolonged linear growth (blue), and eventually slower growth (yellow) and stationary phase (black). The switch from exponential to linear growth depends on the time at which CO_2 and/or light become limiting.

S. obliquus, *C. protothecoides* and *E. gracilis* had similar growth rates in chemically defined medium, with 2 fluorescent lights and CO_2 supplied as air. Biomass production was limited to 1 to 1.8 g L^{-1}. However, supplying additional CO_2 in the sparging gas (5.66 g CO_2 L^{-1} day^{-1}) resulted in an increase in growth rates, as well as production of 4 to 6 g L^{-1} dry biomass (Table 5). *C. protothecoides* was grown in a CO_2 supplemented culture with 3, rather than 2 lights to obtain the higher biomass concentration (6 g L^{-1}). These results demonstrated that CO_2 will be seriously limiting in phototrophic processes, if the system cannot be enriched with e.g. flue gas or a carbonate solution. CO_2 limitation was probably more restrictive than light limitation, but adequate light will be critical to obtain biomass concentrations above 4 g l^{-1}. This is also clear when we consider other published reports of comparable biomass concentrations from phototrophic systems, which are typically obtained only from photobioreactors with short light paths and CO_2 feeding (Gong & Jiang 2011).

Table 5. Growth of *S. obliquus*, *E. gracilis* and *C. protothecoides* in CO_2 and light limited batch cultures in chemically defined medium.

Strain	Light*	CO_2 (%)	Growth rate ($g \cdot L^{-1} d^{-1}$)#
S. obliquus	++	0.03	0.15
E. gracilis	++	2	0.32
C. protothecoides	+++	2	0.41

* 2 or 3 light sources, each ~400 µmol $s^{-1}m^{-2}$ at reactor surface

\# Average linear growth rate

It should be noted that although 5.66 g CO_2 L^{-1} day^{-1} was fed to the culture, at most only 1 g CO_2 L^{-1} day^{-1} was consumed, and most of the time less. Thus, less than 18% of the CO_2 being fed was consumed by the algae and this needs to be taken into account when designing systems for phototrophic algal production. The algae can reduce the CO_2 being released into the atmosphere from a CO_2 generating process, but will not remove all CO_2. This reflects the low solubility of CO_2 in the liquid phase of the culture, which restricts the amount accessible to the algae, and also the decreasing specific growth rate of the algae, as their supply of light becomes increasingly limiting.

Phototrophic continuous flow cultures were established with *S. obliquus*. Biomass concentration in these cultures was low (0.5–1.2 g DW l^{-1}, Table 6) and steady states were not necessarily obtained, but these cultures demonstrated that *S. obliquus* accumulated up to 36% of its biomass as lipid when grown at a low dilution rate with a low N supply (Table 6). In phototrophic conditions, limitation in CO_2 and light penetration resulted in the biomass concentration being dependent on the dilution rate of the mineral salts being added, with less biomass sustained

at higher specific growth rate (D = 0.28 d^{-1}) than at low (D = 0.09 d^{-1}, Table 6). Less lipid accumulated in the biomass at high dilution rate than at low. None-theless, the volumetric productivity for both biomass and lipid was higher or the same at high dilution rate, than at low (Table 8), illustrating that high lipid content is not in itself sufficient to achieve a productive system.

Table 6. Production of biomass and lipid in continuous flow, phototrophic cultures of *S. obliquus* in chemically defined medium at pH 7.5, 23°C, with 0.5 vvm aeration.

Dilution rate (d^{-1})	0.09		0.28	
Feed nitrate concentration (mmol/L)	1.15	2.30	1.15	2.30
Biomass (g L^{-1})	1.0	1.0	0.5	0.4
Total lipid content (%)	36. 5	32.1	27. 7	18.8
Volumetric biomass production rate (g L^{-1} d^{-1})	0.09	0.09	0.13	0.12
Volumetric lipid production rate (g L^{-1} d^{-1})	0.033	0.029	0.035	0.022

4.2 Mixotrophic and heterotrophic batch growth

When *C. protothecoides* (Wang et al. 2013) and *E. gracilis* were grown under mixotrophic or heterotrophic conditions the growth rates were 4 to 10 times higher than in phototrophic conditions. The yield of biomass on glucose in mixotrophic cultures was around 0.5 g [g glucose consumed]$^{-1}$ for both strains, as expected for heterotrophic growth with no ethanol production. The yield of biomass on glucose was slightly higher for mixotrophic growth of *E. gracilis* than for heterotrophic growth, indicating that CO_2 was also being used as a carbon source for biomass production. Biomass degradation occurred during stationary phase of the heterotrophic culture, but did not occur when cells were grown mixotrophically, indicating that the photosynthetic supply of ATP was sufficient to provide maintenance energy.

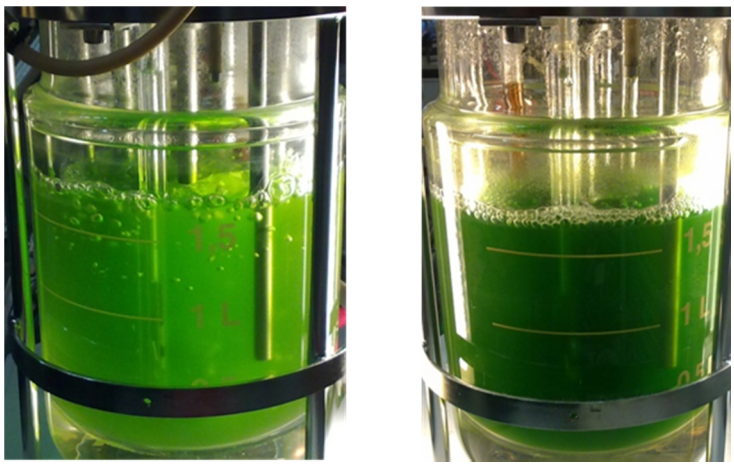

Figure 9. A phototrophic culture of *S. obliquus* (left) and a mixotrophic culture of *E. gracilis* (right) in the Biostat B, growing in defined medium at pH 7.5 and 5.5, respectively, 23°C. Mixotrophic cultures were provided 16 g glucose l^{-1} in addition to mineral salts.

The data confirmed that both *E. gracilis* and *C. protothecoides* were able to utilise glucose as an organic carbon source. Although it was not possible to determine the extent to which individual cells combine photosynthetic and heterotrophic metabolism, it was clear that both strains utilised CO_2 as a carbon source while they adapted to growth on organic carbon, that they reverted to phototrophic growth after the organic carbon had been consumed, and that provision of light to heterotrophic cultures could reduce the total CO_2 output of the cultures (Wang et al. 2013). Considerably higher biomass could be achieved with hetero- or mixotrophic growth than in phototrophic conditions, which would be advantageous for biomass harvesting. Biomass concentrations up to only ~10 g L^{-1} were used here, but biomass concentrations > 100 g l^{-1} have been achieved in heterotrophic algal cultivations (Eriksen 2012), and it should be noted that the relative amount by which CO_2 production can be reduced by providing light in mixotrophic conditions, compared to heterotrophic, will decrease as the total organic carbon provided increases.

Both *E. gracilis* and *C. protothecoides* (Wang et al. 2013) accumulated lipids in N-restricted batch cultures. Lipids were only extracted from samples taken at the end of *E. gracilis* cultivations, when biomass degradation was already occurring in the heterotrophic conditions and thus the lipid content of these cells was unexpectedly low. Storage lipid may have been consumed to provide maintenance energy, but may also have been released to the culture supernatant as a result of cell lysis. Thus we do not conclude that heterotrophic conditions would restrict lipid accumulation, but rather that the time of harvest would be more critical for *E. gracilis* heterotrophic than mixo- or phototrophic conditions.

With *C. protothecoides* we demonstrated the role of N-restriction (or limitation) on lipid accumulation in batch mixotrophic cultures, with more lipid accumulating when less N was provided to the cells (Wang et al. 2013). More lipid (34%) accumulated in the biomass within 4 days when 14 mM N was provided than with 24 mM N (25% lipid; Wang et al. 2013). These experiments were useful in designing suitable conditions for chemostat analyses of CO_2 and lipid production in continuous flow culture (see below).

4.3 Mixotrophic and heterotrophic continuous growth

Mixotrophic lipid production was studied further in chemostat cultivations of *C. protothecoides* at D = 0.44 h^{-1}, with 6, 14 or 24 mM N (Wang et al. 2013). The relationship between N provision and lipid accumulation was similar to that observed in batch cultures with 14 and 24 mM N (40 and 29% lipid in the biomass, respectively). Up to 57% lipid was observed when only 6 mM N was supplied. Surprisingly, *C. protothecoides* consumed all the supplied glucose at both 14 and 24 mM N, and only 1.5 g glucose l^{-1} accumulated in the 6 mM N conditions (i.e. ~90% of the provided glucose was consumed). In non-oleaginous heterotrophs, N-limitation results in high amounts of residual organic carbon in the culture supernatant (e.g. Pleissner & Eriksen 2012) and even oleaginous microbes are unable to consume/ convert to lipid all of the organic carbon supplied, except at very low dilution rates (*cf*. Gill et al. 1977, Meeuwse et al. 2011). Since carbon is thus lost in the waste stream, rather than converted to product, continuous cultures have not generally been considered suitable for biodiesel production. However, *C. protothecoides* was able to sustain lipid production with 36–57% lipid in the biomass and no or low residual organic carbon in N-limited continuous flow culture at D = 0.44 d^{-1} (Wang et al. 2013). This is comparable to the 35–39% lipid accumulation of yeast for N-limited chemostat cultures with C/N ratios similar to that corresponding to 14 mM N here (Gill et al. 1977, Alvarez et al. 1992, Ratledge & Hall 1979), and 45–49% lipid in yeast cultures with higher C/N (Choi et al. 1982, Hassan et al. 1993). Continuous flow cultivation is very interesting for algal systems because they provide a way of sustaining high biomass concentrations, without long periods of waiting while the biomass accumulates. Output would be continuous. Continuous systems would limit the accumulation of excessive inhibitors if an inhibitor containing waste streams would be used, while providing for the continual waste treatment. However, these cultures were maintained for only 25–55 days and the long term stability of lipid production would still need to be assessed.

As with the *S. obliquus* phototrophic continuous flow cultures at different dilution rates, less biomass was produced at high than at low dilution rate in mixotrophic cultures of *C. protothecoides*, but both the biomass and lipid production rates remained high (Wang et al. 2013).

Measurements of the CO_2 evolution rate (CER) and oxygen uptake rates (OUR) in heterotrophic and mixotrophic conditions confirmed that photosynthesis contributed to biomass and lipid production in mixotrophic conditions (Wang et al. 2013).

Providing light with organic carbon (i.e. mixotrophic conditions) reduced CO_2 output by 13 to 35%, depending on the nitrogen concentration in the medium, compared with providing only organic carbon (i.e. only heterotrophic conditions).

4.4 Conclusions and recommendations

Studies of 3 species of algae (*E. gracilis*, *C. protothecoides*, and *S. obliquus*) in photo-, hetero- and mixotrophic conditions have provided insights regarding the potential of these algae for lipid (bio/renewable diesel) production in different conditions. *Euglena* and *Scenedesmus* were shown to be capable of accumulating lipids as > 35% of the biomass, and *Chlorella* > 55% of the biomass (Wang et al. 2013). Nitrogen-limiting conditions for lipid accumulation have been more accurately defined and relationships between lipid content, growth and lipid production rate established, for specific conditions. These should be taken into account when determining the most productive time for harvesting. The data generated have contributed to the techno-economic studies and has provides a reference point for interpreting the more complex growth of algae in waste streams. Although the conditions used were not optimised, the results obtained from these small scale reactors, with pure cultures provided with easily metabolised organic carbon (mixo- and heterotrophic conditions) with no inhibitors represent a 'best-case' scenario, which will not necessarily be achieved at higher scale with complex waste streams. None-the-less they provide both targets and perhaps upper limits for what we should try to achieve in large scale, complex systems.

Key findings:

- Both CO_2 and light will limit phototrophic systems, but CO_2 may be more limiting than light and methods of providing CO_2 should be considered.
- Only a proportion of CO_2 will be consumed from a CO_2 enriched gas stream, therefore phototrophic algal production systems also need to monitor and take into account CO_2 emissions.
- Mixo- and heterotrophic conditions provide the opportunity to achieve higher biomass densities than phototrophic conditions, which would facilitate harvesting.
- Mixotrophic conditions were demonstrated to provide benefits in
    - reducing total CO_2 output, compared to heterotrophic conditions,
    - reducing cell lysis, compared to heterotrophic conditions,
    - providing the high growth rate and biomass concentrations of heterotrophic conditions
    - providing the option of utilising carbon containing waste streams
- Algae are suitable for continuous lipid production in N-limited, mixotrophic continuous flow cultures.

5. Algal cultivation integrated into municipal waste water treatment

Kristian Spilling, Finnish Environment Institute

5.1 Introduction

Use of algae for bioremediation of wastewater was first investigated in the 1950's (Oswald and Gotaas 1957). One of the benefits of using algae in wastewater treatment is that algae produce O_2 during photosynthesis, which promotes aerobic bacterial degradation of the organic components. Bacterial degradation in turn, produces CO_2 which promotes photosynthesis and the algal uptake of inorganic nutrients.

Even though the potential of algal waste water treatment was recognised several decades ago, research efforts in this area have been relatively modest, mainly because other technologies for wastewater treatment were developed. Most of the published literature assesses algal growth in different wastewater streams, ranging from municipal wastewater (e.g. García et al. 2000) to treatment of animal manure (e.g. Mulbry et al. 2008). Generally, algae are able to remove a high percentage of the bioavailable nutrients (e.g. Olguín 2003) at least at lower latitudes, and might also remove other environmental hazardous components, such as heavy metals (e.g. Ahluwalia and Goyal 2007).

Various pond systems have been developed for growing algae. The basic facultative pond has a simple design, and is relatively cheap to build and operate, but is not very efficient. Recent development has focused on high rate algal ponds (HRAPs), which have achieved a high recovery rate of nutrients, in particular when coupled with CO_2 addition (e.g. Park et al. 2011). The HRAPs are relatively shallow raceway ponds that are gently mixed, typically by a paddlewheel. The mixing ensures that algae are circulated around the system and prevents settling to the bottom of the pond. In dense cultures the light does not penetrate far into the water and it is important for algal growth that the algae are circulated into the photic zone. A review of HRAPs can be found in Craggs et al. (2012).

Algal wastewater treatment ponds could be an economically viable option for tertiary level wastewater treatment in locations where climate permits. The co-

benefits of the process would be potential use of algal biomass as biofuel, biogas or bio-fertilizer, recovery of wastewater nutrients, reduction in GHG emission due to low energy wastewater treatment, and substitution of algal biomass for fossil fuel and fertilisers.

Growth of algae at high latitudes, such as in Finland, has not received much attention (Tang et al. 1997, Chevalier et al. 2000). However, an evaluation of algal wastewater treatment in cold climates, using a socio-ecological model, concluded it was very favourable in terms of sustainability criteria (Grönlund et al. 2004). In particular in the Baltic Sea region, where eutrophication is a big concern, the added value of nutrient removal during algal cultivation was obvious. However, with the exception of the theoretical study by Grönlund et al. 2004, very little practical work in this field has been done in the region.

In this publication, we assessed the growth of algae in treated and untreated municipal wastewater. The wastewater originated from the Suomenoja wastewater treatment plant, Espoo (Fig. 10). Untreated wastewater was taken from a separate experimental inlet that originates from ~40 households (i.e. includes no industrial wastewater).

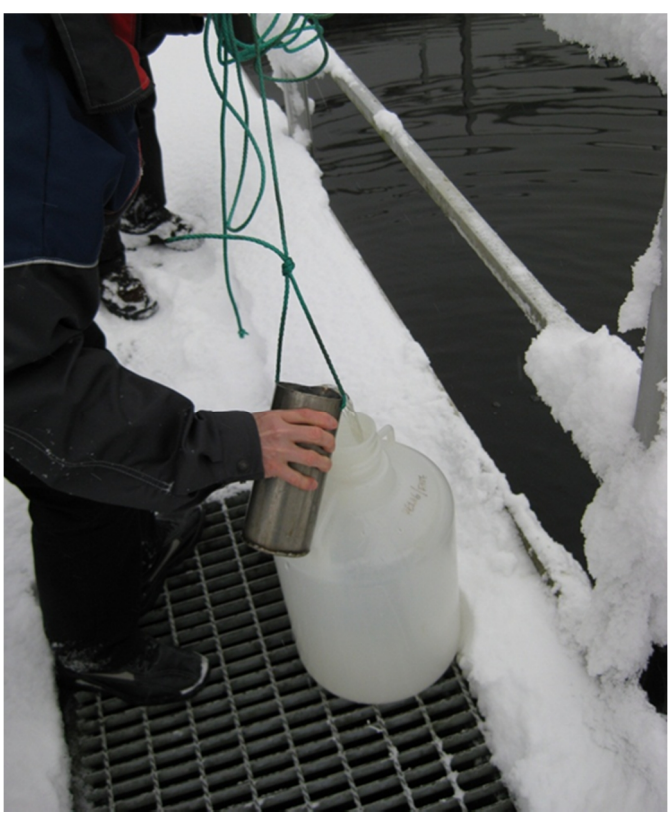

Figure 10. Sampling treated wastewater from Suomenoja wastewater treatment plant.

5.2 Screening for inhibitory effects of treated wastewater

Treated wastewater has a low concentration of nutrients in Finland, and is thus not well suited for algal growth. However, in order to confirm that there were not inhibitory compounds present in the treated wastewater that would affect algal growth, we assessed the growth of six algal species (*Chlorella protothecoides*, *Chlorella pyrenoidosa*, *Euglena gracilis*, *Scenedesmus* sp, *Hematococcus* sp and *Nitzchia* sp.) in the wastewater, in comparison with growth in a reference medium (WC). All species grew as well, or better, in the treated wastewater as in the reference medium, and no inhibitory effects were observed (Fig. 11). Naturally occurring algae in the treated wastewater also grew. This natural community included several species, mainly green algae and cyanobacteria. This algal community grew better than some of the cultured species, and would probably be well suited for use in algal based reclamation of wastewater.

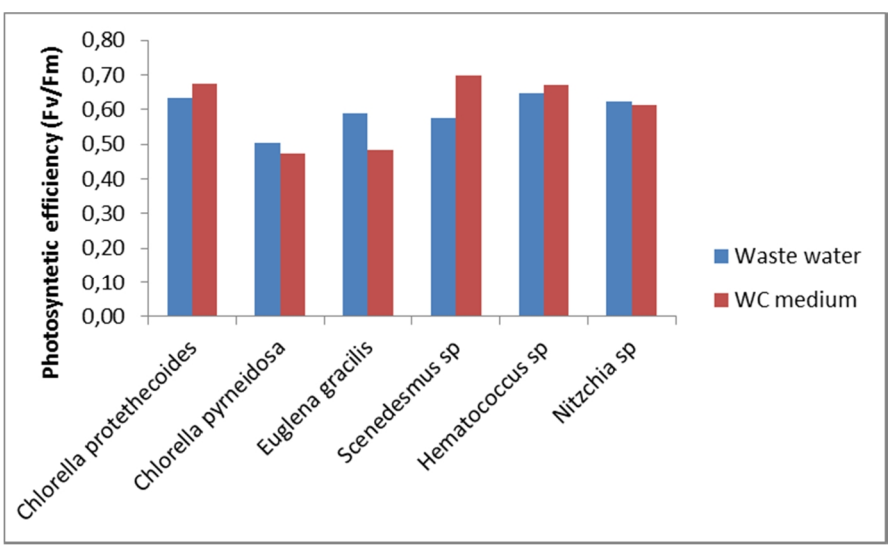

Figure 11. The photosynthetic efficiency (=variable fluorescence (Fv) / maximum fluorescence (Fm)) after 7 days incubation in waste water or WC medium. The photosynthetic efficiency is a parameter that can be used as stress indicator. Values >0.5 indicate viable, growing cells.

5.3 Screening in untreated wastewater

In order to determine the best species (Table 7) for growth in untreated wastewater, the wastewater was filtered through GF/D filters (1.5 µm) to remove particles and the natural community of algae. Algal growth was assessed in 100%, 50% and 10% wastewater, using de-ionized water for the dilutions. The growth of the algae was highly species specific. Some algae/cyanobacteria, like *Anabaena cylindrica*,

grew well in all concentrations of wastewater, whereas other species, e.g. *Cyclotella meneghiniana*, clearly grew less in non-diluted, 100% wastewater, than in diluted wastewater.

The inorganic nutrient concentrations in 100% untreated wastewater after filtration were approximately 5 mg phosphate (PO_4) per liter and 50 mg inorganic nitrogen (N) per liter. The N was mostly in the form of ammonium (NH_4). At high concentration, ammonium can be inhibitory or toxic for some algae, and species specific differences in growth probably reflect differences in tolerance to ammonium.

Table 7. The 18 species screened in filtered, untreated wastewater. The species indicated (*) were also used to assess potential benefits of algal communities (Section 5.4).

Species	Family
Scenedesmus obliquus *	Green algae
Selenastrum capricornutum	Green algae
Desmodesmus subspicatus	Green algae
Golenkinia brevispicula *	Green algae
Staurastrum tetracerum *	Green algae
Haematococcus pluvialis*	Green algae
Pediastrum simplex *	Green algae
Chlorella pyrenoidosa*	Green algae
Synechococcus sp.	Bluegreen algae /Cyanobacteria
Microcystis wesenbergii *	Bluegreen algae /Cyanobacteria
Anabaena cylindrica *	Bluegreen algae /Cyanobacteria
Chroococcus minutus	Bluegreen algae /Cyanobacteria
Planktothrix rubescence *	Bluegreen algae /Cyanobacteria
Synura petersenii *	Golden algae
Fragilaria crotonensis	Diatom
Navicula pelliculosa*	Diatom
Cyclotella meneghiniana *	Diatom
Nitzschia palea *	Diatom

5.4 The community effect

After initially screening the growth of individual algal species in untreated municipal wastewater, we further assessed how biodiversity (multiple algal species) would affect biomass growth and removal of nutrients using 12 species that grew well in wastewater (Table 7). Each specie was grown in monoculture and in random combinations of 3, 5 or 7 species (Fig. 12). Biomass and photosynthetic

efficiency (as a measure of algal health) were measured daily and inorganic nutrients weekly (e.g. Fig. 13).

Our main hypothesis was that high levels of biodiversity would increase the overall growth rate and nutrient uptake, compared to low levels. Different species have different environmental requirements and growth optima, and consequently occupy different biological niches in the system. Different algal groups harvest resources such as nutrients and light differently, for example by having different light harvesting pigments which absorb different wavelengths of the light. Thus some species are able to complement others, providing for more efficient overall use of the resources (i.e. uptake of light and nutrients) and greater overall growth, than any single specie alone.

Populations which contained more species produced more total biomass and removed more nutrients from the water (Fig. 13) than monocultures or populations with fewer species, supporting our original hypothesis. Communities of algae function better as biofilters, than monocultures.

Figure 12. Algal monocultures and populations of 3, 5, and 7 random species growing in untreated wastewater in 0.5 L tissue culture bottles. Cells were kept in suspension and pH was kept stable by bubbling the cultures with prefiltered (0.2 µm) air.

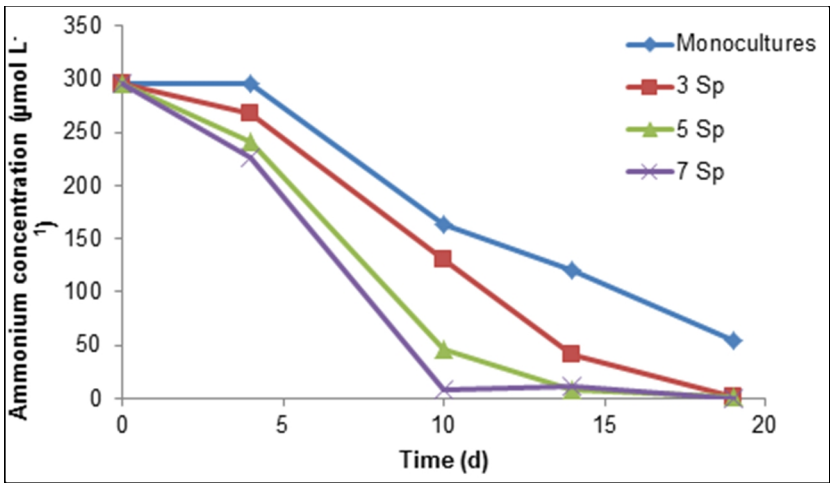

Figure 13. The average concentration of ammonium remaining in untreated wastewater inoculated with monocultures (average from 12 species, Table 7) or random communities of 3, 5 and 7 species (Sp). Ammonium uptake was correlated to increase in biomass (data not shown).

5.5 Potential algal biomass production in municipal wastewater

The nitrogen (~50 mg L^{-1}, NO_3 and NH_4) and phosphate (5 mg P L^{-1}) in the untreated wastewater used in these experiments supported growth of up to 1.5 g L^{-1} algal dry weight, in the best communities (measured during stationary phase). The biomass contained ~3% N and 0.3% P, which is relatively low compared to exponentially growing algae. The average influx of inorganic nutrients to Suomenoja is approximately 4.8 tons N and 0.7 tons P per day. Thus, one might extrapolate that up to 160 tons of algal biomass per day could be supported by the nutrients provided at Suomenoja. However, this probably represents the theoretical maximum achievable biomass production, since it is extrapolated from small, lab-scale experiments. The actual biomass production which could be achieved would be dependent on adequate light and CO_2 provision.

The light provision would also determine the area required to grow 160 tons of algae per day. An optimistic production estimate for southern Finland during summer would be 20–50 g DW m^{-2} d^{-1}, and at these production rates it would therefore require an area of 320–800 ha to grow 160 tons of algae per day.

5.6 Potential wastewater treatment using algae in Finland

Our results and reports in literature clearly show that algae take up nutrients from wastewater quickly and effectively in favorable growing conditions. In addition to reclamation of municipal wastewater, cultivation of algae offers several other benefits.

One benefit of algal cultivation is the algal biomass itself. This could be developed into various products, depending on the quality and composition of the biomass. Algal biomass typically contains high amounts of protein and may also be rich in lipids suitable for food or feed. However, algal uptake of contaminants such as heavy metals and persistent organic pollutants (POPs) would make it unsuitable for consumption, but it would still be suitable for biogas (Section 6) or other biofuel production, since these are not constrained to the same degree by contaminants.

Another benefit of algal cultivation is the capture of CO_2. The uptake of inorganic carbon in the form of CO_2 during photosynthesis causes the pH of the culture to increase until growth is no longer possible. In order to avoid CO_2-limitation and growth inhibiting pH changes, dense algal cultures need to be supplied with more CO_2 than is present in atmospheric air. Municipal wastewater treatment facilities which include algal cultivation should be located near a source of excess CO_2, such as industrial flue gas or a heterotrophic processes such as bacterial degradation of organic waste or a bioethanol plant.

The existing technology used at large scale wastewater treatment plants, such as Suomenoja, removes the nutrients very effectively with a much smaller areal footprint, compared with what algal cultivation would require. Large wastewater treatment plants tend to be close to large cities where the land area available for the process is limited. Algal technologies are not particularly well suited for these areas. However, algal cleaning of municipal wastewater might be suitable for small communities where the building of large scale wastewater treatment plants is too expensive.

One of the main factors that influence algal growth is light availability, and this is clearly an issue during wintertime in Finland. Adding artificial light is currently not a viable option. However, many rural areas have an increase in inhabitants during summer, as city-dwellers spend their holiday in the countryside, causing cyclical generation of wastewater with the peak during summer. Algal treatment of the wastewater could be a good solution, both economically and ecologically in these areas.

Key findings:

- Both treated and untreated municipal wastewater in Finland is suitable for algal cultivation.
- Communities of algae containing several different species would be more productive than single-species cultures.
- Algal wastewater treatment, especially in rural communities, could become a viable method of reducing the mineral content of municipal wastewater if the algal biomass could also be valorised.

6. Anaerobic digestion of algal biomass

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6.1 Introduction

Algae are an interesting feedstock not only for biodiesel production, but also for biogas production by anaerobic digestion (AD), which could be easily integrated in an overall concept, in which the lipids are first extracted for biodiesel and then the algal biomass is converted into biogas. In fact, AD is a key process for improving the economics and environmental effects of producing algae for biofuels, e.g. by providing nutrient recycling through recycling of the reject water from digestion. Although some doubts have been expressed concerning the economics for monodigestion of algae per se (Christi 2007), it is an optional energy production method, especially in cases when algae are grown primarily for the purpose of treating waste water

This section focuses on experimental biogas production from algae. Integrated production of hydrogen and biogas was also considered, with the aim of augmenting energy production in digestion. There are several studies on single-stage CH_4 production from algae (Golueke et al. 1957, Hernández and Córdoba 1993, Mussgnug et al. 2010, Sialve et al. 2009, Yen and Brune 2007) or lipid-extracted algal residues (Ehimen et al. 2009 & 2011) but only few studies on H_2 production from algae by dark fermentation (Carver et al. 2011, Kim et al. 2006, Nguyen et al. 2010, Su et al. 2009, Yang et al. 2010a, b ,c) or combined H_2 and CH_4 production in a two-stage anaerobic digestion process (Arkkola 2012).

The potential of any biomass for biogas, or methane, production depends on its biochemical composition and structure. In general, lipids have the highest methane potential of the organic macromolecules. But, even if the lipids are extracted from algae, the residual (waste) algal biomass is still a good source of proteins and carbohydrates and can be anaerobically digested. However, the cell wall may decrease their bioavailability and, in fact, the biodegradability of algae has been reported to be low in many studies. In this case, pretreatment of the biomass prior to AD could clearly improve biodegradability and increase methane yield. Another

challenge for AD is the high protein, i.e. nitrogen, content of algae, resulting in a potential risk of too high ammonia concentrations in the AD reactor .By co-digestion, that is mixing several complementary biogas substrates, the risk can be better controlled.

Hydrogen gas (H_2) has potential as a sustainable and environmentally friendly fuel, because water is the only by-product when it is combusted, and H_2 has a high energy density of 122 kJ/g (lower heating value). Dark fermentation or digestion can be a low cost strategy for biohydrogen production. However, the H_2 yield in combination with methane production is usually very low, especially in one-stage digestion where the produced hydrogen is quickly consumed by hydrogen utilising bacteria, acetogens and methanogens. In two-stage anaerobic digestion, the substrate is converted to H_2 and organic acids in the first stage, and the hydrogen consuming bacteria are concentrated in the second stage where acids are converted to biogas, which contains mainly methane (CH_4) and carbon dioxide (CO_2). Arkkola (2012) gives an overview of the biochemical reactions, optimal conditions and experimental studies in her master thesis. The two-stage process can be used to enhance the total energy recovery of the anaerobic digestion process, compared with single-stage CH_4 production.

6.2 Materials and methods

For studies of methane generation, microalgal strains were selected based on their availability. Three microalgal strains were used. *Chlorella pyrenoidosa* was available as a dry commercial product or as fresh biomass cultivated at the University of Helsinki (UH, Fig. 14). *Scenedesmus* was provided as freeze dried biomass, produced at Finnish Environment Institute and dried at VTT. *Selenestrum* sp. (the Finnish isolate described in Section 3) was produced at the University of Helsinki. Based on SEM analysis, freeze drying did not disrupt the algae cells (Fig. 15), making the dried algal cells comparable with the fresh algae.

The methane production potential of these algal species and the effects of specific pretreatments and lipid extraction on this potential were evaluated, along with the stability of the AD process when algal biomass was co-digested with biowaste or sewage sludge. *C. pyrenoidosa*, co-digested with fruit and vegetable waste (FVW), was used for combined H_2 and CH_4 production in two-stage anaerobic digestion. The total energy recovery of two-stage hydrogen and methane production from microalgal residues plus fruit and vegetable waste was compared with conventional single-stage methane production.

(a) (b) (c)

Figure 14. Algae as dried product (a) and suspension (b), and the centrifuged algal biomass (c).

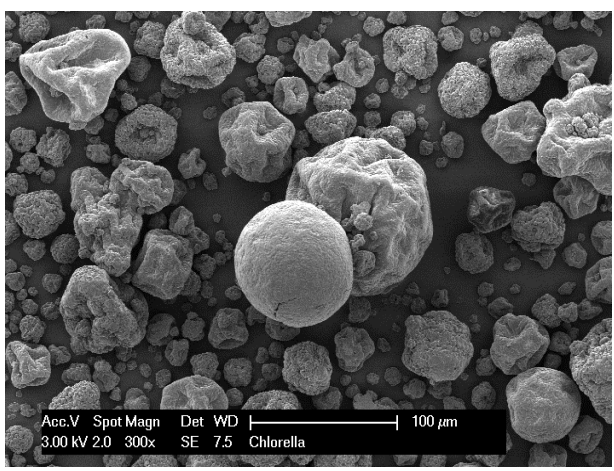
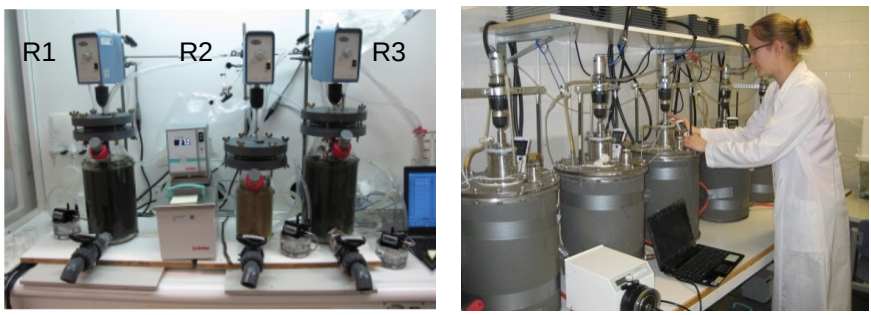


Figure 15. Scanning electron micrograph of the dried commercial alga *Chlorella pyrenoidosa* (courtesy of Kemira Oyj 2012).

Lipid extraction, a challenging step and subject of considerable R&D, was not a focus in this work and therefore a simple, proven, thermal alkaline method was used for bulk lipid extraction (Hiltunen 2011). In addition, the effect of several other pretreatment methods on methane production were tested, including formic acid and hydrogen peroxide (H_2O_2) treatments and supercritical CO_2-extraction (SCE, 80°C, 500 atm, 1 h; carried out at the University of Helsinki) (Viitaja 2012).

The methane production potential of both processed and untreated algal biomass was determined in batch tests with an automatic methane potential test system (AMPTS; Fig. 16). Biogas production and AD process stability were evaluated for algae co-digested with biowaste, glycerol or sewage sludge in mesophilic lab-scale reactors (10 and 3 l) operating semi-continuously for 4 to 12 months (Fig. 16b). The effect of chemical addition (BDP product, a trace element mixture supplied by Kemira Oyj) on AD process behavior was also assessed.

Semi-continuous, two-stage anaerobic digestion for biohydrogen production used two 4-L reactors in parallel with conventional single-stage anaerobic digestion (Fig. 16a). The experiment was started with fruit and vegetable waste as the sole substrate and later algal residues were added as 5–12% of the volatile solids (VS). Both the gas composition and individual VFA:s were analyzed (GC) for process optimization.



(a) (b)

Figure 16. Experimental systems, a) The CSTR reactor configurations: H_2-producing stage (R1) in the middle,CH_4-producing stage (R2) on the right, and reference single-stage CH_4 reactor (R3) on the left. (VTT/Arkkola) B) 10 liters CSTRs for AD application testing (HAMK/Kymäläinen 2012).

6.3 Methane production

Methane production from the algal species studied in this work varied from 220 to 280 ml gVS^{-1} (Fig. 17). The limited data on CH_4 yields from microalgal species suggest values between 100 and 400 ml gVS^{-1} could be expected. Typical conversions of algal organic matter have varied between 20–80% in AD. The high variation, both in methane yield and in organic matter conversion, has been explained by the differences in algal composition, the cell wall structure and its degradation in AD. Because of the strong, sturdy cell wall structure of the algae species studied here, a pretreatment may be needed to increase their biodegradability and thus the methane production rate in the AD process.

Thermo-alkaline lipid extraction clearly increased (by 33–34%) and accelerated CH_4-production (Fig. 17, Hiltunen 2011). Correspondingly, VS-conversion in AD increased from *ca*. 40% (original algae) to 60% (pretreated algae). However, washing (for lipid removal/extraction) of the algal biomass after thermo-alkaline treatment resulted in a high biomass loss (ca 46–50% of total solids), thus decreasing the CH_4 yield when calculated based on the original algal biomass (Fig. 17). More methane could be produced from the organic matter left after extraction (301 and 283 liters CH_4 kg $^{-1}VS^{-1}$, for *Chlorella* and *Scenedesmus*, respectively) than from untreated algal cells, but taking into account the mass loss, the methane

yields (148 and 138 liters CH_4 kg $^{-1}VS^{-1}$) were only 63–64% compared to the original biomass specific yield. If AD is to be integrated with a biodiesel production process, the method of lipid extraction should avoid this loss of other organic matter or design a system for recycling the non-lipid organic components back into the AD process.

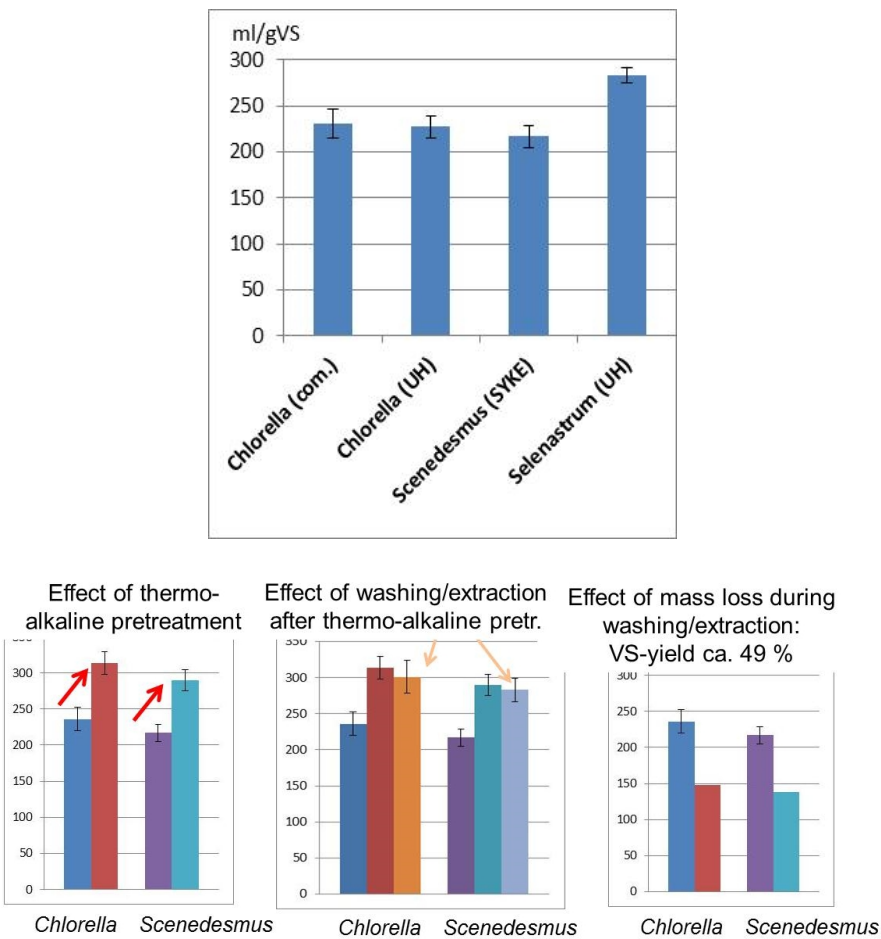


Figure 17. CH_4- production using biomass from different algal species, and the effect of lipid extraction on methane production.

Treatment with formic acid at room temperature had no effect on methane production (Viitaja 2012). H_2O_2-treatment together with BDP-chemical addition increased CH_4 production by ca 10%. The best results were achieved when the algal biomass was treated with supercritical fluid extraction, resulting in a 22% increase in specific methane generation. The CH_4 production rate was also clearly increased

by SCE treatment (Fig. 18). The extremely high pressure (500 atm) used in SCE was apparently effective in making organic matter of algal cells more bioavailable and biodegradable for methane production. An advantage of SCE is its selectivity for neutral lipids with minor losses of other components of the algal biomass. However, it should be noted that, in contrast to the tests with thermo-alkaline extracted biomass, the tests carried out on SCE treated biomass did not involve removal of biomass (i.e. no lipids were extracted).

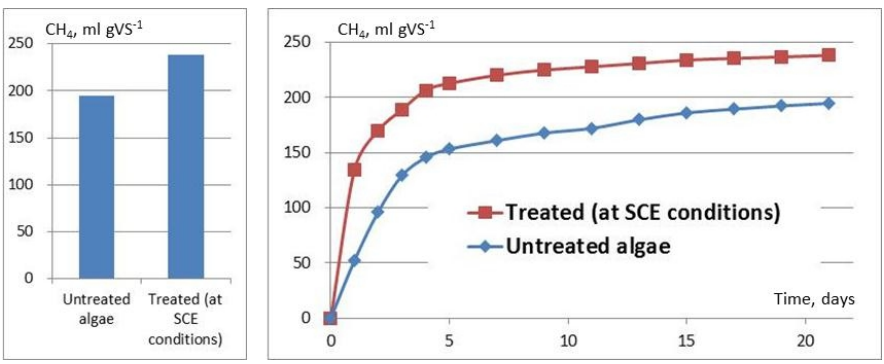
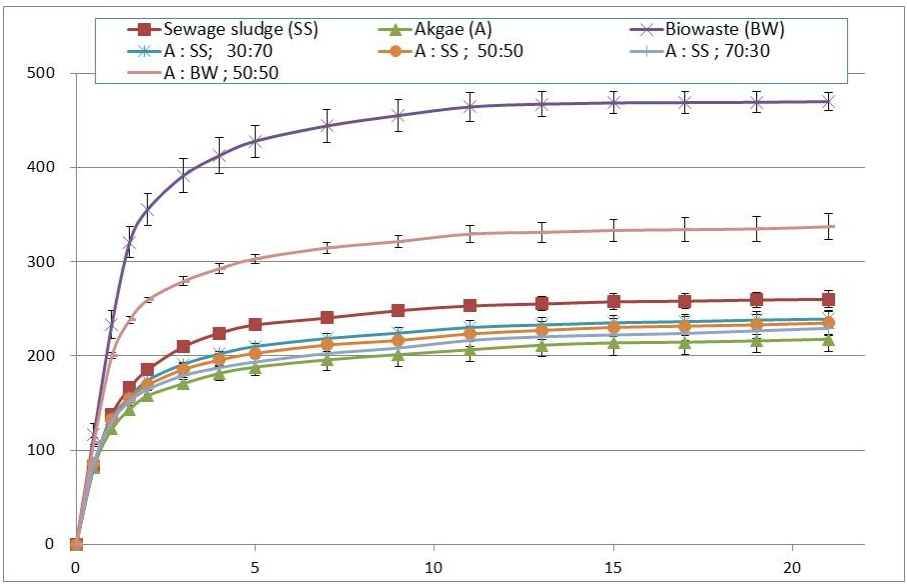


Figure 18. Comparison between methane production of SCE treated and untreated *Chlorella pyrenoidosa*.

When *C. pyrenoidosa* biomass was co-digestioned with municipal biowaste or sewage sludge the amount of methane generated by the mixtures corresponded well with the values calculated based on the composition of the mixture and previously measured production from each of the components (biowaste / sludge and algae). No additional increase in methane production was allocated to the inclusion of algae as a nitrogen-rich co-substrate (Fig. 19).



	ml CH_4 gVS^{-1}	
	Measured	Calculated
Biowaste (BW)	470	
Sewage sludge (SS)	260	
Algae (A)	218	
A : SS, 30:70	239	247
A : SS, 50:50	235	239
A : SS, 70:30	229	231
A : BW, 50:50	337	344

Figure 19. CH_4- production from biowaste and sewage sludge and mixtures of these with *Chlorella pyrenoidosa*. The table compares measured values with those calculated for the mixtures based on the individual components.

6.4 Biogas (AD) process performance

Co-digestion, rather than mono-fermentation, is recommended for algae because of the relatively low C/N-ratio of the biomass. The alga *C. pyrenoidosa,* with a C/N ratio of ca. 6, was co-digested with municipal biowaste (in a 1:1 mixture, based on VS) in long term lab-scale CSTR (completely stirred tank reactor) tests (Table 8). Mixing with biowaste increased the C/N ratio to ca 10. In some tests, the carbon/nitrogen ratio was further increased up to 20 by adding glycerol. The algae were used as such, or were first lipid-depleted using the thermo alkaline method. In addition, co-digestion with sewage sludge, which also has a low C/N, was studied (Table 8).

Table 8. Substrates co-digested in AD.

Reactor	Substrates
A	untreated *Chlorella* algae + biowaste, 1:1 (VS-ratio)
B	*Chlorella* non- lipid residue + biowaste, 1:1 (VS-ratio)
C	as in A, but with addition of a BDP-trace element mixture
D	as in A, but with addition of glycerol (-> C/N = 20)
E	as in B, but with addition of glycerol (-> C/N = 20)
F	municipal sewage sludge
G	sewage sludge + *Chlorella* algae, 1:1 (VS-ratio)
H	*Chlorella* algae

The co-digestion processes with biowaste (reactors A–E) performed well up to an organic loading rate (OLR) of 5 kgVS $m^{-3}d^{-1}$, and a hydraulic retention time (HRT) of 20 days (Fig. 20). Signs of process instability, for example increases in VFA concentrations, were noticed first in reactor (B), co-digesting treated algae with biowaste. At loads < 5 kgVS $m^{-3}d^{-1}$ this reactor demonstrated ca 10% higher biogas production and methane content compared to biowaste with untreated algae (B vs. A), but the ammonium concentrations were also the highest of all conditions tested, up to *ca*. 5 g $liter^{-1}$. Thus, at loadings over 5 kgVS $m^{-3}d^{-1}$, lower biogas yields were obtained and biodegradation occurred. This was also observed in the processes supplemented with glycerol in the feed (D and E). BDP-chemical addition clearly stabilized the AD process (C vs. A), as well as helping to recover the process after a severe instability. Fig. 20 shows the VFA- and ammonium concentrations in the reactors during a test period of *ca.* 10 months.

Because of the time limits of the project, the co-digestion process with sewage sludge (reactors G vs. F and H) was not tested at higher OLR than 4 kgVS $m^{-3}d^{-1}$, with a HRT of 20 days. All reactors generated *ca*. 300 l biogas $kgVS^{-1}$ with a CH_4 content of *ca*. 60% for each reactor. Mono- digestion of algae (H) resulted in quite high CH_4 production but the process became instable, that is the VFA concentrations in the reactor started to increase after the load was increased from 2 to 3 kgVS $m^{-3}d^{-1}$.

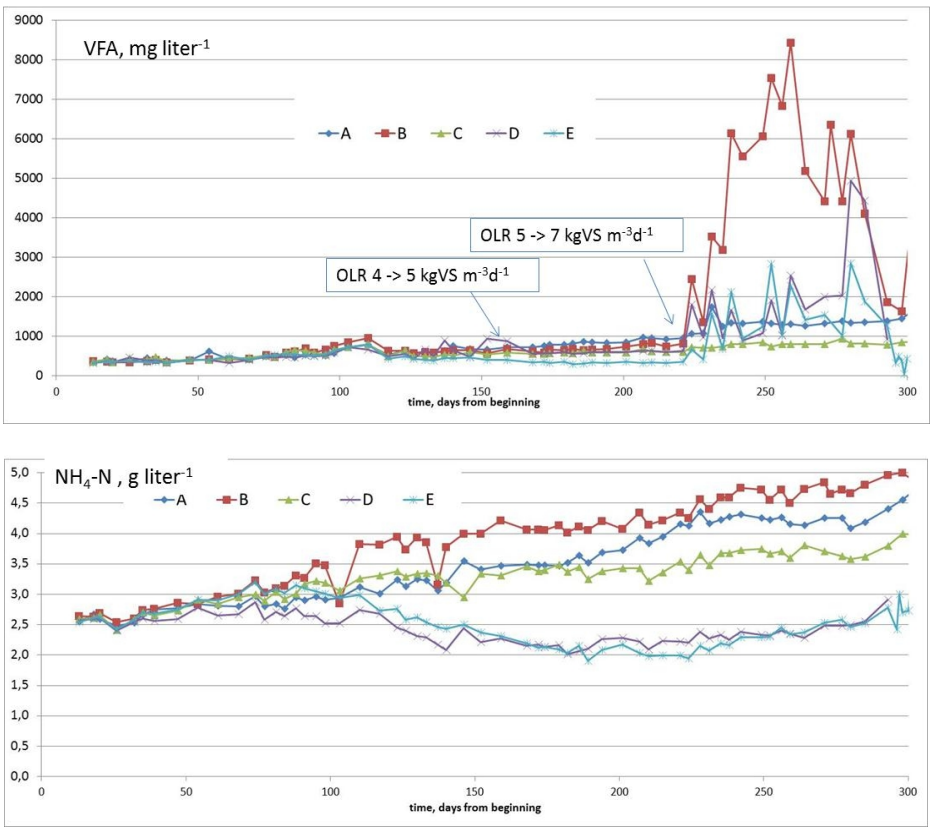


Figure 20. VFA- and ammonium concentrations in reactors A, B, C, D and E (Table 8).

6.5 Combined biohydrogen and methane production

Hydrogen could be produced from FVW waste when co-digested with lipid-extracted algal residues. The maximum H_2 yield (48 ml/g-VS) obtained in co-digestion was similar to that reported in the literature. However, it was significantly lower than the theoretical maximum yield and the maximum H_2 content of the gas produced was only 19% (Fig. 21).

The CH_4 yields obtained both in the two-stage and in the single-stage processes were low compared with literature results obtained with similar substrates (Arkkola 2012). The low methane production was probably not caused by the addition of algae, but by unstable process conditions in the first stage. Fluctuating pH and effluent composition in the first stage caused accumulation of acetate in the second stage, which probably inhibited methane production.

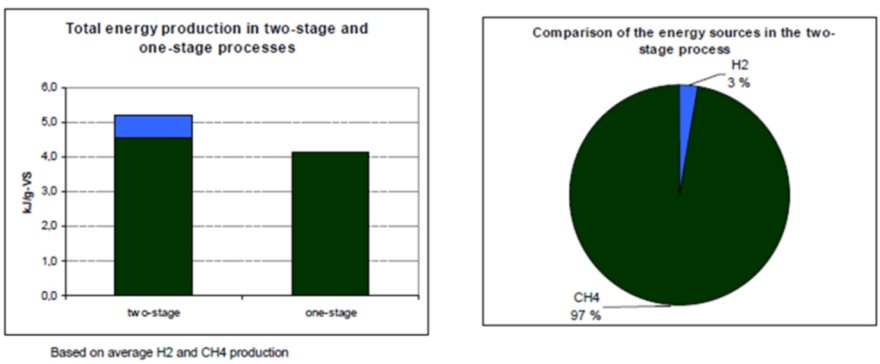


Figure 21. Comparison of the average total energy production in two-stage and one-stage anaerobic digestion processes.

6.6 Conclusions

The algal strains studied are good sources for methane production, producing 220–280 liters CH_4 per kg organic matter (VS), corresponding to an energy content of 2.2–2.8 kWh $kgVS^{-1}$. CH_4 production from algae is similar to that of several cultivated crops. Thus, the difference in biogas energy yield per hectar will be determined by the difference in biomass productivity of the algae and plants.

Because of the strong, sturdy structure of the cell wall of these algal species, we recommend that pretreatment should be used to increase the biodegradability and methane production rate in the AD process. Indeed, we found that the lipid extraction process for biodiesel production may serve as such a pretreatment, enhancing the biodegradability of the residual algal biomass from the process. A clear increase in methane production amount and rate was found by both super critical CO_2 (SCE) and thermo-alkaline treatment. The SCE method would be more beneficial than the thermo-alkaline extraction, since it has greater selectivity for extracting neutral lipids and minor loss of other organic matter. Oxidative hydrogen peroxide treatment plus supplementation with some trace elements (BDP product of Kemira) also slightly (*ca*. 10%) increased CH_4 production.

The AD process experiments verified that algal biomass was a suitable co-substrate in AD plants, but demonstrated that it requires careful monitoring of ammonium nitrogen concentration in the AD reactor. The risk is higher in processes using treated (lipid-extracted) algal biomass because of the greater bioavailability of organic matter, and thus the increased release of ammonium nitrogen, coupled with the reduction in the total carbon available with the removal of the lipids. Trace element addition (Co-Fe-Ni) to AD process was found to be beneficial when municipal biowaste was used as co-substrate with the algae. This may indicate that even though a substrate like biowaste contains sufficient concentrations of trace elements, these may not be in a bioavailable form.

The aim of the biohydrogen generation study was to evaluate the total energy recovery of the two-stage H_2 and CH_4 production, compared with a conventional single-stage CH_4 production. The suitability of the selected substrates for H_2 production was also assessed. The two stage processes using fruit and food waste with algae showed that combined hydrogen and methane production was possible. However, the process was sensitive to process changes. Careful control of pH would be essential when processing high sugar and nitrogen containing substrates. Maintaining constant pH was unfortunately very difficult for these tests, since automatic pH control was not available.

In an algal based biorefinery, multiple biofuels can be produced in a combined process concept, together with co-production of some value added compounds. Anaerobic digestion can play a key role, providing synergistic benefits within the concept.

Key findings:

- 220–280 L $kgVS^{-1}$ methane can be produced from algal biomass.
- Pretreated algal biomass is more digestable and generates more methane in the AD process, but the pretreatment should be designed to preserve as much organic content as possible for conversion in the AD.
- The methane generated in co-digestion processes is determined by the potential of each of the components of the mixture.
- The high nitrogen content of algae, particularly after lipid has been extracted, may lead to process instability an AD plant and should be carefully monitored.
    - Addition of trace elements to the process may improve both process stability and total methane production.
- Two stage processes which include hydrogen production should be further developed to enhance energy output from AD plants.

Acknowledgements: We thank project engineer Laura Kannisto (HAMK) for her skillful practical work and exchange student Miriam Meyer from the Hamburg University of Applied Sciences for her help in practical research. Heli Hiltunen and Tuomo Viitaja are acknowledged for the research they carried out within this project for their Bachelor theses.

7. Techno-economic feasibility of microalgae-based energy in Finland and globally

Eemeli Hytönen, VTT

7.1 Introduction

Various forms of bioenergy can be produced from algae. These include biodiesel through transesterification of the algal lipids, biogas from anaerobic digestion of the algal biomass (after or without lipid extraction), and heat and electricity from combustion of the algal biomass or burning of biogas produced from it. Suitable sources of carbon, nutrients and energy are key requirements for the growth of algae. The source of carbon can be CO_2 or sugars originating, for example, from agricultural side streams. Nutrients for growth can be either purchased (commercial fertilizers) or waste-dervied, such as from waste water. Energy can be in the form of sunlight or chemical energy from consumption of organic compounds. An overall scheme of some microalgae-based energy production alternatives is shown in Fig. 22.

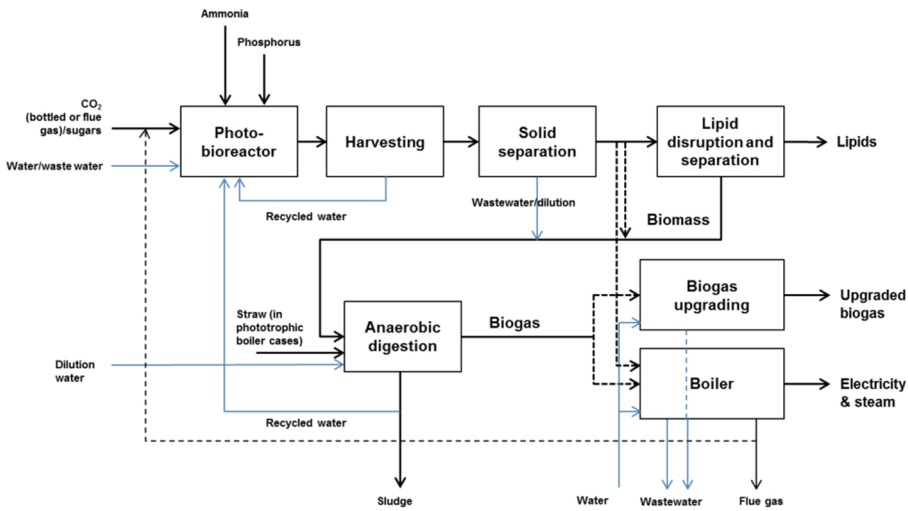


Figure 22. Block-flow diagram of microalgae-based energy production. Dashed lines represent alternative configurations.

7.1.1 Objective

The goal of the techno-economic and greenhouse gas emission analysis was to compare different concepts of microalgae based energy using mill level economic and environmental criteria. The basic designs and the process variables were defined with other work packages, i.e. the economic potential of experimental research from the ALDIGA project was assessed in selected process contexts. The research results used were specifically related to waste water characterization, algal growth in waste waters, lipid accumulation in the selected algal strains, growth condition optimization, and biogas production using algal biomass.

7.1.2 Algae based energy – processes

The main process steps in microalgae-based energy production are cultivation, harvesting, lipid extraction, and residual biomass processing. In addition, various systems for supply of feedstock and utilities, and recycling of water and nutrients are required – these can also be parts of the residual biomass processing step, such as anaerobic digestion for water and nutrient recycling, or boiler for CO_2 supply.

Algal cultivation can be carried out in an open system (pond), a closed system (photobioreactor – PBR) or in a combination of them. Most of the operational and biological factors favour closed systems over open systems, but open systems have lower investment and operation costs compared to closed systems (Mata et al. 2010, Caravalho et al. 2006, Pulz 2001). The main benefit of closed systems is

their better operational control (e.g. contamination, mixing, light, CO_2 losses, and algal specie flexibility).

There are also various methods available for harvesting (Table 9). All of the methods are applicable to both cultivation techniques; however some of them need a pre-harvesting step to increase the biomass concentration so that the input concentration is suitable for the main separation technique, as defined either by physical requirements of the technique or the capital costs of the separation equipment.

Table 9. Comparison of algal harvesting methods (Udumann et al. 2010). TSS = total suspended solids.

Dewatering process	Highest possible yield	Energy usage
Centrifugation	>22% TSS	Very high - 8 kWh/m^3
Flocculation	>95% removal of algae	Low for slow mixing: varies largely
Natural filtration	1-6% TSS	Low (vibrating screen) - 0.4 kWh/m^3
Pressure filtration	5-27% TSS	Moderate (chamber filter press) - 0.88 kWh/m^3
Tangential flow filtration	70-89% removal of algae	High - 2.06 kWh/m^3
Gravity sedimentation	0.5-1.5% TSS	Low (lamella separator) - 0.1 kWh/m^3
Dissolved air flotation	1-6% TSS	High - 10-20 kWh/m^3
Dispersed air flotation	90% removal of algae	High
Electrocoagulation	99.5% TSS	Medium to high - 0.8-1.5 kWh/m^3
Electroflotation	3-5% TSS	Very high
Electrolytic flocculation	>90% removal of algae	Low to medium - 0.33 kWh/m^3

The most common methods for lipid extraction are mechanical separation (70–75% lipid extraction can be obtained) and solvent or oil extraction (over 95% extraction efficiencies reported). More recently supercritical fluid extraction using CO_2 or other fluids has also been studied. It should be noted that solvent extraction requires drying of the biomass (up-to 95% solids content needed; spray, drum or sun drying can be used) prior to the extraction. On the other hand, mechanical disruption can be carried out with wet biomass (~20% solids content).

Residual algal biomass processing can include anaerobic digestion (algal biomass alone or co-digested with other waste/biomass streams) from which the main product is biogas. The digestate is also valuable: the liquid can be recycled to algal cultivation to provide nutrients. Another alternative is to dry the residual biomass, mix it with other solid fuels and combust it, or if the quality is sufficient, to sell it as fertilizer or animal feed.

7.2 Techno-economics and greenhouse gas emissions of selected microalgae-based biofuel concepts in Finnish conditions

7.2.1 Case study definition

Four main cases were defined to evaluate the economic and environmental benefits resulting from the technology development and experimental work of the project. The cases represent different overall energy production concepts:

1) Phototrophic production (using purchased CO_2) to lipids and upgraded gas
2) Phototrophic production (using self-produced CO_2) to lipids and electricity
3) Heterotrophic production (using glucose and xylose from straw as carbon source) to lipids and upgraded gas
4) Heterotrophic production (using glucose and xylose from straw as carbon source) to electricity.

Several subcases were analysed to test other research concepts: no lipid extraction, integration into wastewater treatment, combustion of the alga without lipid extraction. The configurations are illustrated in Fig. 22.

In the techno-economic analysis, mass and energy balances were calculated using Balas (http://balas.vtt.fi/). These were used for a) the main equipment dimensioning for investment cost estimation, and b) manufacturing cost and revenue calculation. These costs and revenues were further converted to investment project profitability estimates, using return on investment (ROI) as the measure. Finally, the sensitivity analysis focused on the main process and cost variables.

In the life cycle assessment, the greenhouse gas emissions of the concepts were assessed using KCL-ECO (http://www.vtt.fi/research/technology/kcl_eco_software.jsp). A cradle to gate approach was used. The common emissions were allocated to all products using their heating values. Process emissions directly related to a specific product were allocated 100% to that product. A sensitivity analysis of the GHG emissions on the key system parameters was also conducted.

7.2.2 Assumptions

A 32500 dry ton algal biomass/year facility using photobioreactor-based cultivation was considered in all cases. The facility was assumed to be located in Finland and to be operational 214 day/year, based on availability of sunlight. The nutrient demand was calculated from a generic algal biomass formula $CO_{0.48}H_{1.82}N_{0.11}P_{0.01}$ (Christi 2007). The productivity of anaerobic digestion was defined, based on project results, to be between 0.2 and 0.25 m^3 CH_4/ton dry solids (60% CH_4). The specific electricity consumption of the process operations were based on literature that used similar process condition assumptions: Cultivation 0.020 MWh/t flow

(AlgaeLink 2007) harvesting and extraction 0.056 MWh/t flow (Molina Grima et al. 2003, Benemann and Oswald 1996, Green et al. 1995), anaerobic digestion 0.0005 MWh/t biomass (Humbird et al. 2011) and gas upgrading 0.3 kWh/m^3 (Electrigas Technologies 2008).

The equipment investment cost estimates were scaled to the study capacity from the same literature sources that were used for electricity consumption. This ensures better traceability of the results. The total delivered equipment costs were converted to total project investment using a Lang factor of 4. The capital costs were annualized, using a capital recovery factor of 10%; different project financing alternatives were not considered.

Prices used in the techno-economic analysis are shown in Table 10.

Table 10. Prices.

Hydrolysis	**Price**	**Unit**	**Reference**
Straw	52,5	€/t	von Weymarn (2007)
H_2SO_4	120	€/t	von Weymarn (2007)
NaOH (pH control)	250	€/t	von Weymarn (2007)
Enzymes	148	€/t	von Weymarn (2007)
Algal cultivation			
CO_2 (pure)	20	€/t	Ruohonen and Tamminen (2009)
Ammonia	375	€/t	Hemming (2011)
Diammonium phosphate	430	€/t	Hemming (2011)
Make-up algae	100	€/t	Estimate
Waste biomass	20	€/t	Estimate
Utilities			
Clean water	0,8	€/m^3	von Weymarn (2007)
Cooling water	0,03	€/m^3	von Weymarn (2007)
Steam	35	€/MWh	von Weymarn (2007)
Electricity	45	€/MWh	von Weymarn (2007)
Products			
Lipids (pure)	400	€/t	Ruohonen and Tamminen (2009)
Biogas (upgraded)	450	€/t	Energiamarkkinavirasto (2012)
Lignin residue	25	€/t	Estimate

7.2.3 Techno-economic feasibility

The detailed operation and maintenance, and investment cost breakdowns of all cases studied can be found in Anna Leino's master thesis (Leino 2012). As an example, only the four main cases are shown in Fig. 23 (delivered equipment costs) and Fig. 24 (total production costs).

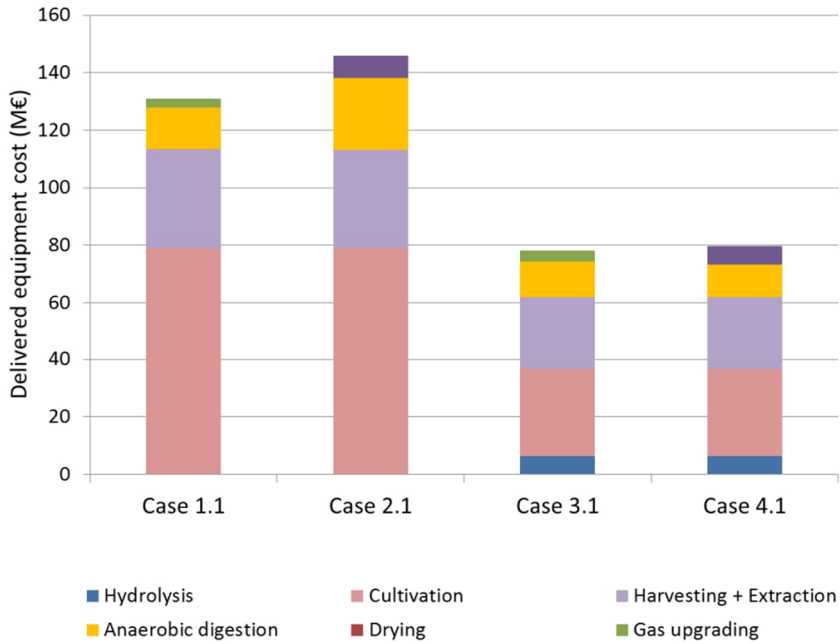


Figure 23. Delivered equipment cost estimates in the four main cases.

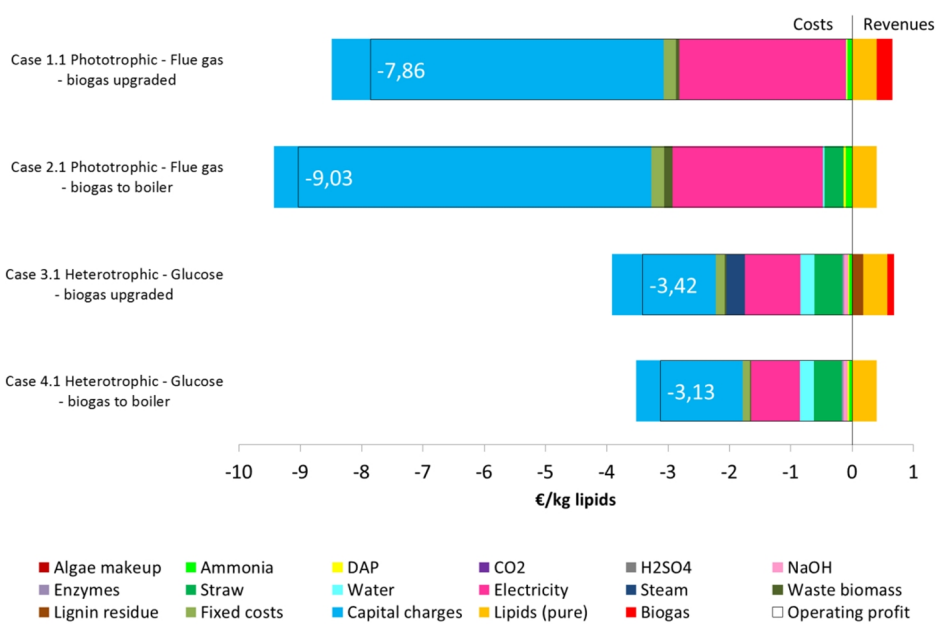


Figure 24. Production costs in the four main cases.

It was clear from the investment costs that the total project investments of the designed plant would be in the range of 300 to 450 M€. These translate into very high capital cost per kg of lipids. In addition to the high capital charges, electricity costs contribute a major part of the production costs. Clearly, none of the cases presented would have positive profitability.

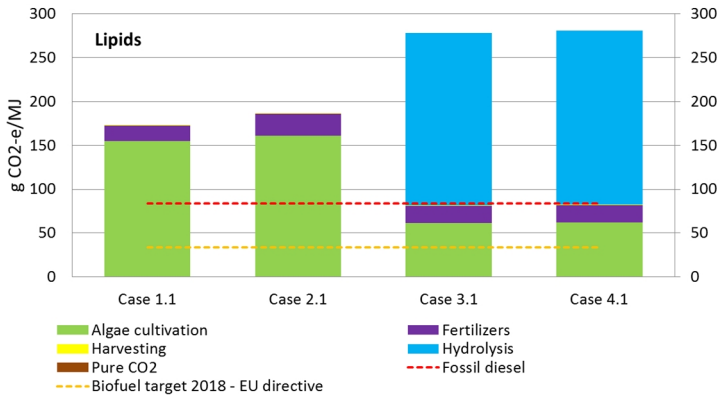
Other subcases showed similarly low economic performance: The cases producing only electricity would have no revenues because the selected designs would consume more electricity than it would be possible to generate from the algal biomass. Cases producing only biogas have a higher gas production rate than the two-product cases presented here, but due to the relatively low market price, the revenues still remain low compared to the production costs.

Economic performance is most sensitive to algal productivity. However, a significant improvement would be required to make any of these designs profitable.

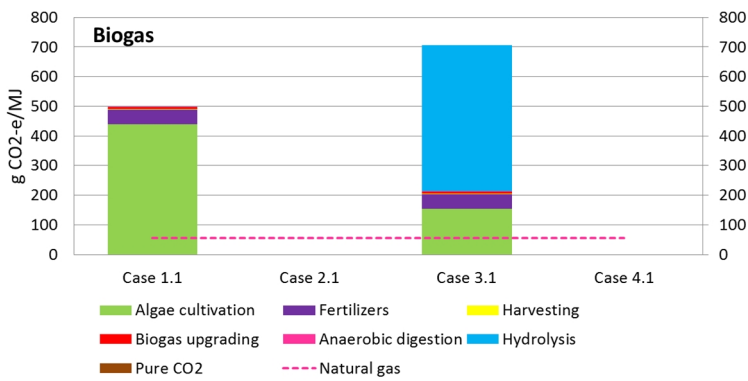
7.2.4 Environmental assessment

Detailed description of the LCA analysis process, selections regarding the goal and scope, methods and data used, and all results are given by Leino (2012). Fig. 25 shows the environmental performance of the same cases as for economic analysis.

Cultivation (including all processing steps shown in Fig. 22) and, in heterotrophic cases, sugar production (hydrolysis) have the largest impact on GHG gas emissions. These emissions can be further traced to electricity consumption for cultivation and enzyme manufacturing. (Database values were used for emissions from Finnish electricity mix and enzyme manufacturing). The high electricity consumption that was assumed in this work lowers both the economic and environmental performance. Overall, none of the products perform as well as their corresponding fossil energy product, even though the emissions from the last step of lipids-to-diesel have been excluded from this analysis. The EU biofuel GHG emission level target for 2018 is even more challenging to achieve with the selected designs.



a)



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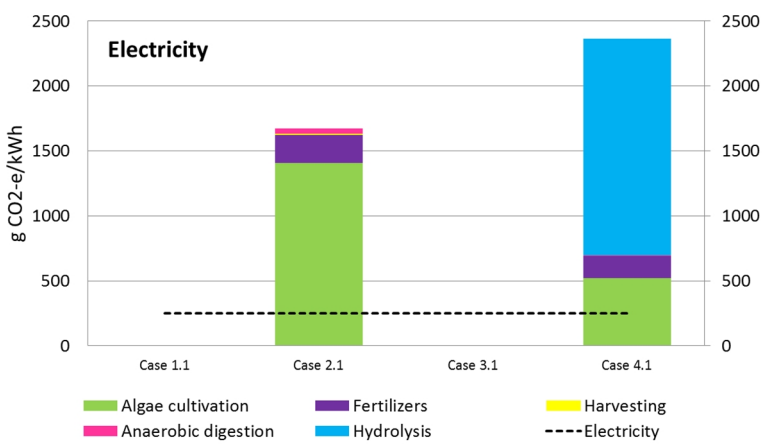

c)

Figure 25. GHG emission breakdown by life cycle phase in the four main cases. Emissions are allocated to all products of the case: a) lipids, b) biogas, and c) electricity.

7.3 Conclusions

Several process concepts for algae-based energy production (PBR as cultivation system) were analysed to demonstrate the potential of the research results in the project. Using the experimental results and the assumptions presented above, none of the selected concepts showed overall good economic or environmental performance. The high capital cost requirements, high electricity demands of the concepts, and the GHG emissions of sugar production from lignocellulosic feedstock are particularly pronounced in the analysis. On the other hand, similar production cost and GHG emission levels have also been reported in the literature as current "state-of-the-art" cost of production (Davis et al. 2011) and environmental impacts (Aquafuel 2011). Moreover, the technologies are still in developmental stages and therefore such performance could be partly expected.

Higher productivity, meaning higher growth rate and higher end-biomass density from the cultivation, in heterotrophic cases lowers the investment cost requirement of the cultivation system significantly. At the same time, higher cell density in the cultivation in the heterotrophic case lowers the electricity demand for harvesting and the GHG emissions from harvesting. Thus, improving algal productivity and optimal, high PBR consistency are key technical factors directly impacting economic and environmental performance.

Algae-based energy developmental efforts around the world are focused on cultivation system design, algal specie selection, biomass harvesting and lipid separation techniques, integration into waste management, potential for carbon capture, and identification of suitable product portfolios (by-product selection/design), along with other factors. The cultivation system is of paramount importance for the economics and various systems are under development, including open pond systems, photobioreactors and dark fermentation (heterotrophic) systems. For example, some companies are pursuing a heterotrophic, dark fermentation approach (e.g. Solazyme, and Neste Oil), utilising algae (or other microbes) which can grow without energy from sunlight and thus avoiding the density constraints for sufficient sunlight penetration. This approach can potentially offer more significant cost and environmental impact reduction than the phototrophic cases analysed in this work. On the other hand, cost and emissions associated with sugar production might increase the impact, since the energy needed to grow the algae is obtained from sugar, which is obtained from crops with lower biomass yield per unit area than algae. Different PBR designs, including plastic bags of different shapes and sizes, tubular pipes, and flat plates/panels, also offer different benefits from the low construction costs of plastic bag designs to the long lifetime of tubular pipe systems. Sunlight energy is a key benefit for these cultivation methods. The lowest cost cultivation system, the open pond, could also provide a solution if contamination and evaporation issues are solved, for example by suitable location of the plant and selection of the algal specie or algal multiculture.

The target of algae-based energy R&D has been the development of a sustainable domestic transportation biofuels sector. Significant public and private investments

have been made by companies in the USA, aiming at technology demonstration (including companies such as Sapphier Energy, Solazyme, Algenol, Heliae and Cellana). Similarly, development of transportation fuels has also been in focus in Europe and Asia. E.g. Neste Oil and A4F in Europe, and World Health Energy Holdings in India have biofuels as the main product in their technology development. However, other products from algae have also been studied, developed and commercialized e.g. applications for aquacultures, cosmetics and other purposes. The economic and environmental performance of the algae-based energy systems can be expected to improve as more understanding about the most suitable, local overall concepts and the technological solutions is obtained.

Key findings:

- Technological developments are still needed if algal biofuels are to become profitable.
- Strategies are needed to reduce high electirical costs associated with harvesting algal biomass and with cultivation (particularly if photobioreactors are used).
- Investment costs should also be lowered by improving the productivity of the algae used.
- Using current technologies (with photobioreactors), the green house gas emission from an integrated algal process would be higher than could be achieved from fossil fuel sources.

Acknowledgement: The case study evaluation summarized in this section is mainly the work of Anna Leino, and has been presented in her Master's Thesis (Leino 2012).

8. Fractionation of residual algal biomass – potential for value-added products

Jaakko Pere, Maija Mattinen, Taina Ohra-aho and Tiina Liitiä, VTT

8.1 Introduction and motivation

Demand for plant proteins has steadily increased around the world and is expected to explode in the near future, due to a lack of sufficient meat proteins. Technologies for protein isolation and enrichment will be crucial to provide adequate nutrition. In addition to, e.g. rapeseed press cakes and cereal cell wall materials, substantial amounts of proteins could be available from algae. The functional and nutritional value of these protein fractions may be utilized for several purposes, if successfully fractionated from the cell matrix. In addition, algal polysaccharides may provide an interesting raw material for the development of novel sustainable biomaterials for replacement of oil-based synthetic materials. Hence, fast and reliable analytical methods are required to for biomass fractionation, especially those which preserve the protein and polysaccharide components present.

Defatted micro-algal biomass could become available in vast quantities as a by-product from biofuel production. By targeting added value for the residual biomass the cost-effectiveness of the algal biofuel concept would be improved.

The objectives of the algal fractionation study were:

- to **fractionate** algal biomass for alternative end uses within material science,
- to isolate **polymeric constituents** of algal biomass from the fractions, and
- to develop a tool kit for the **analysis** of the chemical composition and structural features of algal biomass.

8.2 Fractionation

Dried *Scenedesmus* sp biomass, produced at the Finnish Environment Institute in an earlier project and dried at VTT, was used as the raw material for the study. The extraction procedure is shown in Figure 26. High shear mechanical disintegra-

tion for efficient cell wall disruption was achieved by passing an algal suspension through a pair of chambers at high pressure. Half of the disrupted sample thus attained would be subjected to protease treatment, prior to successive extractions in water, alkaline and acidic conditions. After each step the insoluble fraction was separated, washed and used in the next treatment. Chemical composition and analysis of molar mass distribution was carried out for the insoluble and soluble fractions thus obtained.

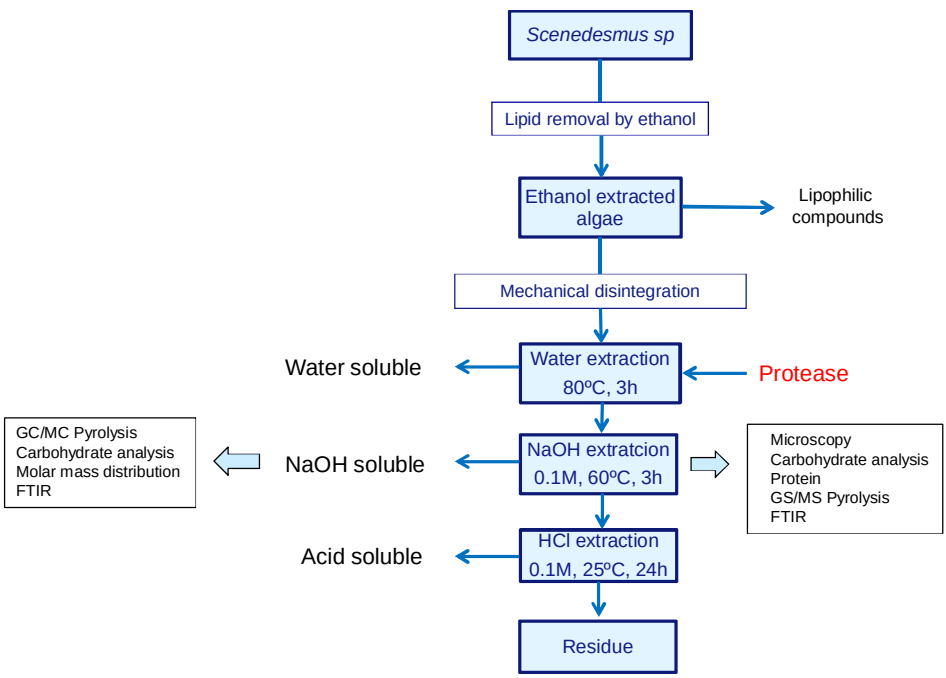

Figure 26. The fractionation procedure for *Scenedesmus* sp. biomass.

The cell wall of the microalga *Scenedesmus* was quite resistant to mechanical and chemical extraction. After successive extractions in water, 0.1 M NaOH and 0.1 M HCl, only about 48% of the algal biomass had been dissolved (Fig. 27a). Including a protease treatment in the fractionation procedure increased solubilisation substantially: the yield of the water-soluble fraction increased from 34% to 56%, compared with the untreated reference, with a total extraction of 67% after all steps. Mechanical treatment further enhanced solubilisation of biomass components, especially when combined with the protease treatment (Fig. 27b). Protein analysis of the biomass residues after water extraction demonstrated that the protein content was reduced from 45.4% (prior to extraction) to 27.6% when protease was present during the extraction, but only to 44.0% when it was not.

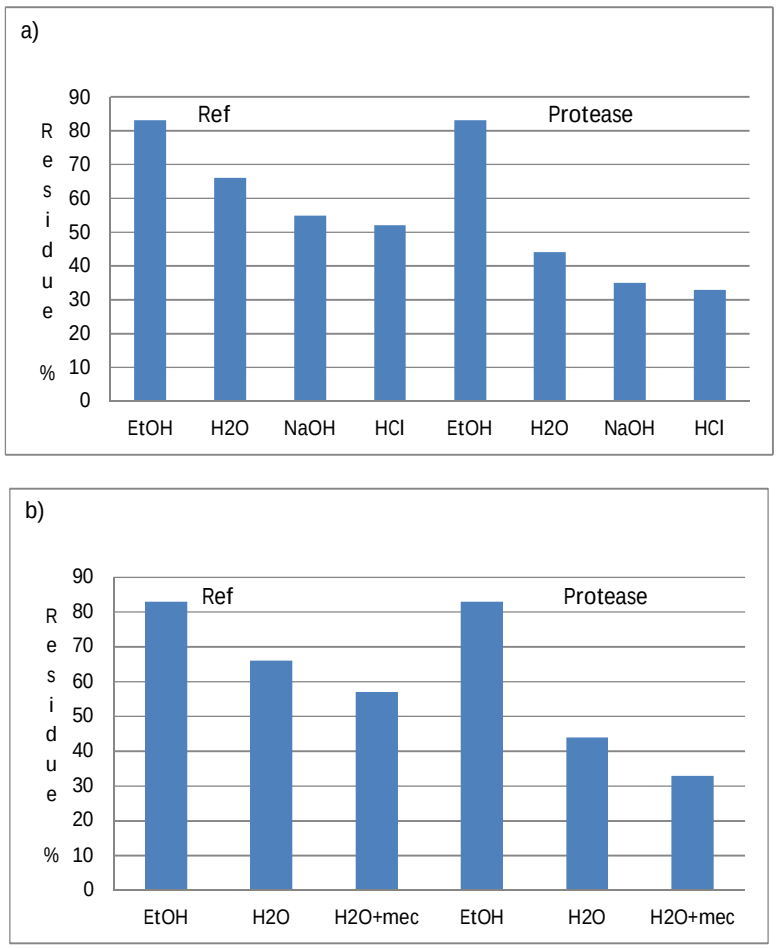

Figure 27. The fractionation efficiency of *Scenedesmus* sp. biomass after different processing steps, expressed as insoluble residual biomass (wt-%). Biomass residues from fractionated algal biomass after ethanol, water, alkali and acid extraction steps, with and without protease treatment (a), and the effect of mechanical treatment on the yield of the water extraction (b).

8.2.1 Microscopy

Intact *Scenedesmus* cells were visible microscopically as green single cells and clusters (Fig. 28a). Although autoflurescence interfered with the analysis, epifluorescent microscopy and differential staining of thin sections revealed that lipids and protein were present in abundance (Fig. 28a and 28b). β-Glucan and related polysaccharides were detected as cell wall constituents by staining with Calcofluor (Fig. 28c). Starch was observed in some of the cells.

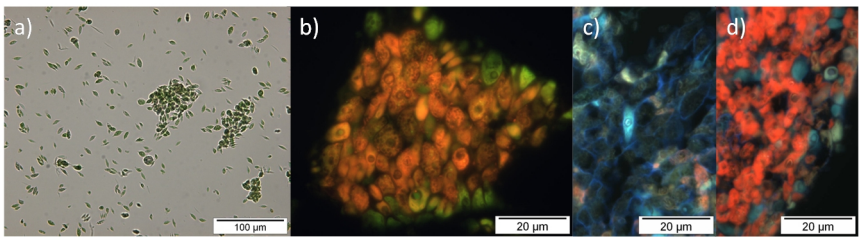

Figure 28. Light (a) and fluroescent (b, c, d) microscopy were used to visualise *Scenedesmus* sp. cells. Cells were stained to reveal the presence of lipids (b), glucan (c) or protein (d).

8.2.2 FTIR spectroscopy

Fourier transform infrared (FTIR, Mecozzi et al. 2011) photoacoustic spectroscopy (PAS) was used as a fast and efficient method to assess changes in the gross composition of algal fractions during the fractionation procedure. FTIR spectra of model compounds (protein, carbohydrate, lipid) were used to verify the identification of the main components in the algal fractions.

Fig. 29 shows the FTIR-PAS spectra of unfractionated (raw algae) and ethanol fractionated algal samples.

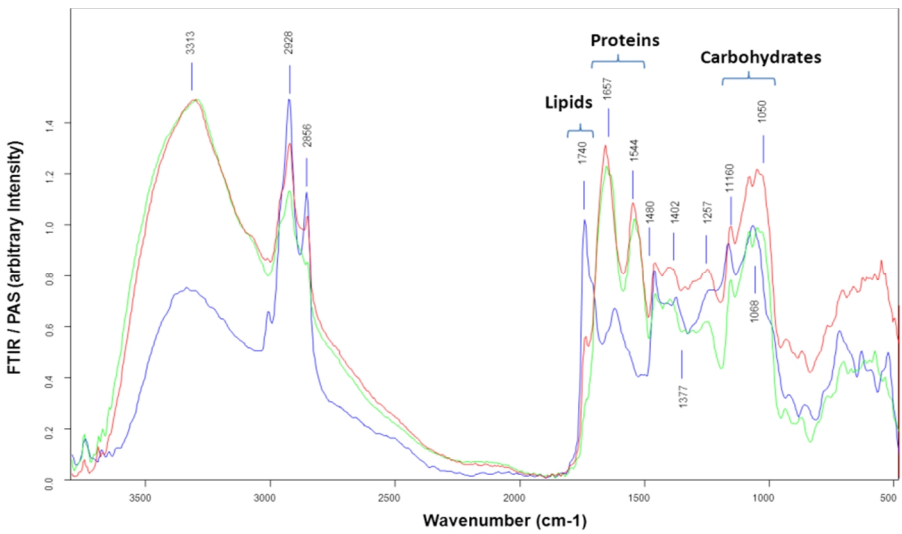

Figure 29. FTIR-PAS spectrum of unfractionated (red) (40.9 m-%), ethanol extract (blue), and residual, ethanol-extracted (green) (45.4 m-%) biomass of *Scenedesmus* sp. Protein concentration as analysed by Kjeldhal method is shown in the parenthesis. The typical bands of biocompounds are marked above the bands (proteins: amide I 1 657 cm^{-1} and II 1 544 cm^{-1}; lipids: ester 1 740 cm^{-1} and ca. 1 160 cm^{-1}, carbohydrates: ether ca. 1050 cm^{-1}; lignin: several overlapping bands between 1 480–1 257 cm^{-1}). These bands allow identification of the main polymeric components (lipids, proteins and carbohydrates) of algae down to 5 m-% concentration.

8.2.3 SDS-PAGE and Mass spectroscopy

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to visualise proteins in the various fractions from the *Scenedesmus* sp. biomass. Figure 30 shows samples from one of the extracts (labelled as E1, water extracted biomass) which contained relatively pure protein, as well as the corresponding sample which had been treated with protease (labelled as F1) are shown. The sample labelled E1 contained protein of mainly *ca*. 30 kDa size. When the protease-treated sample contained larger protein particles (MW *ca*. 100 kDa), which had apparently been loosened from the algal matrix by the treatment.

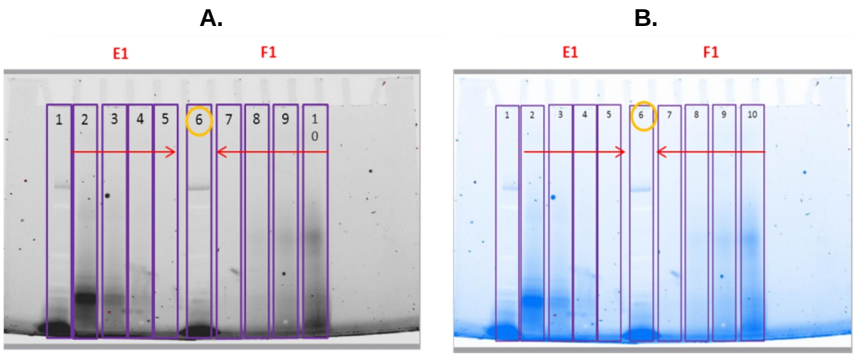

Figure 30. SDS-PAGE gels from *Scenedesmus* sp. biomass extracts (E1 without protease treatment, F1 with protease treatment) with (A) Griterion TGX stain-free (4–20%) and (B) stained with Coomasie blue. Molecular weight markers are shown in lanes 1 and 6: 6.9, 20, 29, 37, 55, 98, 117, 210 Da. Other lanes: 2. 1E (20 µg), 3. 1E (10 µg), 4. 1E (5 µg), 5. 1E (1 µg), 7. 1F (1 µg), 8. 1F (5 µg), 9. 1F (10 µg), 10. 1F (20 µg).

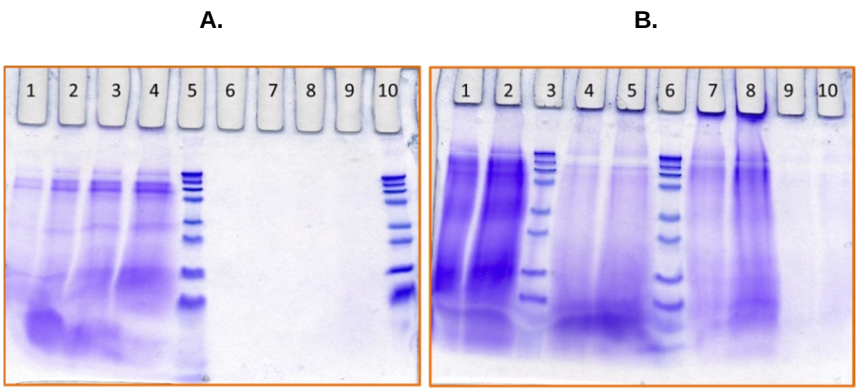

Figure 31. SDS-PAGE gels from residues of water and protease treated *Scenedesmus* sp. biomass. A. 1. 1EL (0.2 mg), 2. 1EL (0.4 mg), 3. 1EL (0.6 mg), 4. 1EL (1 mg), 5. Molecular weight marker (6.9, 20, 29, 37, 55, 98, 117, 210 Da), 6. 1FL (0.2 mg), 7. 1FL (0.4 mg), 8. 1FL (0.6 mg), 9. 1FL (1 mg), 10. The same MW marker as in lane 6.

B. 1. 1C (0.4 mg), 2. 1C (0.8 mg), 3. Molecular weight marker (6.9, 20, 29, 37, 55, 98, 117, 210 Da), 4. 1D (0.4 mg), 5. 1D (0.8 mg), 6. Molecular weight marker (6.9, 20, 29, 37, 55, 98, 117, 210 Da), 7. 1FS (0.4 mg), 8. 1FS (0.8 mg), 9. 1FS (0.4 mg), 10. 1FS (0.8 mg).

Some freeze dried and precipitated solid fractions (1C, 1D, 1ES and 1FS) were also analysed by SDS-PAGE (Figure 31). Some of these fractions (e.g. 1EL and 1C) contained proteins of *ca.* 200 kDa size. Another fraction (1ES) contained such large proteins that they did not migrate into the gel. Protease treatment of the samples resulted in hydrolysis of proteins into much smaller proteins, which in some cases (e.g. 1FL and 1FS) were so small that they could not be analysed by SDS-PAGE, even though FTIR spectroscopy had shown that these fractions contained proteineaceous material.

MALDI-TOF MS was used to further characterise peptides in the extracts labelled E1 and F1 (protease untreated and treated, *cf.* Fig. 30). These extracts contained only few peptides of 1 000–4 000 Da. Peptides of less than 1000 Da could not be analysed by this technique.

These analyses demonstrated that FTIR-PAS was suitable for detection of proteineaceous material in fractionated and unfractionated algal biomass and that SDS-PAGE and MALDI-TOF MS could be used to identify the sizes proteins and peptides (1 000–4 000 Da) in the fractions. This provides a relatively rapid and simple set of methods for assessing chemical and enzymatic fractionation of algal biomass.

8.3 Pyrolysis GC/MS analysis of fractionated algal biomass

Pyrolysis (platinum foil pulse pyrolyzer, Pyrolab2000®) GC/MS (Varian 3800 GC-Varian 2000 MS) (Py-GC/MS) measurements of the original and fractionated algal biomass, with and without a derivatisation reagent (tetramethyl ammonium hydroxide), were used to determine polymer composition and fatty acid content. Heneicosanoic acid was used as internal standard for fatty acid determination. Degradation products formed were identified using data from literature and the commercial NIST05 library.

Degradation products of protein, polysaccharides and chlorophyll were detected in the pyrogram of untreated algal biomass, measured without derivatisation (Fig. 32). The polysaccharides consisted mainly of glucose, but some mannose, xylose and galactose were also identified (Fig. 33). Lipids cannot be detected without derivatisation. Unidentified peaks in the chromatogram originated from chlorophyll, as verified using chlorophyll model compounds. Changes in the protein, polysaccharide and chlorophyll composition were observed after the various extraction steps (ethanol, water, alkali, acid), although Py-GC/MS is not quantitative (Fig. 33, 34).

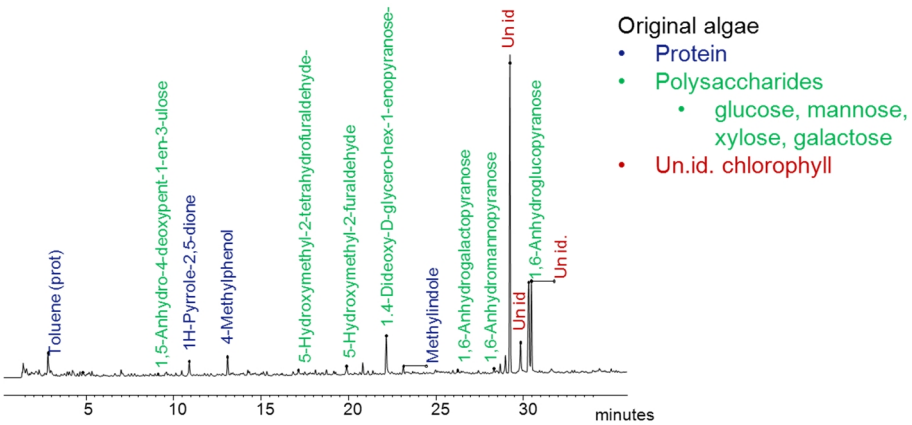

Figure 32. Pyrogram of untreated *Scenedesmus* sp. biomass, measured at 600 for 2 s. Degradation products of protein: toluene; 1H-Pyrrole-2,5-dione; 4-Methylphenol; Methylindole. Degradation products of polysaccharides: 1,5-Anhydro-4-deoxypent-1-en-3-ulose; 5-Hydroxymethyl-2-tetrahydrofuraldehyde; 5-Hydroxymethyl-2-furaldehyde; 1,4-Dideoxy-D-glycerol-hex-1-enopyranose; 1,6-Anhydrogalactopyranose, 1,6-Anhydromannopyranose; 1,6-Anhydroglucopyranose. Degradation products of chlorophyll marked as un. id.

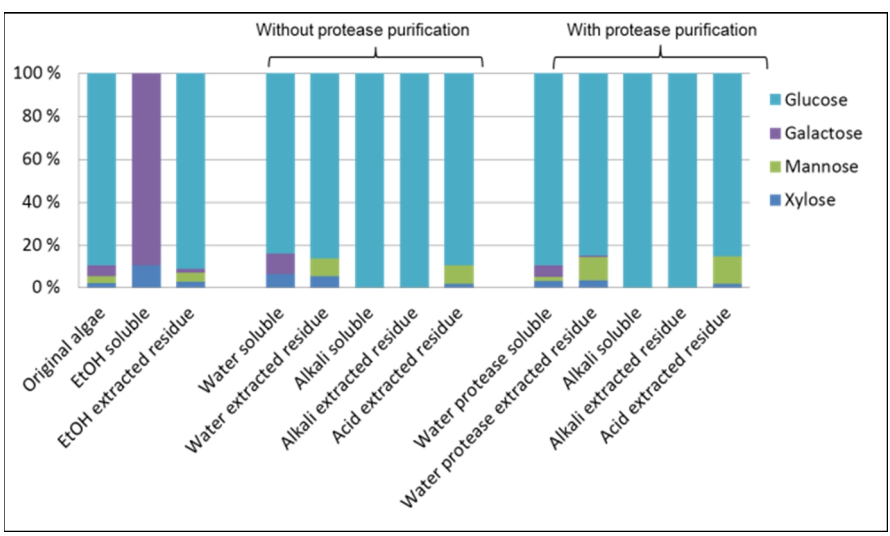

Figure 33. Composition of polysaccharides (after normalization of peak areas to 100%) in different fractions, determined by Py-GC/MS.

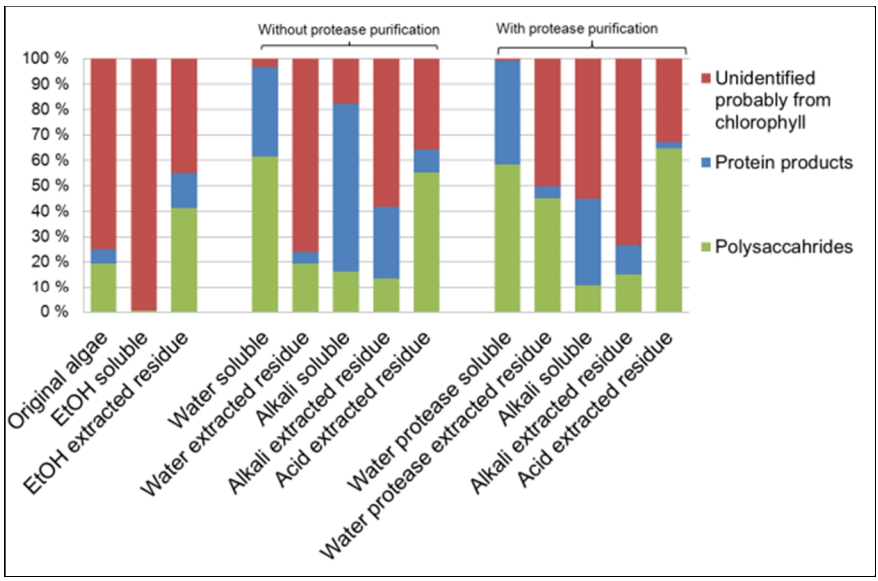

Figure 34. Proportion of chlorophyll, protein and polysaccharide in various fractions of algal biomass, determined by Py-GC/MS.

The ethanol extraction stage, which was used to remove lipids from the biomass, also removed about half of the chlorophyll (Fig. 34) as well as lipids (Fig. 35). Both protein and polysaccharides were enriched in the ethanol-extracted residue. Water, with or without addition of protease, removed both protein and polysaccharides. Galactose was removed in both ethanol and water extraction stages (Fig. 33). After water extraction, the proportion of chlorophyll in the residue was increased. Alkali dissolved both polysaccharides and protein, but chlorophyll was also removed (Fig. 34). More protein was dissolved into alkali from the fraction which had not been treated with protease than from the fraction which had been. This fraction probably contained more protein than the protease treated fraction. Alkali extracted residue not treated with protease also contained a bit more protein than the protease treated residue. The final residue, which had been acid extracted, was enriched with polysaccharides, which were enriched in glucose and mannose (Fig. 33). Compared with the composition of the unfractioned biomass, the fractionated residue still contained a rather high amount of chlorophyll and protein. More protein was removed when protease was used in the water extraction stage.

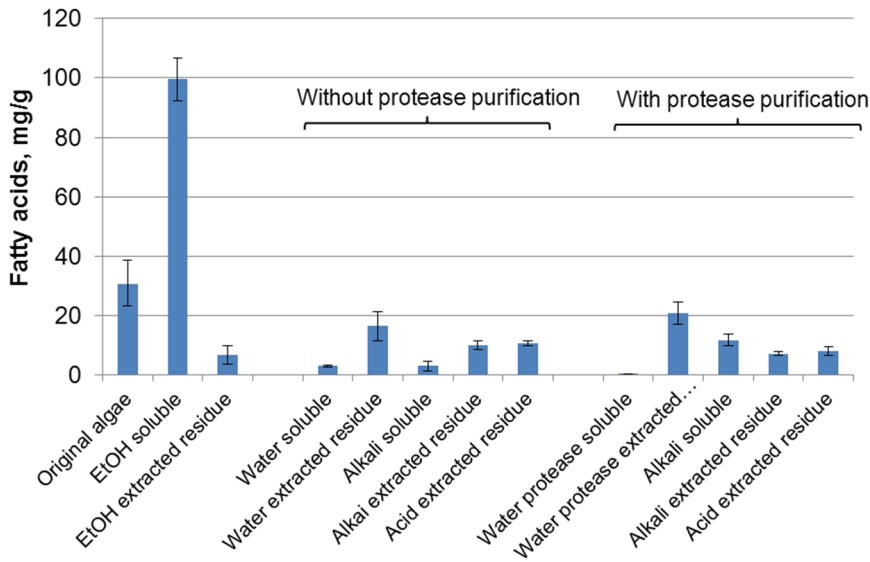

Figure 35. Amount of lipids, measured as fatty acids, in fractions of Scenedesmus sp. biomass, as determined by methylation Py-GC/MS.

Both the composition and the amount of lipids were determined for the different fractions of extracted algal biomass. Octadecenoic acid (C18:1) and hexadecanoic acid (C16:0) were the main fatty acids present, but tetradecanoic acid, palmitoleic, octadecanoic, linoleic and docosanoic acids were also detected. The unfractionated biomass contained the highest amount of lipids (Fig. 35). Only part of the lipids was removed with ethanol extraction. The residue after ethanol extraction contained less lipid than the residue after water extraction with or without protease treatment. The reason might be that the lipids were not uniformly distributed in the biomass. Since the sample size was small, homogenisation prior to measurement was inadequate. With larger samples homogenisation should be considered. A small amount of lipids was removed in the alkali extraction but not in the acid extraction. The result showed that two thirds of the extracted lipids were removed during the fractionation. The extraction efficiency of lipids might be improved by using a different extraction solvent.

8.4 Molar mass determination of polymers

Molar mass is one of the most important structural features which affects the potential utilisation of dissolved and recovered polymers. Molar mass determination was performed by size exclusion chromatography (SEC) using MCX columns and distributions were calculated relative to pullulan (5 900–708 000 g/mol).

Hot water extracted mainly small molecular material (< 5 000 g/mol). Protease treatment increased the amount of material dissolved in the water extract, but the proportion of oligomeric material (~5 000 g/mol) was lower, the main fraction being ~1 000–1 500 g/mol (Fig. 36).

Alkali extraction enhanced the dissolution of oligomeric and polymeric fractions of ~5 000, 25 000 and >100 000 g/mol. Without protease, the main fraction was oligomeric (~5 000 g/mol), whereas after protease treatment the proportion of polymeric fractions was higher. Due to the limited yield of the acidic extraction, these were not analysed.

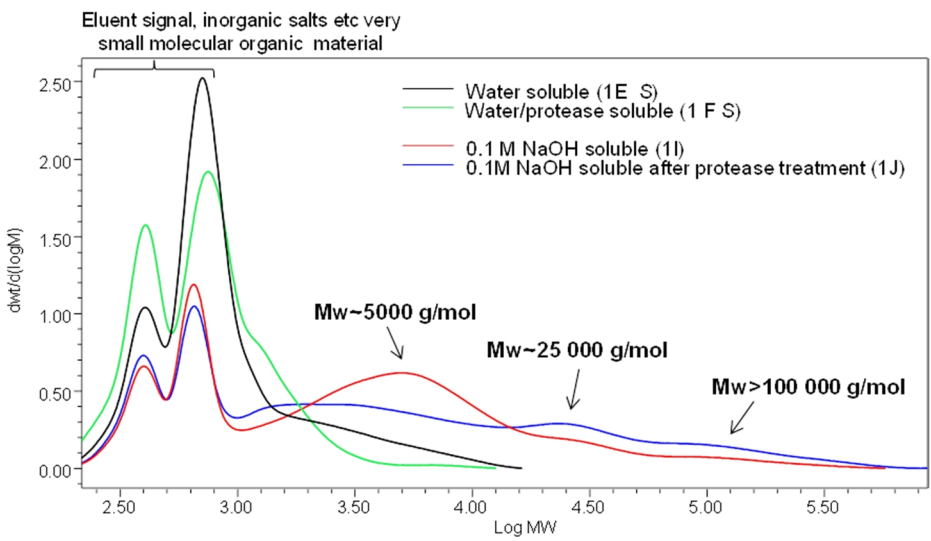

Figure 36. The molar mass distributions of oligomers and polymers in the dissolved fractions extracted from algal biomass.

To evaluate the molar mass of the polymeric components still remaining in the solid residue, the solid samples were dissolved in DMAc/8% LiCl with the solvent exchange method used for cellulose and pulp samples. Unfortunately, the algal residues only partially dissolved in DMAC/LiCl and the results are thus not conclusive. PL MiniMix columns in 0.8% DMAc/LiCl were used and in all cases, the solubilized material of the solid residues contains two fractions: MP ~ 5 000 Da (Molar mass range 1–40 kDa) and MP ~ 300 000 Da (Molar mass range 30–2 500 kDa). However, because of the limited solubility in DMAc/LiCl, it could be expected that the average molar mass of the whole insoluble residue would be significantly higher. Differences between the samples could not be evaluated reliably because of the low solubility (Fig. 37).

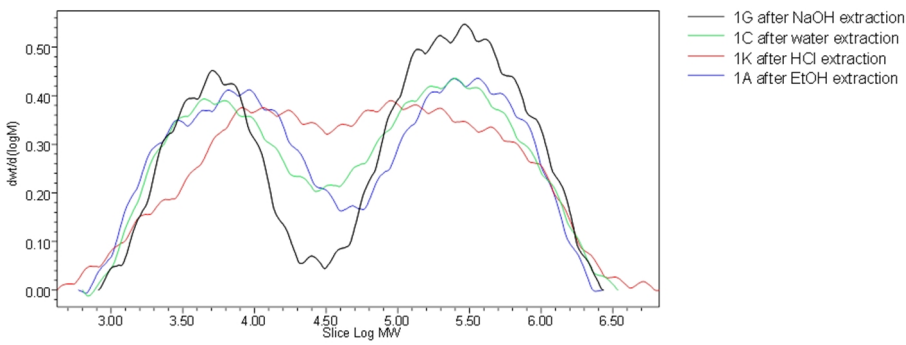

Figure 37. The molar mass distributions of insoluble residues (SEC DMAc/0.8% LiCl, pullulan calibration).

8.5 Conclusions

To define the composition of algal biomass requires a set of complementary analytical methods. FTIR-PAS, SDS-PAGE, pyrolysis-GC/MS, and size exclusion chromatography are useful tools for analysing the composition of algal biomass.

Extraction of high value biocompounds from defatted algal biomass residual could improve the cost-efficiency of algal cultivation for biofuels. Simple (water based) fractionation methods, which would minimize fractionation costs, solubilised about 48% of *Scenedesmus* sp. biomass. Enzymatic treatment (e.g. protease) could be used to enhance fractionation, increasing the extraction to 67% of the biomass, and to obtain enriched polymeric fractions.

Key findings:

- FTIR-PAS, SDS-PAGE, pyrolysis-GC/MS, and size exclusion chromatography were good tools for analysing the composition of algal biomass. Algal biomass is diverse, the composition being dependent on both the species and the environmental conditions, and a good tool kit for assessing the composition is essential for future valorisation of the biomass.
- Aqueous and ethanol extractions solubilised approximately half of algal biomass.
- pH affected the relative proportion of carbohydrates and proteins extracted.
- Protease treatment was effective in releasing larger proteins from the biomass and resulted in an overall improvement in biomass extraction, but with some loss of proteins as smaller peptides.

9. General conclusions

Algae are expected to play an important role in tomorrow's bioeconomy. This multifaceted project showed the potential for integrating algal biomass with waste and wastewater management. However it also confirmed that, to realize profitable, unsubsidised production systems, the costs of cultivation must be reduced. Technological development is still needed in this sector. Other useful biocompounds, such as proteins and carbohydrates, must also be extracted from the algae, in addition to oil for biofuel, and both the extraction processes and fractions thus derived need to be commercialised.

It is apparent that an economically viable algae-to-biodiesel commercialization will initially depend on government subsidies and the price of oil, in addition to optimizing the biomass yields of the algae. However, algae-to-biofuels is a globally topical sector with a high interest from several stakeholders. The markets are likewise global. From a biofuel point of view, fuel for air traffic is particularly interesting, as this sector will probably need to rely on liquid fuel still during the next decade.

10. Summary

Industrial and municipal waste streams as a source of nutrients for algal growth

The feasibility assessment of various liquid waste streams for algal cultivation included waste flows from biowaste digestion, fish cultivation, and composting plants. The highest algal biomass growth was obtained with liquid fractions from composting processes and both *Selenastrum* sp. and *C. pyrenoides* were effective in removing nitrogen and phosphorus from these fractions. The concentrations of nitrogen, phosphorous or organic carbon in the other waste sources tested was either too low to support mixotrophic or heterotrophic growth, or they contained components that were toxic for the algae. In contrast, composting waste water needed to be diluted because nutrient concentrations were too high. We also observed that other pretreatments, e.g. to reduce bacterial contamination, may be needed if industrial waste waters are to be used for algal cultivation. None-theless, it was possible to generate up to 2.7 g L^{-1} *Selenastrum* sp. biomass in composting waste water at pilot scale.

Municipal wastewaters also sustained good algal biomass production. The results indicated that algal based wastewater technology could be used as a single-stage, post treatment for nutrient removal. We also found that a community of algae would function better in removing nutrients from wastewater than a mono-culture. This work has continued with the growing of algal communities on algal turf filters, using only available sunlight. The potential for using this technology in biomass production and cleaning of wastewater under Finnish summer conditions will be evaluated.

Physiological considerations

Light intensity studies showed that there is sufficient light for algal growth in Southern Finland from February to October. During winter time, dark fermentation could be implemented using waste carbohydrates as the carbon source for the algae.

Lipid and biomass yields and productivities were determined in controlled photo-, hetero- and mixotrophic cultivations, in order to assess the process kinetics. The algal species tested used both CO_2 and organic carbon sources in mixotrophic cultures, which were shown to be as productive (or better) as heterotrophic cultures,

but with lower CO_2 production. Our results suggested that continuous, nitrogen-limited, mixotrophic algal cultivation is feasible and could be a useful strategy for producing lipid as biofuel feedstock. Caution is needed in extrapolating lab scale results to large scale, but these experiments are essential for scaling up, process techno-economic modelling and life cycle analysis and provide data on the upper limits of the productivity feasible for the algal species studied.

Algae as a feed for biogas production

Algal biomass was a suitable co-substrate for anaerobic digestion, but required careful control of the ammonia concentration, which affects the process stability. Biogas production and the stability of the digestion process were evaluated with algae alone (untreated or using residuals from which lipid had been extracted) and with algae co-digested with biowaste, glycerol or sewage sludge. Lipid extraction was found to be a good pre-treatment of algal biomass, increasing the bioavailability of the nutrients remaining in the residual algal biomass and making this a good feed for biogas production. The methane production rate from lipid-extracted biomass was higher than that of untreated, native algal biomass. Addition of trace elements (Kemira) was found to stabilize the biogas production process and also appeared to increase biogas production. Two stage fermentation, including biohydrogen production, is promising way to increase biogas based energy production, but would require careful pH control, especially in the first stage.

Techno-economic evaluation

Techno-economic performance and greenhouse gas emissions of twenty integrated algae-to-biofuel process concepts were evaluated using process simulation and LCA analysis. Input data was delivered by the project, including biomass and lipid yields, methane yields from biogas production, waste water characterizations and process conditions for cultivation and biogas production. The cases included photoautotrophic algal production using power plant flue gas or purchased CO_2, and heterotrophic algal production using straw based sugars as carbon source. Integration into waste water treatment was also considered. The outputs were lipids and electricity or purified biogas, or biogas only. Under the process and economic assumptions used, none of the considered cases were profitable, mainly due to high electricity consumption during cultivation and harvesting, combined with the high capital investment costs. The GHG emission estimates of all concepts considered clearly exceeded the target values set in the Biofuel target 2018 – EU directive.

Biopolymers from algal biomass

FTIR-PAS, SDS-PAGE, pyrolysis-GC/MS, and size exclusion chromatography were useful tools for analysing the composition of algal biomass. Algal biomass was fractionated using simple, cost effective, extraction techniques which were

able to remove 48–67% of compounds from the biomass of *Scenedesmus* sp. Ethanol extracted primarily lipids, whereas aqueous treatments (neutral, alkali or acidic) extracted proteins and carbohydrates. Protease treatment could be used to increase the extraction of other components, including enriched polymeric material, but resulted in some loss of large proteins, with a corresponding increase in smaller peptides. The tools developed here form a valuable resource for valorisation of polymers in residual algal biomass in the future.

Impact of the results

Algae are expected to play an important role in tomorrow's bioeconomy. This multifaceted project demonstrated the potential of integrating algal biomass generation with waste and waste water management. However, it also confirmed that there is still a need to reduce costs, especially those of cultivation, in order to realize profitable, unsubsidised production systems. Technology development is still needed in this sector. In addition, other useful substances, such as proteins, should be extracted from the algae and these fractions need to be commercialised.

The business model concepts based on this project's results are being further developed in a continuing project ALGIND Algae energy business opportunities for Finnish companies 2011–2014 (Tekes Groove programme).

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Series title and number
VTT Technology 147

Title	**Sustainable algal biomass products by cultivation in waste water flows**
Author(s)	Mona Arnold (Ed.)
Abstract	Algae are predicted to play an important role in tomorrow's bioeconomy. The technical goal of the project was to develop enhanced algal cultivation processes utilising waste flows and to increase the overall material and energy efficiency of algal processing for biodiesel and biogas production. The project produced new knowledge on the boundary conditions for cost efficient algal cultivation and productivity. The use of algae as a tertiary treatment of municipal waste water, such as the utilisation of waste water flows from biowaste handling, was assessed with positive results. Process concepts based both on CO_2 uptake and (waste) organic carbon were assessed. In a Nordic climate the utilisation of spill heat is requisite and with restricted available daylight in the winter time, an alga's ability to shift from autotrophic to heterotrophic growth provides a potential strategy for algal cultivation in Nordic circumstances. The fractionation of algal residuals for biopolymers is a new research area with potential long term impact in the bioeconomy sector. Cost efficient Integrated production concepts still need to be developed, as premises to successful business models. It is apparent that an economically viable algae-to-biodiesel commercialization will initially depend on government subsidies and the future price of oil, in addition to optimized biomass yields. However, algae to biofuels is globally a topical sector with a high interest from several stakeholders. The markets are likewise global. From the biofuel point of view, air traffic is particularly interesting, as this sector will probably need to rely on liquid fuel still during the next decade.
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Sustainable algal biomass products by cultivation in waste water flows

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VTT TECHNOLOGY 147 Sustainable algal biomass products by cultivation in waste water flows