BIOGASIFICATION POTENTIAL OF SOME ALGAE SPECIES

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Heli Hiltunen

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Author Heli Hiltunen

Supervised by Maritta Kymäläinen

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ABSTRACT

The obligation to reduce greenhouse gases motivates to replace fossil fuels with renewable energy. Algae have increased their interest to be used as a raw material for bioenergy recently. The thesis was part of a public project ALDIGA, which was funded by Finnish Funding Agency for Technology and Innovation (Tekes). Algae cultivation, converting algae to biofuels, and algae biogasification is discussed in the theory part of the thesis. In the practical part of the thesis, the methane potential of the selected algae species was studied. What effects the lipid extraction and pre-treatment had on the methane yield was also researched.

Two different algae species *Chlorella*, and *Scenedesmus* were selected for methane tests. Alkali heat treatment was used as a pre-treatment method. Lipids were extracted from the pre-treated algal biomass by washing the biomass with water. The methane yields were measured from the original, pre-treated, and algae of which lipids were extracted. In addition, it was found out how TS, VS, lipids, proteins, and sugars were divided between the different phases of lipid extraction.

The methane yields of original algae (approximately 220-240 litre/kgVS) studied in this thesis were similar to the yields of other algae species mentioned in literature. The methane yields were about the same as with plant biomasses (for example, clover 280-300 litre/kgVS) in general.

The methane yield of algae can be improved significantly with alkali and heat treatment without extracting the lipids. The improvement was approximately 33-34 %. Higher yields possibly result from weakened cell wall and organic material becoming more bio-available. About half of the organic matter is lost in lipid extraction. The alkali heat treatment is recommended as a pre-treatment method for improving the methane yield but for lipid extraction a more specific method is needed.

Keywords Algae, methane, biogas

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TIIVISTELMÄ

Päästöjen vähentämisvelvoitteet motivoivat fossiilisten polttoaineiden korvaamisen uusiutuvilla energialähteillä. Levien käyttö bioenergian raaka-aineeksi on kasvattanut kiinnostustaan viime aikoina. Opinnäytetyö tehtiin osana meneillään olevaa levien kokonaisvaltaista materiaalihyödyntämistä käsittelevää Tekes -rahoitteista ALDIGA-hanketta. Työn kirjallisuusosassa käsitellään tiivistetysti levien tuottamista ja jalostamista biopolttoaineiksi ja tarkemmin levien biokaasuttamista. Käytännön osuudessa selvitettiin valittujen levälajien metaanituottoja, sekä esikäsittelyn ja biodiesel-rasvajakeiden erottamisen vaikutusta metaanituottoon.

Metaanituottotesteihin valittiin kaksi eri levälajia, *Chlorella* ja *Scenedesmus*. Levien esikäsittelynä käytettiin alkalista lämpökäsittelyä. Esikäsitellystä levämassasta uutettiin rasvat vesipesulla. Metaanituotot mitattiin alkuperäisestä, esikäsitellystä ja rasvaerotuksen jälkeisestä levästä. Lisäksi selvitettiin kuiva-aineen ja orgaanisen aineen, rasvojen, proteiinien ja sokerien jakaantuminen rasvan erotusvaiheissa.

Tässä tutkittujen alkuperäisten levien metaanituotot (noin 220-240 litraa/kgVS) olivat hyvin vastaavia kirjallisuudessa leville esitettyjen tuottojen kanssa, ja samalla tasolla kuin kasvibiomassat (esimerkiksi apila 280-300 litraa/kgVS) yleensä.

Esikäsittely, ilman rasvan erotusta, paransi metaanituottoa molemmilla levillä merkittävästi noin 33-34 %. Suuremmat metaanisaannot selittynevät osaltaan sillä, että levän soluseinärakenne heikentyi esikäsittelyssä ja enemmän orgaanista ainesta saatiin liukenemaan. Toisaalta, rasvaerotusvaiheessa menetettiin noin puolet kaikesta orgaanisesta aineesta. Näin ollen alkalinen lämpökäsittely on metaanituoton kannalta suositeltava esikäsittelymenetelmä, mutta rasvajakeiden erottamiseen tarvitaan spesifisempi menetelmä.

Avainsanat Levä, metaani, biokaasu

Sivut 46 s. + liitteet 7 s.

CONTENTS

PREFACE

I would like to thank Mrs. Maritta Kymäläinen for being my supervisor in this thesis and given me valuable information and guidance throughout the project. Her vast knowledge of the anaerobic digestion field is priceless. I am also grateful for being given this opportunity to write my thesis for this ALDIGA project and being able to continue work with process modelling in the project. In addition, I need to say a big thank you to my family who made it possible for me to go to Waterloo.

The companies involved in this project are Kemira Oyj, Gasum, Ekokem Oy, Wärtsilä Finland Oy, Bioste Oy, Neste Oil, Biovakka Suomi Oy, Päijät-Häme Jätehuolto Oy, Kujalan Komposti Oy, Clewer Ltd, Hartwall Oy, Sybimar Oy, Envor Group Oy, LHJ Group and Lahti Science and Business Park.

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Waterloo 31 May 2011
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Heli Hiltunen

1 INTRODUCTION

The pressure to find out favourable biofuel to meet the growing energy demand in terms of investments and resources are at all time high. EU obliges all of its member countries to increase the share of biofuels used in transportation to 10 percent by 2020. Finland has defined its own goal to increase the share to 20 percent. Partly because of this commitment, companies are eager to find out new ways of making biofuels and optimising available yield.

Biofuels are fuels that are made of renewable energy sources and do not pollute in the same way as fossil fuels. The ambition is to develop sustainable biofuels production with comprehensive utilisation of materials and side streams.

Biofuels are derived from agriculture or forestry. For example Finnish forest and paper industry use black liquor (also called black lye) as an energy source and because of that, statistically about 25 percent of all electricity consumed in Finland comes from renewable energy sources. Conventional biofuel sources are wood (forest converted chips, pellets etc.) and biomasses from arable land. These include reed canary grass, straw, sugar beet and sugar cane, maize, rape seed and jathropa. Biomasses can be utilised directly as energy by burning or they can be processed into ethanol, biodiesel or biogas.

One thing in favour for using microalgae as a source material for biofuels is that the alga is not a common food plant. In addition it does not require a large cultivation area or potable water, and fuel production would not compete with food production. In that sense, food crops would not be turned into fuels and there is not an ethical dilemma involved. If and when growing and harvesting of algae can be optimised, algae can seriously challenge fossil fuels.

The aim of this thesis is to make a written review of the research done in anaerobic digestion field with algae. The theory part of the work is to pull together a concise text about algae biogasification and view results that have been received from studies conducted earlier.

The experimental part of the project was done on laboratory scale on HAMK premises. In order to find out selected algae's methane potential a series of batch tests was run with parallel samples. The samples included non-treated algae and algae in which lipids had been extracted for biodiesel production.

The thesis is part of Algae from Waste for Combined Biodiesel and Biogas Production (ALDIGA) project, which is funded by the Finnish Funding Agency for Technology and Innovation (Tekes). The project's main objective is to implement an integrated operations model exploiting algae, biodiesel, and biogas production with the use of nutrients of waste origin for example

reject waters and biowaste. The endeavour is to produce a way to gain all the potential from algae including side streams with as little external energy as possible.

To sum up the project, the purpose is to make a comprehensive use of algae with material and energy efficiency and benefit from the nutrient cycle. The research partners involved in the project are Technical Research Centre of Finland (VTT), Finnish Environment Institute (SYKE), University of Helsinki (UH), Lahti University of Applied Sciences (LAMK) and HAMK University of Applied Sciences (HAMK). In addition there are 15 companies in total involved in the project.

2 GENERAL ABOUT ALGAE USED IN THE BIOFUEL FIELD

Algae are primitive organisms, which produce oxygen when photosynthesising. Algae exist in all kinds of water systems, lakes, rivers, seas and even on moist surfaces. In addition, microalgae can be found also non-aqueous, terrestrial ecological system (Mata, Martins & Caetano 2009). Algae do not have vascular tissue, roots, stalks or leaves as other plants and thus algae belong to the thallophytes. Algae have membrane-bound cell organelles and a nucleus (Figure 1). Some species do not contain any cell wall but a pellicle, which is a thick elastic membrane. (Maier, Pepper & Gerba 2000, 34; Finnish Environment Institute 2010.)

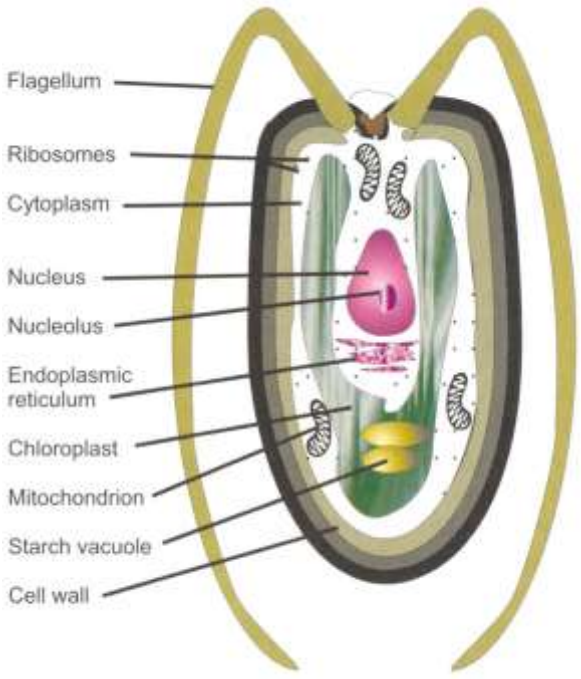

Figure 1 Algal cell (Maier et al. 2000)

Algae can be autotrophs, mixotrophs, or heterotophs. Autotrophic algae are base producers, in other words, self-sufficient and take carbon dioxide from the atmosphere when photosynthesising. Some of the species are mixotrophs, which means that those species can change from being autotrophs to being heterotrophs depending on the environment. Heterotrophs cannot use carbon dioxide from the atmosphere but they saturate their carbon need by degrading organic compounds. (Finnish Environment Institute 2010.)

Growth rate and productivity of algae is much higher than those in conventionally cultivated plants. In addition, the alga is not a competitor for food crops and can grow on marginal land, which therefore also reduces cultivated area usage. (Mata et al. 2009.)

Generally microalgae double their biomass within 24 hours. During exponential growth phase doubling can take only 3.5 hours. (Chisti 2007.) Figure 2 illustrates algal growth rates of different species according to their cell size.

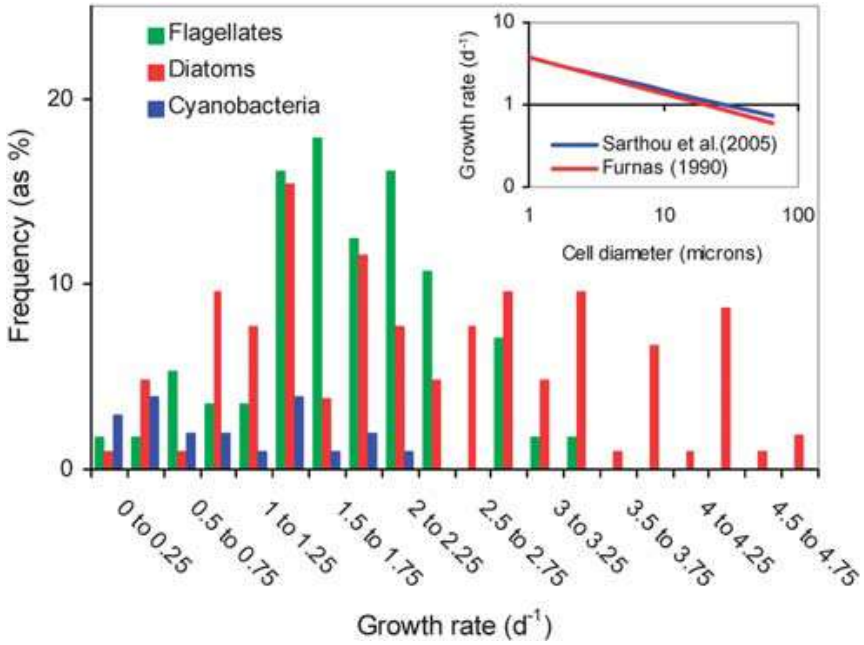

Figure 2 Frequency analysis of algal growth rates and algal growth-size relationship (Williams & Laurens 2010)

Reynolds has pulled together an informative table of various algae species' growth rates, which includes information from different studies (table 1).

Table 1 Maximun specific growth rates (r'_{20} d^{-1}) reported for some freshwater species of phytoplankton in laboratory cultures, under continuous energy and resource saturation at 20 °C, table modified (Reynolds 2006).

Species	r'20 d^{-1}	Ref.
Synechococcus sp.	1.72[a]	Kratz and Myers (1955)
Planktothrix agardhii	0.86	Van Liere (1979)
Anabaena flos-aquae	0.78	Foy et al. (1976)
aphanizomenon flos-aquae	0.98	Foy et al. (1976)
Microcystis aeruginosa [b]	1.11	Kappers (1984)
Microcystis aeruginosa [c]	0.48	Reynolds et al. (1981)
Chlorella strain 221	1.84	Reynolds (1990)
Ankistrodesmus braunii	1.59[a]	Hoogenhout and Amesz (1965)
Eudorina unicocca	0.62	Reynolds and Rodgers (1983)
Volvox aureus	0.46	Reynols (1983b)
Cryptomonas ovata	0.81[a]	Cloern (1977)
Monodus subterraneus	0.64[a]	Hoogenhout and Amesz (1965)
Dinobryon divergens	1.00	Saxby (1990), Saxby-Rouen et al. (1997)
Stephanodiscus hantzschii	1.18	Hoogenhout and Amesz (1965)
Asterionella Formosa	1.78	Lund (1949)
Fragilaria crotonensis	1.37	Jaworski, in Reynolds (1983a)
Tabellaria flocculosa var. asterionelloides	0.66	G. H. M. Jaworski (unpublished data)
Ceratium hirundinella	0.21	G. H. M. Jaworski (unpublished data)

[a] Rate extrapolated to 20 °C

[b] Unicellular culture

[c] Colonial culture

When choosing algae for the biorefinery concept there are certain criteria algae should meet. The species selected should have a high lipid content (for biodiesel application) and a high growth rate, an adequate fatty acid composition for biodiesel and be effortless to culture, harvest and extract. So far, diatoms and secondly green algae are regarded as the potential ones (Demirbas & Demirbas 2010; 149.)

3 GROWING, HARVESTING AND DEGRADING OF ALGAE

The alga has a better energy balance for the use of biofuel production than for example field crops. Algae can thrive on residue nutrient streams and no fertilisers are necessarily needed. No herbicides or pesticides are need to be used, either. Furthermore, when algae are digested anaerobically nutrients are concentrated into digestate, which could be used as a nutrient substance (Brennan & Owende 2010.) In summary, microalgae give a chance to obtain a carbon neutral biofuel and a closed nutrient cycle.

There is a possibility to try and grow algae in industrial waste water (ponds) and provide the necessary heat from condensate water and the carbon dioxide needed from combustion gas (i.e. flue gas). However, flue gases need to be cooled down and possibly purified to some extent from SO_x and NO_x so that concentrations would not disturb the growth of the algal biomass. (Brennan & Owende, 2010). So far, cultivating and harvesting of algae have been a bit of a hindrance. Singh & Gu have illustrated what algal biorefinery concept could look like (Figure 3).

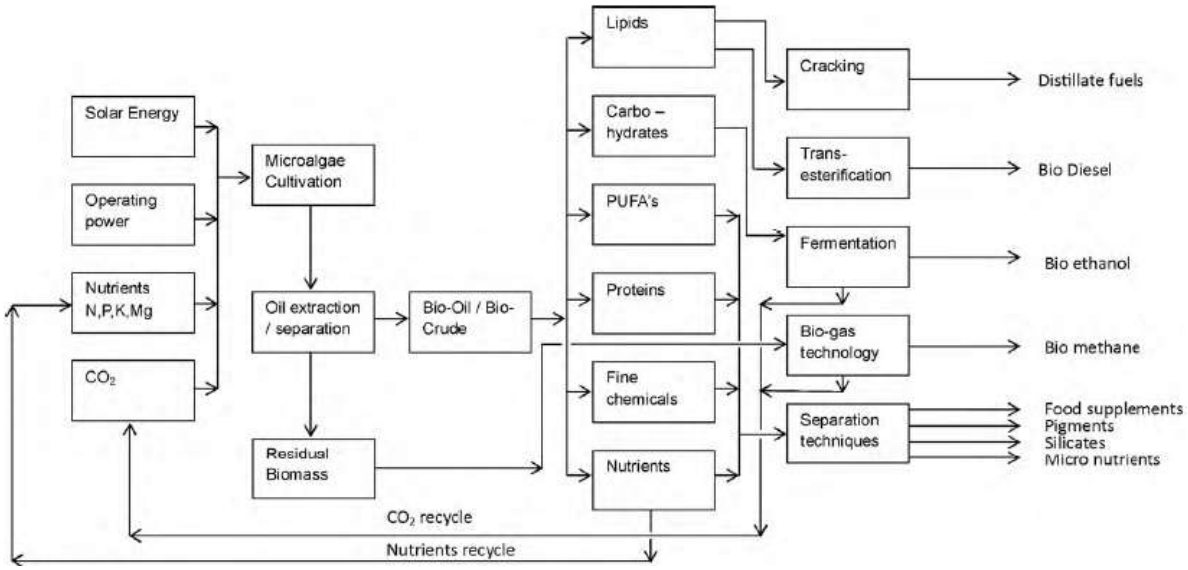

Figure 3 The biorefinery flow chart (Singh & Gu 2010)

3.1 Growing of algae

Algae request considerable amounts of water as they grow in rather weak solution. The typical artificial way of growing algae is either in an open raceway pond (Figure 4) or in a closed photobioreactor (Figure 5).

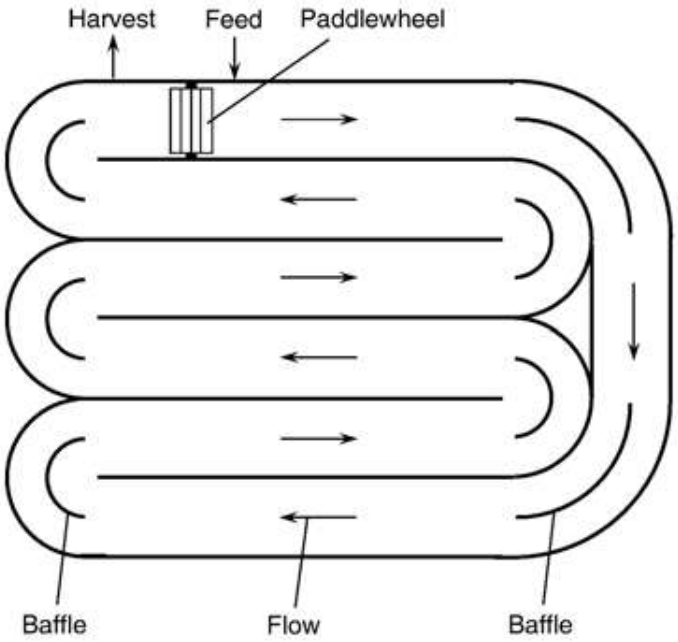

Figure 4 The raceway pond for algae growth (Chisti 2007)

The raceway ponds are meandering closed circles and rather shallow, about 0.3 m deep. Circulation and agitating are provided by paddlewheel at one end of the pond, which is constantly on so that growing biomass does not settle. In curves, stream is directed by baffles. Cultivation is fed in front of the paddlewheel continuously during daylight. Harvesting takes place on completing the circle just behind the paddlewheel. (Chisti 2007.)

The challenges with raceway ponds are contamination of other algae species and microbes that subsist on algae, which obviously affects the productivity. There are no cooling systems in the raceway ponds and temperature varies depending on time of the day and season. Any cooling happening is because of the evaporation which can be notable. Besides, usage of carbon dioxide is not as optimal as with the photobioreactors. However start-up and operational costs are lower than with the photobioreactors, but then again so is productivity (table 2). (Chisti 2007.)

Table 2 Feature comparison of algae cultivating systems

Open pond	Photobioreactor
Risk of contamination	Contamination not likely
No cooling available	Temperature can be adjusted
Biomass productivity lower	Biomass productivity higher
Water loss very high	Water loss low
CO_2 use not optimal	Low CO_2 loss
Lower start-up and operational costs	More expensive

Disadvantages present with raceway ponds are eliminated when growing algae in tubular photobioreactors. There is no contamination of unwanted species involved, so large amounts of biomass can be cultured. Required factors needed for algal growth are supplied in a controlled way. Oxygen is created in photosynthesis. When level of dissolved oxygen reaches much higher than air saturation level, it starts to inhibit photosynthesis and together with strong sunlight causes photooxidative harm to cells. To avoid inhibition and harm, culture is circulated to a degassing zone from time to time and oxygen is stripped out from process by using air. Sunlight is kept in so called solar collector, which consists of collector plastic or glass tubes that are typically less than 0.05 m in radius. There is no need for tubes to be any larger since sunlight does not get too deep into culture medium. So that biomass would not settle, there is a turbulent flow inside the tubes. The flow is kept constant either by a mechanical or an airlift pump, the latter being more tender to biomass. (Chisti 2007.)

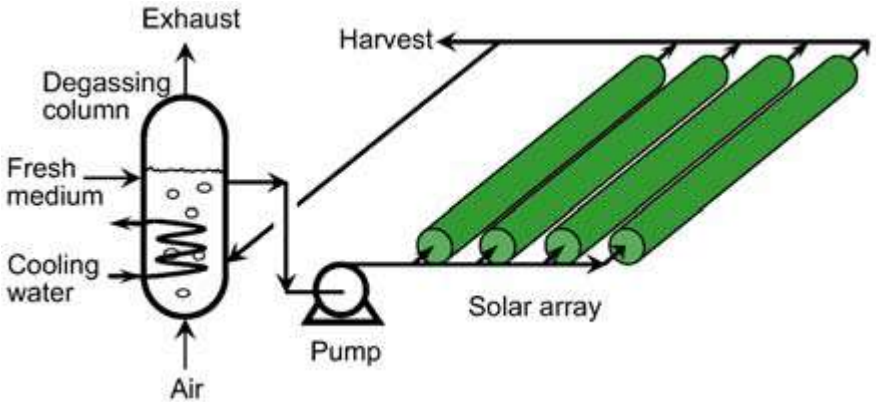

Figure 5 A tubular photobioreactor with parallel run horizontal tubes (Chisti 2007)

According to Williams and Laurens, a combination of these two systems has been successfully used. In the combined system, a photobioreactor acts as a pre-culture phase and from there the biomass is transferred to an open pond to growing phase. (Williams & Laurens 2010.)

3.2 Harvesting of algae

At the moment means of algae harvesting are flocculation, filtration, flotation and centrifugal sedimentation. Certain strains are easier to recover than others and the choice of harvesting depends on the nature of the algae. (Brennan & Owende 2010.) In Richmond's view important decisive criterion when selecting a harvesting method is the quality desired for product. Another starting point for the harvesting method selection can be its prospect of controlling the density or moisture content of the product. (Mata et al. 2010.) Demirbas & Demirbas write that finding a suitable method depends on the species, cell density and culture conditions. (Demirbas & Demirbas 2010, 79-80)

Flocculation can be used to form clusters from algae and increasing the particle size thus making sedimentation and filtration easier. As algae cells have a negative charge, they do not aggregate without the help of polyvalent cations. To revoke the charge of the cells chemicals, such as ferric chloride ($FeCl_3$) or aluminium sulphate ($Al_2(SO_4)_3$), can be added. (Brennan & Owende 2010.)

The traditional filtration cannot separate the smallest microalgae such as *Chlorella*, but filtration can be justified if size of the alga is rather large. For smaller algae, membrane microfiltration or ultrafiltration can be used. However these filtration methods come with a higher price because of the cost of pumping and replacing membranes. (Brennan & Owende 2010; Mata et al. 2010.)

3.3 Degrading of algae

Lipid extraction from the alga can be done by chemical or physical methods or the combination of these two. Table 3 combines the extraction methods mentioned in literature. These extraction methods include oil press or expeller, solvent extraction, supercritical extraction and ultrasound. When thinking of biodiesel application, selecting the best extraction method available is critical for the process. The method should be such that it is easy to scale-up, it should not damage the lipids and at the same time it should be efficient and swiftly done. (Rawat et al. 2010.)

Table 3 Comparison of extraction methods, table modified (Harun et al. 2010).

Extraction methods	Benefits	Limitations	References
Oil press	User-friendly,no solvent needed	Large amount of biomass needed, slow process	Popoola & Yangomodou 2006
Solvent extraction	Solvents used are relatively low-cost; reproducible	Most organic solvents are highly flammable and/or poisonous;solvent recovery is costly and energy intensive; large volume of solvent needed	Herrero, Ibanez, Senorans & Cifuentes 2004; Galloway, Koester, Paasch & Macosko 2004
Microwave-integrated extraction with solvents	Consumption of solvent and time needed for extraction is reduced with microwave treatment	See above; solvents are harsh chemicals	Mahesar et al. 2008; Lee et al. 2010
Supercritical fluid extraction	Non-toxicity (absence of organic solvent in residue or extracts), 'green solvent' used; non-flammable, and simple in operation	Often fails in quantitative extraction of polar analytes from solid matrices, insufficient interaction between supercritical CO_2 and the samples	Macias-Sanchez, Mantell, Rodriguez, Martinez De La Ossa, Lubian & Montero 2005; Pawliszyn 1993
Ultrasound	Reduced extraction time; reduced solvent consumption; greater penetration of solvent into cellular materials; improves the release of cell contents into the bulk medium	Energy intensive; difficult scale-up	Luque-Garcia & Luque De Castro 2003; Martin 1993

Similarly to the harvesting, the solvent selected for solvent extraction will depend on the nature of the algae species grown. Solvent should come at low-cost and be atoxic, volatile and non-polar. In addition, solvent should not extract too much of the other cellurar components. (Rawat et al. 2010.)

Usually solvents break down the cell structure but in some cases algae might have to be pre-treated to rupture cells for lipid extraction. This can be done by sonication, homogenisation, grinding, bead beating, freezing, autoclaving, osmotic shock, microwaving or freeze-drying. (Rawat et al. 2010.)

4 BIOFUELS FROM ALGAE

Biofuel can be defined as solid, liquid or gaseous fuel that is produced directly or indirectly from renewable feedstock. Ideally in the foreseeable future fossil fuels will be superseded by biofuels (Demirbas & Demirbas 2010, 49, 99.)

There are different ways to process algae biomass to biofuels. Methods can be categorised as three types, those where biofuels are produced from algal extracts, for example lipids and carbohydrates. Then there are those where whole biomass is processed in order to receive fuel molecules and those methods where recoverable fuel molecules are received from algal production without any extraction, for example hydrogen. (Gouveia 2011, 5.)

As with everything, there are some benefits and drawbacks when using microalgae as a raw material for the biofuel production (table 4). Instead of processing conventional crops to biofuels, (such as sugar beet or sugarcane to process bioethanol, oleaginous plants such as soybean, colza or rapeseed to refine biodiesel) they could be used as food or fodder. Microalgae, on the other hand, would not compromise food production. (Brennan & Owende 2010.)

An exponent of using microalgae as a feedstock is their fast growth rate. What makes it even more lucrative is the ability to mitigate carbon dioxide which could be taken advantage of by building algae cultivation integrated to factories. Another opportunity to integrated cultivation systems would be waste water treatment plants. Waste water could provide phosphorus, nitrogen and other nutrients needed for algae growth. (Demirbas & Demirbas 2010, 98, Brennan & Owende 2010.)

Algae's consumption of fresh water is less than with terrestrial crops and there is no need for herbicides or pesticides either. Then again, algae's low biomass concentration sets its challenges to harvesting, and capital costs are higher than with conventional crops. Species selection has to meet the requirements for biofuel production, but at the same time the potential of valuable co-products need to be taken into account. There is also a possibility of receiving a negative energy balance when all water pumping, CO_2 transfer, harvesting and extraction are added up. (Demirbas & Demirbas 2010, 98, Brennan & Owende 2010.)

Table 4 Benefits and drawbacks of using microalgae in biofuel production, table modified (Demirbas & Demirbas 2010, Brennan & Owende 2010).

Benefits	Challenges
Growth rate	Low biomass concentration
Water consumption	Capital costs
CO_2 mitigation	Species selection
No need for herbicides or pesticides	Chance of negative energy balance
Possibility to receive nutrients from waste water	Scrubbing of flue gas from toxic compounds

4.1 Biodiesel

4.1.1 General about biodiesel production

There are different ways of making biodiesel. Maybe the most commonly known is transestherification to achieve FAME, Fatty Acid Methyl Esther (Figure 6). In transestherification triglycerides of vegetable oils or animal fats are converted into biodiesel by adding alcohol (e.g. methanol) in the presence of an alkali catalyst such as potassium hydroxide (KOH) or sodium hydroxide (NaOH). Catalyst can also be an acid or an alkalimetal alkoxide. (Demirbas & Demirbas 2010, 102; 139-147.)

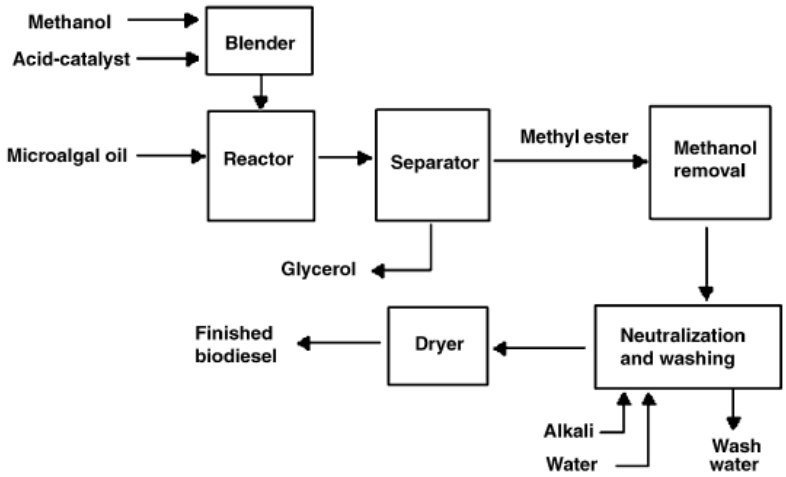

Figure 6 Biodiesel (FAME) from microalgae process flow chart (Xu et al. 2006)

Neste Oil uses a different kind of approach compared to traditional transesterification process to produce biodiesel. Neste Oil's NExBTL is manufactured by hydrotreating vegetable or waste oils. Hydrotreating means that molecules' reactive bonds (unsaturated) are combined with hydrogen. (Neste Oil 2011.) Quality of hydrotreated biodiesel is better than that of FAME.

UPM is in the process of making biodiesel by gasification from energy wood, which would consist of logging residues, wood chips, stumps and bark. It is planned that forthcoming biorefinery would be situated nearby existing pulp or paper mill. UPM has been developing needed gasification technology in cooperation with Andritz/Carbona. (UPM 2010.) In addition, in Finland, Metsäliitto has joined forces with VAPO and they are planning to produce biodiesel by gasification. (Metsäliiton ja Vapon BioDiesel-hanke n.d.)

4.1.2 Algal oils

Algae's potential for biofuel production is widely recognised. According to research conducted earlier, algae can contain up to 70 % of lipids per dry weight (Chisti 2007). Figures are listed in table 5. With minor modifications oil can be used in biodiesel production. What this means is that algal biodiesel could be treated in already available refineries and used in existing service network. Biodiesel is usable in diesel engines and mixed with fossil diesel at any ratio (Singh & Gu 2010).

Table 5 Oil content of some microalgae species, table modified (Chisti 2007).

Microalga	Oil content (% dry weight)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Dunaliella primolecta	23
Nannochloris sp.	20-35
Nitzschia sp.	45-47
Schizochytrium sp.	50-77
Tetraselmis sueica	15-23

Oil yield is far better than in food crops, in fact, algae are the most effective oil producers that nature has to offer. In table 6 there are some field crops' oil yields that have been compared to those of algae. (Gouveia & Oliveira, 2009.)

Table 6 Arable land crops' versus algae's oil yield, table modified (Chisti 2007)

Crop	Oil yield (L ha)
Corn	172
Soybean	446
Canola	1,19
Jatropha	1,892
Coconut	2,689
Palm	5,95
Microalgae, 30 % oil by dry wt	58,7
Microalgae, 50 % oil by dry wt	97,8
Microalgae, 70 % oil by dry wt	136,9

4.2 Bioethanol and hydrogen

Usability of microalgae as a raw material for bioethanol production has been studied to some extent and there have been some hopeful results. Bioethanol is produced by firstly using an enzyme treatment (i.e. enzymatic hydrolysis) to biomass and then adding some yeast, bacteria or fungi to the mass to commence the fermentation. (Demirbas 2010). Nonetheless, as Singh & Gu (2010) state, commercialisation of producing bioethanol from microalgae is further researched and still under development. (Singh & Gu 2010.)

Table 7 shows the share of carbohydrates in certain microalgae species. Harun et al. conclude that the ethanol yield depends on pre-treatment, hydrolysis and fermentation. (Harun et al. 2010.)

Table 7 Amount of carbohydrates of various microalgae, table modified (Harun et al. 2010)

Species	Carbohydrates (%)
Scenedesmus obliquus	10-17
Scenedesmus dimorphus	21-52
Chlamydomonas rheinhardii	17
Chlorella vulgaris	12-17
Chlorella pyrenoidosa	26
Spirogyra sp.	33-64
Dunaliella bioculata	4
Dunaliella salina	32
Euglena gracilis	14-18
Prymnesium parvum	25-33
Tetraselmis maculate	15
Porphyridium cruentum	40-57
Spirulina platensis	8-14
Spirulina maxima	13-16
Synechoccus sp.	15
Anabaena cylindrical	25-30

Table 8 shows the potential ethanol yields from different biomasses on a weight basis. Harun et al. (2010) point out that the advantage compared to other cultivated crops is not the yield on a weight basis but the land, cost and time needed. (Harun et al. 2010.)

Table 8 Ethanol yield from different biomass sources, table modified (Harun et al. 2010)

Raw material	Conversion to sugar or starch (%)	Ethanol yield (gEtOH/gbiomass)
Sugar cane	12.5	0.055
Sweet sorghum	14	0.063
Sugar beet	-	0.079
Cane bagasse	-	0.111
Cassava	25	0.118
Microalgae	57	0.235
Corn stover	-	0.260
Wheat	66	0.308
Corn	69	0.324

VTT and SYKE have run a project, called Microbes and Algae for Biodiesel Production, Microfuel (in 2007-2009), the goals of which were among others to optimise algal and fungal lipid yields and analyse residual biomass after lipid extraction. One of the project scenarios was to produce ethanol from residual biomass by using fungi.

Figure 7 illustrates simplified picture of algal resource allocation. In case of all nutrients being available and an adequate amount of light energy to drive carbon fixation, resources go to algal vegetative growth, which usually means cell division. When there is a deficiency of an inorganic nutrient like nitrogen and carbon, fixation is on-going, and excess carbon is to be stored in the cell. The form of stored carbon depends on the species; some algae store it mostly as carbohydrates whereas others store it as lipids. (Biorefine yearbook 2009.)

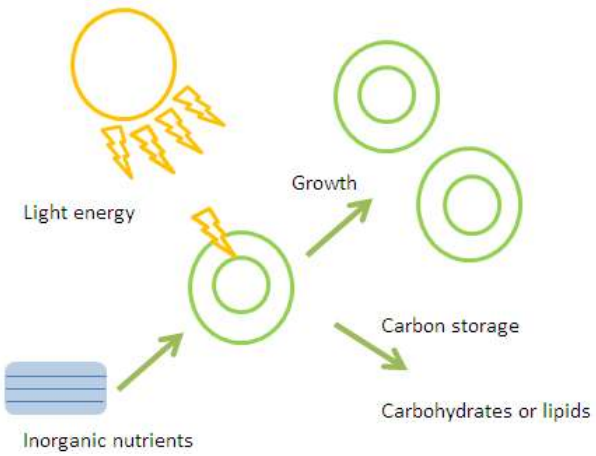

Figure 7 Simplified algal allocation of resources (Microfuel, Biorefine yearbook 2009)

There are also species of microalgae which have the capability to produce hydrogen in light via water hydrolysis. After hydrogen production the algal biomass could be further utilised in biogas generation. (Mussgnug, J.H., Klassen, V. & Schlüter, A. & Kruse, O. 2010.)

5 BIOGAS FROM ALGAE

5.1 Anaerobic digestion in general

There are four phases in the anaerobic digestion, hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the first phase the initial material is broken down to smaller pieces. Proteins and carbohydrates are degraded into amino acids and sugars whereas lipids turn into long chain fatty acids and alcohols. Secondly comes the acidogenesis and as the name suggests, acids are formed in this phase. The phase is generated by the fermentative bacteria and products from the hydrolysis are broken down to ever smaller pieces. When process is stable, sugars, amino acids and fatty acids become acetate, carbon dioxide and hydrogen while volatile fatty acids (VFA) and alcohols have a minor role. In the acetogenesis compounds from previous phase are converted to methanogenic substrates (acetate, hydrogen) and it typically runs parallel with the methanogenesis in which the methane is formed.

Typically biogas contains approximately 60 % methane and approximately 40 % carbon dioxide. Low concentration of hydrogen sulphide and ammonia can be detected as well as some trace compounds like silicates, VOCs and so on (Cheng, 2010; 152.)

Primarily the anaerobic digestion has been a treatment method for sludge and solid wastes to achieve less of a waste in a preferable quality for possible further treatment. Nowadays, the anaerobic digestion is getting more accredited as a good renewable energy provider. The residual digestate from biogasification can be used as a fertiliser, nutrient additive for cattle or it can be for example burned.

5.2 Biogasification research on algae

In order to get a picture of microalgae methane potential, it is worthwhile to look at their biochemical composition. A study conducted by Brown et al. researched the nutritive characteristics of microalgae. The overall composition differs between the species. In their study they report protein content of 6-52 %, carbohydrates 5-23 % and lipids 7-23 %. (Brown et al., 1997.) Sialve et al. (2009) have put together a table of algae's theoretical methane potentials (table 9) by using organic compounds as a basis of their calculations. The formula they have used is $C_{6.0}H_{13.1}O_1N_{0.6}$. Heaven et al. (2011) commented that preferable formula to use would be $C_{1.9}H_{3.8}O_1N_{0.5}$.

Table 9 Certain algae species' composition and theoretical methane potential, table modified (Sialve et al. 2009; Heaven et al. 2011)

Species	Proteins (%)	Lipids (%)	Carbohydrates (%)	CH_4 ($mlCH_4/gVS$)[a]	CH_4 ($mlCH_4/gVS$)[b]
Euglena gracilis	39-61	14-20	14-18	530-800	555-558
Chlamydomonas reinhardtii	48	21	17	690	579
Chlorella pyrenodoisa	57	2	26	800	450
Chlorella vulgaris	51-58	14-22	12-17	630-790	544-569
Dunaliella salina	57	6	32	680	471
Spirulina maxima	60-71	6-7	13-16	630-740	483-484
Spirulina platensis	46-63	4-9	8-14	470-690	481-500
Scenedesmus obliquus	50-56	12-14	10-17	590-690	531-536

[a] Formula by Sialve et al. 2009

[b] Formula by Heaven et al. 2011

In general lipids have the highest methane producing potential of the organic macromolecules (table 10). However, feedstock for the anaerobic digestion cannot consist only of fat but other ingredients are needed as well. Even if the lipids are to be extracted from algae and to be used for biodiesel production, the waste algal biomass is still a good source of proteins and carbohydrates to be anaerobically digested.

Table 10 Stoichiometric theoretical biogas potential of macromolecules

Biogas potential of macromolecules			
1 kg	m^3biogas/kg	CH_4 (%)	CO_2 (%)
Lipids	1,25	68	32
Carbohydrates	0,79	50	50
Proteins	0,7	71	29

To optimise the continuous biogas yield, a low organic loading rate (OLR) and a high hydraulic retention time (HRT) should be kept in the process. When using mesophilic conditions (temperature about 35 °C) hydraulic retention times vary around 30 days. As a rule decomposition improves with the longer retention time and the volume load declines in compliance with it. (Deublein & Steinhauser 2008, 274-275.)

Brune, Schwartz, Massingill, Benemann and Weissman (2006) and Yen (2004) have studied the effects of the carbon nitrogen ratio in biomass to efficacy anaerobic digestion. Algal biomass ratio is about 6:1, which means too high nitrogen content. When the biomass has high nitrogen content, it leads to an increase in ammonia in anaerobic digestion and can become an inhibitory factor in methane output. The studies have shown that optimal ratio between carbon and nitrogen varies around 12-20:1. Deublein & Steinhauser have written that the functional ratio is somewhere between 16:1 and 25:1. To improve the C/N ratio extra cellulose, or other carbon sources, needs to be added. In addition, the biomass can be mixed with wastes having higher car-

bon content, such as animal manures, sludges or waste paper. (Brune, Lundquist & Benemann 2009; Deublein & Steinhauser 2008.)

Ehimen et al. (in press 2010) concluded in their study with semi-continuous reactors that HRT is a major single factor when digesting algal residues from biodiesel production. Methane yields improved when biomasses were digested for periods over 5 days. They suggested that C/N ratio of 12.44 was optimal for biogasification of algal residues when HRT was 15 d. However, surprisingly, no longer retention times were applied in their study, even when study was performed under mesophilic conditions. With such short retention times there is a chance that functional methanogenic bacteria have been washed away from the reactor. Had the retention time been longer, there might be slight differences in the methane yields. Obviously when C/N ratio was 5.4 the methane yields were significantly lower. They co-digested residual algal biomass with different amounts of glycerol temperature being at 35 ±0.5 °C. (Ehimen et al. 2010.)

Mussgnug et al. studied the methane potential of six different algal species by digesting them anaerobically for a period of 32 days. They concluded in their research that the yield of biogas was pronouncedly dependent on the species and should be tested separately. The methane yields of those species that had a robust cell wall structure were lower than of those that had an easily degradable cell wall or no cell wall at all. (Mussgnug et al. 2010.)

Table 11 Comparison of methane yields of different algal species according to studies

Substrates	T	HRT	C/N ratio	ml/gVS	St dev	CH_4 content	CH_4 yield ml/gVS	CH_4 yield ml/gVSS	Ref
Chlorella sp. residues and glycerol	35	15	12.44	N/A	N/A	65.3	295	N/A	Ehimen et al. 2010
Chlorella sp. residues and glycerol	40	15	12.44	N/A	N/A	63.1	265	N/A	Ehimen et al. 2010
Arthrospira platensis	38	32	N/A	481	14	61	293	N/A	Mussgnug et al. 2010
Chlamydomonas reinhardtii	38	32	N/A	587	9	66	387	N/A	Mussgnug et al. 2010
Chlorella kessleri	38	32	N/A	335	8	65	218	N/A	Mussgnug et al. 2010
Dunaliella salina	38	32	N/A	505	25	64	323	N/A	Mussgnug et al. 2010
Euglena gracilis	38	32	N/A	485	3	67	325	N/A	Mussgnug et al. 2010
Scenedesmus obliquus	38	32	N/A	287	10	62	178	N/A	Mussgnug et al. 2010
Zea mays	38	32	N/A	653	38	54	353	N/A	Mussgnug et al. 2010
Chlorella vulgaris	35	16	est. 6	N/A	N/A	N/A	N/A	147	M. Ras et al. 2011
Chlorella vulgaris	35	28	est. 6	N/A	N/A	N/A	NA	240	M. Ras et al. 2011

5.3 Case Anjalankoski, Finland

Within the project "Kaakosta voimaa", which focused on developing wind energy and bioenergy know how in southeast Finland, two open circle ponds (Figure 8) for algae growing was built during summer 2010. The ponds were built nearby an incinerator so that heat from combustion gases, carbon dioxide emissions and effluents could be utilised in algae growth. Ponds were approximately 0.5 meter deep and had an area of about 400 m^2, accounting for a total volume of about 400 m^3. (Vatanen 2010.)

Algae were harvested by using flocculation and fed directly to biogasification process. Process was conducted in two stages, in two consecutive biogas reactors. According to Vatanent the size of the biogasification equipment was 30 tonnes. The process HRT was 26 days. Unfortunately algae could not be grown and fed uniformly to the process so the yield of the biogas was low. In addition the methane content of the biogas varied a lot.

Vatanen mentions in his thesis that at the end of the summer algae growth was so fast that animal plankton started to eat them. There was also some sort of contamination to be seen, and because of it algae growth was disturbed and thus the quality of the biogas was not uniform enough. (Vatanen 2010.)

Figure 8 Open pond for algae growth in Anjalankoski, Finland (Vatanen 2010).

6 MATERIALS AND METHODS

6.1 Substrates and inoculum

Digestate from Envor Biotech Oy's bioreactor was used as an inoculum, i.e seed sludge. The sludge was received a day or two days before the start of tests and was kept in a water bath for starvation overnight at 36 °C.

Since not enough ALDIGA's focus strains algal biomass was available when starting batch reactors in January, species used were *Scenedesmus* and *Chlorella* (Figure 9). *Scenedesmus* was cultivated in Finnish Environment Institute (SYKE) in 2008-2009 and then freeze-dried. Lipid content was estimated to be approximately 7-11 %. Powdered *Chlorella* was bought from health food shop and did not include specific table of ingredients. By request a list of ingredients was received, in which lipid content of *Chlorella* was 6.7 %, however it did not say whether it was as per dry weight or by fresh weight.

Figure 9 *Scenedesmus* (left) and *Chlorella* (right)

6.2 Pre-treatment of substrates

Certain amount of substrates was pre-processed before starting each of the batch tests. Methods that were used are explained later in the text.

6.2.1 Lipid extraction by alkali method

Lipids were extracted from *Chlorella* and *Scenedesmus* in order to imitate what is left in algal biomass after fractions have been taken out for biodiesel production. Alkali extraction method, which was received from VTT, was used to perform extraction (Figure 10).

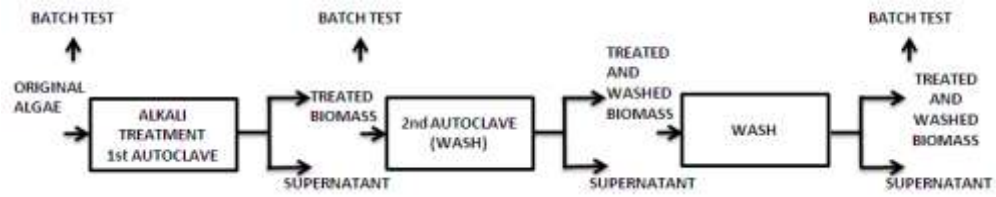

Figure 10 Simplified flow chart of pre-treatment of substrates

Dry algae were dissolved in 0.9 % KOH-solution [~150g algae/l KOH-vol]. Since algae were elutriated, pH was checked to be over 7. Both solutions were then autoclaved for 20 minutes at 121 °C and centrifuged. Supernatants were removed and kept for lipid analysis to see how well extraction had succeeded.

Figure 11 Alkali treated centrifuged algal biomass

To wash the extracted lipids out from the centrifuged biomasses (Figure 11), they were re-elutriated to half a litre of water and autoclaved again. After the compounds had been cooled a bit they were centrifuged. From this batch, the supernatant were kept for analyse purposes, too.

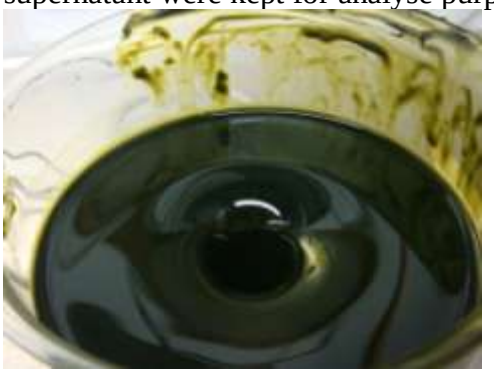

Figure 12 Re-elutriated algal biomass

After twice autoclaved and centrifuged, the algal biomasses were once again re-elutriated to half a litre of water. Instead of autoclaving, the masses were just stirred with a magnetic stirrer for half an hour and centrifuged (Figure 12). The supernatants were kept for analyses and the biomasses were recovered.

In order to receive sufficiently alkali treated biomass for subsequent batch tests another 100 grams of both *Chlorella* and *Scenedesmus* were treated with alkali. However, biomasses were freeze-dried to achieve as homogenous biomass as possible for the second batch test. For the third batch test 50 grams of both algae were treated with alkali and autoclaved but not centrifuged and washed. Instead, liquefied biomasses were freeze-dried.

To find out how the total solids divide between biomass and supernatants consecutive lipid extractions were carried out. The lipid extraction was carried out six times to *Chlorella* and twice to *Scenedesmus*. Afterwards TS, VS and ash analysis was performed.

6.2.2 Freeze-drying

Freeze-drying was performed with Heto Drywinner. First batch of treated wet biomasses were frozen in small plastic bags overnight. After freezing plastic bags were perforated and put into freeze dryer for sufficient amount of time, around 2-3 days. Another batch was freeze-dried without plastic bags on top of aluminium foil to speed-up the process. Alkali treated freeze-dried algae are illustrated in figure 13.

Figure 13 Freeze-dried algal biomass

6.3 Methane formation potential tests

In the batch tests, certain amount of the feedstock together with anaerobic inoculum is put into a test bottle at the beginning of the experiment and is not fed after bottling during test period. In this way the biogas or the methane potential of the initial material can be determined. The tests are done with parallel samples so that the average potential can be checked and a standard deviation calculated. The amount of formed biogas can be measured frequently.

The methane potential of algae was measured with two Bioprocess Control's Automatic Methane Potential Test System (AMPTS), each including 15 sample bottles. The system measures the methane as it is formed (online) and saves results to the computer. Formed carbon dioxide is scrubbed in the process and therefore not taken into account. The system is illustrated in figure 14.

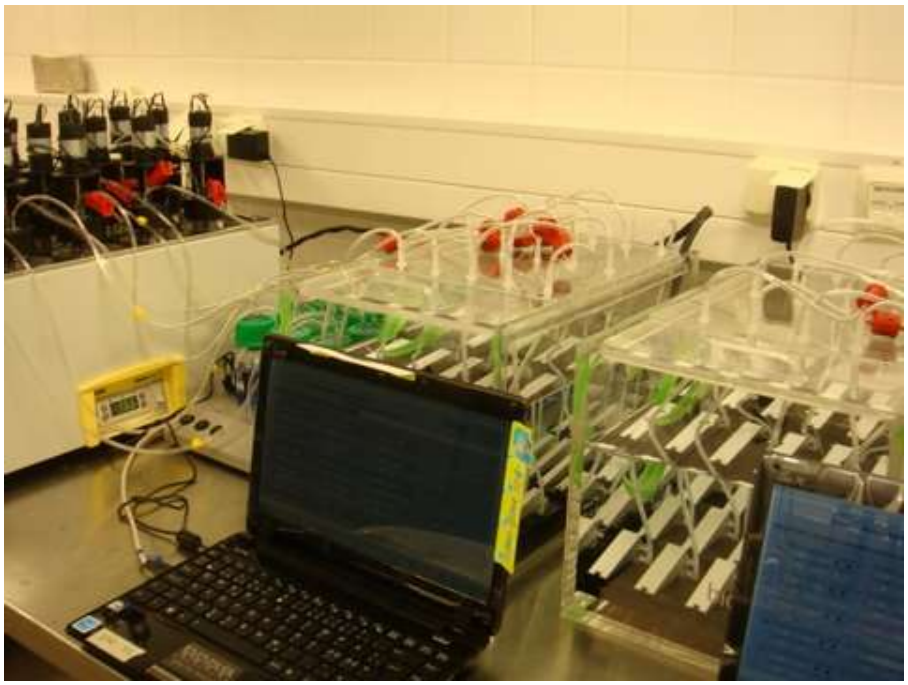

Figure 14 Automatic Methane Potential System (AMPTS)

6.3.1 Preliminary preparations

Each calibration volume of the gas measuring device was entered onto computer (Figure 15). After that about 80 ml of 12 % sodium hydroxide (NaOH) was measured to 100 ml measuring bottles and a magnet was put into each bottle. The bottles were put onto a magnetic stirrer and closed with rubber stoppers with two metal tubing and plastic screw caps with a hole. The tubes were attached to bottles and to a gas measuring array. Water baths were filled with deionised water.

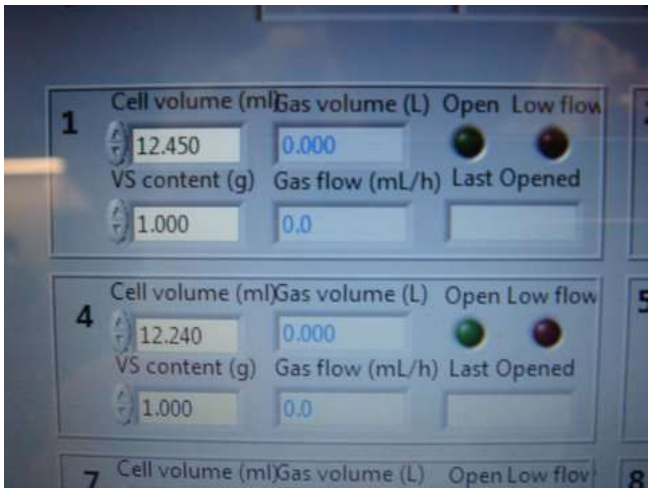

Figure 15 Gas collecting cell volumes (AMPTS programme)

6.3.2 The first test

Plan for the batch tests was done based on VS content of samples and inoculum. The plan is attached as an appendix 1. The actual TS and VS analysis to determine dry matter was begun on the same day as batch test was begun in order to get representative samples. In this way the degradation that occurs in the inoculum is minimised.

Water was weighed first to each reactor bottle. After water addition samples of dry *Chlorella* and *Scenedesmus* and wet alkali treated algae biomasses from both algae were measured according to the batch plan and bottles were gently shaken in order to get the samples to dissolve. Lastly inoculum was weighed. pH was measured only on few bottles, since there were several parallel samples. Reactor bottles were closed with rubber stoppers with two metal tubings, a rotating shaft and an agitator. Wires were connected to the power array.

When every water bath was filled, all bottles and tubes were put in place, nitrogen (N_2) was added to the bottles so that there was no room left for oxygen. After purging the samples with N_2, gas measuring cell arrays were opened underwater that no nitrogen bubbles were left inside the arrays to distort the measuring results. Folders were created on both computers for data collecting. Finally magnetic stirrers were turned on, temperature-controlled water baths were set to 36 ±1 °C and data collecting was initiated.

6.3.3 The second and third test

The second batch test was done similarly to the first one but contrary to first batch test alkali treated algae biomasses were freeze-dried and measured to sample bottles as dry (appendix 1). The test was performed similarly as the first batch test.

As with the first and second batch tests preliminary preparations were done likewise for the third test. What was special in the third test was that alkali treated and autoclaved algae were freeze-dried without washing the lipids off. The plan of the third test can be seen in appendix 1. In this test the objective was to observe what kind of effect alkali and heat as a pre-treatment had on methane yield.

6.4 Analysis methods

6.4.1 TS, VS and ash

To specify the total solids (TS) i.e. dry matter, volatile solids (VS) i.e. organic matter and ash i.e. inorganic matter of the algae and seed sludge TS and VS analysis was performed. Firstly crucibles were tared for two hours in a muffle

furnace at 550 °C then cooled in desiccators and weighed. About four grams of samples were weighed into the crucibles after which, total weight was measured and the crucibles were put into heating chamber for 22 hours for drying (105 °C). Dried samples were once again cooled in desiccators and weighed before putting into the muffle furnace for two hours at 550 °C.

6.4.2 Lipid analysis

Different kinds of lipid extractions were performed for the purpose of lipid analysis. First lipid analysis was performed by extracting lipids with petroleum ether. The purpose of the lipid analysis was to check if alkali extraction method had been successful. In order to find that out, lipid analysis was done both for non-treated and treated algae and dried supernatants.

Evaporating dishes were tared at 105 °C, cooled in desiccators and weighed. After weighing the dishes about five grams of sample were measured into evaporating dishes. The remaining water of the samples was evaporated by using a heat plate.

50 ml of petroleum ether was added with measuring glass and samples were stirred with a glass rod to achieve a homogenous mixture. The mixtures were left on a slant for fifteen minutes so that existing lipids were extracted (Figure 16). After some time had passed, sediment and supernatant were separated by decanting the petroleum ether.

Figure 16 *Chlorella* and alkali treated freeze-dried *Chlorella* in petroleum ether

Because the first extraction does not elute all lipids, adding of petroleum ether needs to be repeated a few times. Additional 30 ml of petrol ether was measured and added to the sample. The samples were again left tilted for ten minutes and petroleum ether was decanted. Once more 30 ml of petroleum ether was added and samples were left to separate for another ten minutes and afterwards decanted. Wet sample was then evaporated on a heat plate and gravimetric analysis was performed to remaining residues.

Another lipid analysis was done in the University of Helsinki. Lipids were extracted after acid hydrolysis and fatty acids were determined as methyl esters. Lipid content of the samples was then estimated based on the fatty acid content.

6.4.3 Carbohydrates and lignin by acid hydrolysis

Two different strengths of acid hydrolysis were tried for analysing carbohydrates and lignin. With strong acid hydrolysis the lignin content of the sample can be defined. However, it is assumed that algae do not contain any lignin, and strong hydrolysis was done more because of trying out the method and double checking if milder acid hydrolysis is enough to define structural carbohydrates. The acid hydrolysis was performed only to parallel samples of *Chlorella*.

In the beginning two filtering crucibles (which had no glazing in the bottom) were tared for two hours in a muffle furnace at 550 °C and two glass filters were tared for two hours in the muffle furnace at 470 °C (higher temperature will damage the glass). All dishes were then cooled in desiccators and weighed.

About 300 mg of *Chlorella* was weighed to four test tubes and 3 ml of 72 % sulphuric acid was added to two of the tubes in order to perform two-step acid hydrolysis. Those samples with sulphuric acid were stirred with glass rod and put into water bath for one hour (30 °C). The samples were stirred every now and then.

After one hour 84 ± 0.75 ml of deionised water was added with measuring glass and the samples were poured into 100 ml measuring flask. The two remaining samples were added 72 % sulphuric acid and diluted immediately with 84 ± 0.75 ml of deionised water to achieve milder acid hydrolysis with 4 % sulphuric acid content. Liquids were mixed thoroughly to avoid developing of different phases. Aluminium foil was put on top of the flasks to seal them. All four samples were then autoclaved for one hour at 121 °C.

After the samples were cooled down, deionised water was added to total volume of 100 ml. The samples with stronger acid hydrolysis treatment were vacuum filtered to filtering glass by using tared crucibles (Figure 17). The samples with milder acid treatment were vacuum filtered by using tare glass filters.

Figure 17 Filtering of sample

The filtered liquid was taken to small test tubes and acid soluble lignin was analysed with a spectrophotometer. Furthermore about 25 ml of the filtered liquid was kept and neutralised by using 20 % NaOH and 0.5M HCl. The samples from neutralised liquid were kept for later spectrophotometer analysis. The crucibles and the glass filters were rinsed with warm deionised water.

The filtering crucibles and the glass filters were put into heating chamber for drying at 105 °C and they were left overnight. After drying items were cooled in desiccators and weighed. The crucibles were put in the muffle furnace for two hours at 550 °C and cooled in the desiccators afterwards. The glass filters were placed in the muffle furnace for two hours at 470 °C. The remaining residues were weighted.

6.4.4 Defining of acid soluble lignin content by using spectrophotometer

Lignin is after cellulose the second most ample organic polymer. It is a complex biopolymer, which can be found from the cell walls of higher plants. (Keshwani, 2010; 29). Supposedly, algae should not contain any of the lignin.

After acid hydrolysis, samples were diluted with a ratio of 1:2. Acid soluble lignin was measured by using wavelength 320 nm.

6.4.5 Sugar analyses

Sugars were defined by using both a spectrophotometric and a HPLC method. In the spectrophotometric method four different glucose standards were made with concentrations of 0.1 g/l, 0.2 g/l, 0.4 g/l and 0.5 g/l to receive a standard curve. The spectrophotometer was set to wavelength 540 nm.

Algae samples were first diluted at ratio 1:2. 0.5 ml of diluted sample was added to test tube with a 0.75 ml of DNS reagent, mixed and boiled vigorously for five minutes. With a dilution ratio of 1:2, results were not within the standard curve. Another set of algae samples were diluted at 1:5 ratio.

In addition to spectrophotometer, sugars were determined by using HPLC. Analysis was done at University of Helsinki.

7 RESULTS AND DISCUSSION

7.1 Composition of substrates

On average, dry matter content of both algae species was very high as can be seen from table 12. The dry matter content of the original, non-treated samples did not differ much. For the first batch test, both alkali-treated samples were used as wet biomasses, whereas for the second and third batch test alkali-treated algae were freeze-dried.

Table 12 TS, VS and ash analysis of the substrates for batch tests

	Substrate	TS (%)	St dev	VS (%)	St dev	Ash (%)	VS/TS (%)	Ash/TS (%)
First batch test	Chlorella	94,6	0	81,3	0,7	13,3	85,9	14,1
	Alkali treated C	14,6	0,1	13,9	0,1	0,7	95,2	4,8
	Scenedesmus	97,1	0	84,5	0,9	12,6	87	13,0
	Alkali treated S	18,3	0,5	17	0,5	1,2	93,3	6,7
Second batch test	Chlorella	94,2	0	84,4	1,1	9,8	89,6	10,4
	Alkali treated C	72,9	0,9	68,9	0,9	3,9	94,6	5,4
	Scenedesmus	96,8	0,1	86,3	0,7	10,5	89,1	10,9
	Alkali treated S	81,7	0,3	75,6	0,5	6,2	92,5	7,5
Third batch test	Chlorella	95	0,2	84,7	0,2	10,3	89,2	10,8
	Alkali treated C	98,8	0,2	93	0,2	5,8	94,2	5,8
	Alkali treated C, n w	94,1	0,1	83	0,7	11,1	88,2	11,8
	Scenedesmus	97,5	0,1	85,7	0,3	11,8	87,9	12,1
	Alkali treated S	99,1	0,2	92,2	0,2	6,9	93	7,0
	Alkali treated S, n w	90,3	0,9	77,7	1,0	12,6	86	14,0

What makes TS, VS and ash results from all three batch tests interesting is the ratio of ash and total solids after alkali treatment. It was assumed that the ratio of organic matter and dry matter was to be decreased after the alkali treatment because of lipids removal. However, results clearly show that the alkali treatment removes not only the lipids but even more inorganic matter and maybe some other organic matter thus increasing the ratio of VS/TS.

How the organic macromolecules of the non-treated algae have been divided can be seen from table 13. The carbon and nitrogen content of the algae was analysed in Vantaa, Metla laboratories. The lipid content of *Chlorella* according to importer was 6.7 % and *Scenedesmus* according to VTT was assumed to be approximately 7-11 %.

Table 13 Composition of algae species used in this project % in TS

Algae	C (%)	N (%)	TKN* (%)	Proteins** (%)	Sugars (%)	Lipids*** (%)	C/N
Chlorella	52.3	10.4	9.9	65.0	10.4	5.3	5.1
Alkali treated *Chlorella*	54.4	9.36	9.7	58.8	11.35	2.0	5.8
Scenedesmus	50.0	7.0	6.5	43.8	N/A	5.1	7.1
Alkali treated *Scenedesmus*	53.1	5.8	5.8	38.9	N/A	3.3	8.5

*performed twice
**factor 6.25
***factor 1.046

The average Total Kjeldahl Nitrogen has been calculated from two analyses, both of which had parallel samples (Figure 18). Proteins have been calculated based on 6.25 times N-content. Sugar content has been taken from the HPLC analysis, which is more accurate than results after acid hydrolysis by using the spectrophotometer. According to importer, sugar content of *Chlorella* is 11.2 %. The lipid analysis was done in the University of Helsinki. The total fatty acid composition has been multiplied by correction coefficient of 1.046.

Insoluble lignin accounted for 0.6 to 0.7 % but total lignin content seemed to be too high, varying from 21.9 to 22.9 %. Most likely the high protein content of *Chlorella* distorted the result.

Figure 18 Kjeltech tubes filled with variable amount of algae for TKN analysis

7.2 Yields of dry and organic matter in an alkali pre-treatment

TS yields from alkali pre-treatment were less than 50 % of the total biomass, 46 % on average (appendix 2). First wash after the alkali treatment extracts about 20-30 % of the total solids. The second wash extracts about 10 % of the total solids and third wash only a slight amount. VS yields of alkali pre-treatment were slightly higher, about 49 % on both algae.

Looking at results from the TKN analysis and taking into account biomass yields from the alkali treatment of *Chlorella*, nitrogen content of biomass varies from 40-50 % of the original (table 14).

Table 14 Nitrogen yields and losses of alkali treated *Chlorella*

TKN	gN/kgTS	Compared to original (TS yield 46.25 %) gN/kgTS	Yield of N (%)	Loss of N (%)
Chlorella	101.3			
Alkali treated *Chlorella*	109.4	50.6	50.0	50.0
Chlorella	97.7			
Alkali treated *Chlorella*	84.4	39.6	40.0	60.0

The nitrogen content for supernatants of *Chlorella* have been calculated similarly (table 15). The results for *Scenedesmus* are attached (appendix 2).

Table 15 Nitrogen yields and losses of *Chlorella* supernatants

TKN	gN/kgTS	Compared to original (TS yield 53.75 %) gN/kgTS	Yield of N (%)	Loss of N (%)
Chlorella	97.7			
Chlorella supernatant (first wash)	103.7	55.7	57.1	42.9
Chlorella supernatant (first wash)	90.3	48.5	49.7	50.3
Chlorella supernatant (pooled)	103.1	55.4	56.7	43.3

7.3 Lipid extraction

After the alkali treatment and freeze-drying, biomasses became brittle and surface area was larger. The method did extract the lipids but at the same time some of the sugars and proteins were lost in the process. The biomass recovery accounted for a little less than half of the original total solids. The average biomass recovery for both *Chlorella* and *Scenedesmus* was 46 % meaning that over half of the total solids were lost in lipid extraction. Since lipid content of both algae was around 7 %, some of the sugars and proteins were lost in the process. Total nitrogen analysis supports the reflection that some of the proteins were lost in the process, as do the carbon and nitrogen results from Metla's laboratory.

The lipid analyses performed with petroleum ether did not give reliable results. The analysis was repeated 5 times with variable results. The analysis was based on a gravimetric method, which in this case cannot be trusted. In addition, parallel results varied more than the 0.4% allowed in the given me-

thod. Already the TKN analysis showed that some of the proteins were lost in lipid extraction and not all were lipids. The gravimetric method does not take into account that within sample there could be some other matter, too.

7.4 Methane production

7.4.1 Methane formation of the first test

The first test was kept going for 24 days in order to imitate about three weeks' retention time. Some algae ended their methane production sooner than this and therefore were not taken into account in the average and standard deviation calculations. The alkali treated algae had higher variation in standard deviation whereas the non-treated algae had more similar yield curves. The second and third batch tests were carried on for 21 days.

During the batch tests the methane production continued until finishing but slowed down from the beginning. The result tables from the other tests can be seen in appendices 3 and 4. The alkali treatment improved both algae's specific methane yields based on organic matter. One reason for the better methane yield after the alkali treatment could be that the treatment took off some of the inorganic (and possibly inhibiting) compounds as shown in TS, VS and ash analysis. Presumably the alkali treatment combined with autoclaving could have made some of the substrate more biodegradable.

During the first batch test the methane yield of *Scenedesmus* did not improve after alkali treatment, on the contrary. However, the wet biomasses were not homogenous enough and not enough organic matter may have been available.

In figure 19 the cumulative methane yields are described one by one from each alkali treated algae sample. The average yield of seed sludge has been taken off from the results. The x-axis shows the time per hours and the y-axis the methane yield in millilitres per one gram volatile solids, i.e. organic matter. From the chart it can clearly be detected that two bottles of *Scenedesmus*, numbers 16 and 18 have stopped producing the methane after two days as the yield curve declines towards the end because of the subtracted yield of the seed sludge.

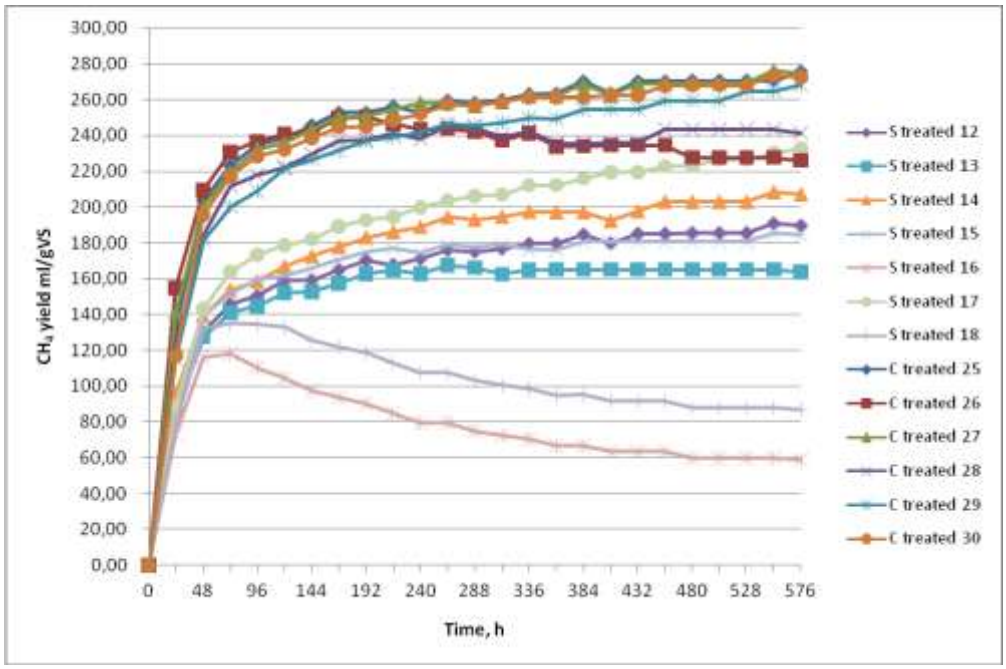

Figure 19 Parallel results of alkali treated algae's methane yields from first batch test (S being *Scenedesmus* and C being *Chlorella*, numbers following are to identify different sample bottles)

Figure 20 shows parallel samples' average methane yields of the first batch test in millilitres per one gram volatile solids and with standard deviation bars. Some of the sample bottles were left out from the calculations because of too high deviation from the average yield. The time in hours can be seen on the x-axis. Both alkali treated and non-treated *Chlorella* generates more methane than *Scenedesmus*. Yields of the alkali treated *Chlorella* are the highest whereas the alkali treated *Scenedesmus* scores at the lowest of all samples. The treated *Scenedesmus* has the highest variation of the results, which can easily be seen from the standard deviation bars illustrated in the figure. As discussed before, big differences in results and the lower methane production of the alkali treated *Scenedesmus* were most likely due to not homogenous enough samples.

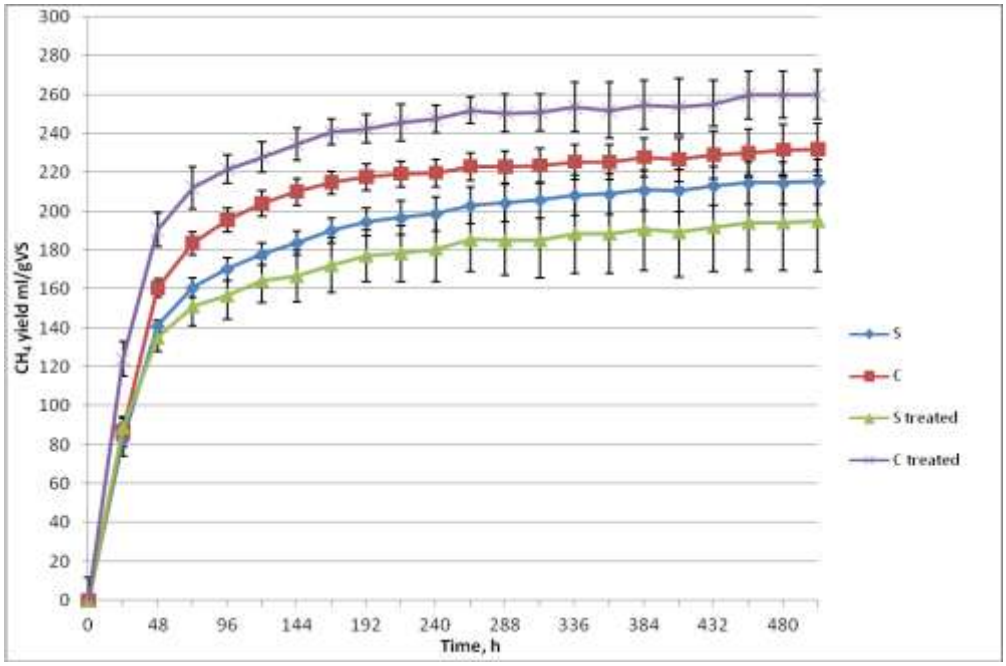

Figure 20 First batch test's average methane yields with standard deviation bars (S being *Scenedesmus* and C being *Chlorella*)

7.4.2 Methane formation of the second and third test

Figure 21 shows the average methane yields of the second batch test. As with the first test, also in this calculation some of the sample bottles have been excluded from the results, otherwise the average curves would be skewed. As in the first test, also in this second one the alkali treated *Chlorella* produced most methane of the four different substrates, nonetheless it had highest standard deviation, too.

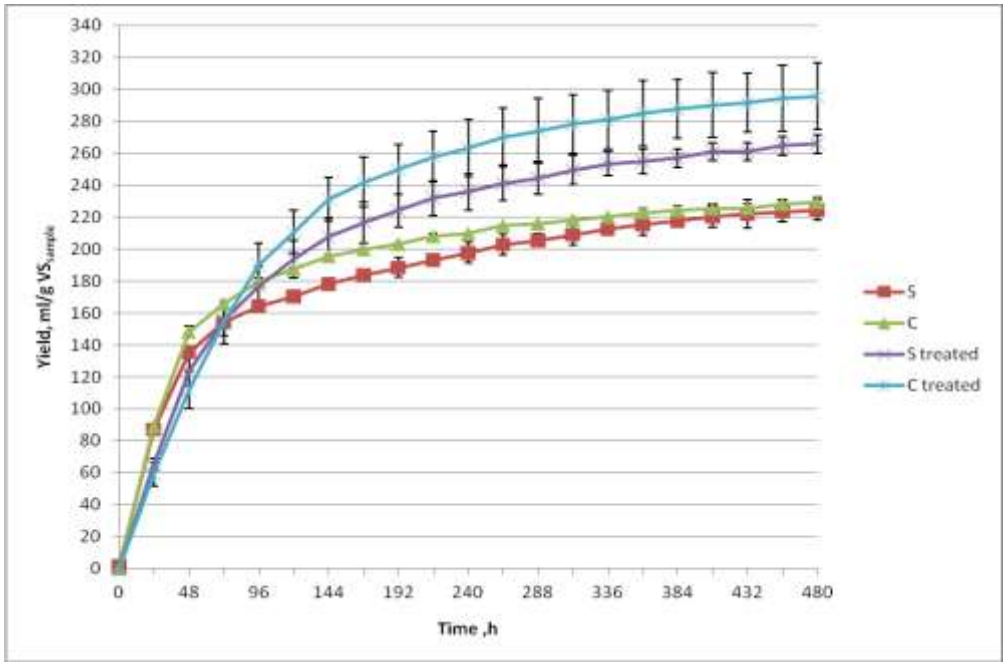

Figure 21 Second batch test's average methane yields with standard deviation bars (S being *Scenedesmus* and C being *Chlorella*)

Similarly to the first and second test, the methane yield curves of the substrates (excluding some aliquot bottles) from the third test are illustrated in figure 22. The interesting thing is that both alkali treated algae without wash after treatment start to produce methane considerably faster than non-treated substrates. This supports the idea that the alkali combined heat treatment breaks down or weakens the cell structure, making algae more biodegradable for methane producing microbes.

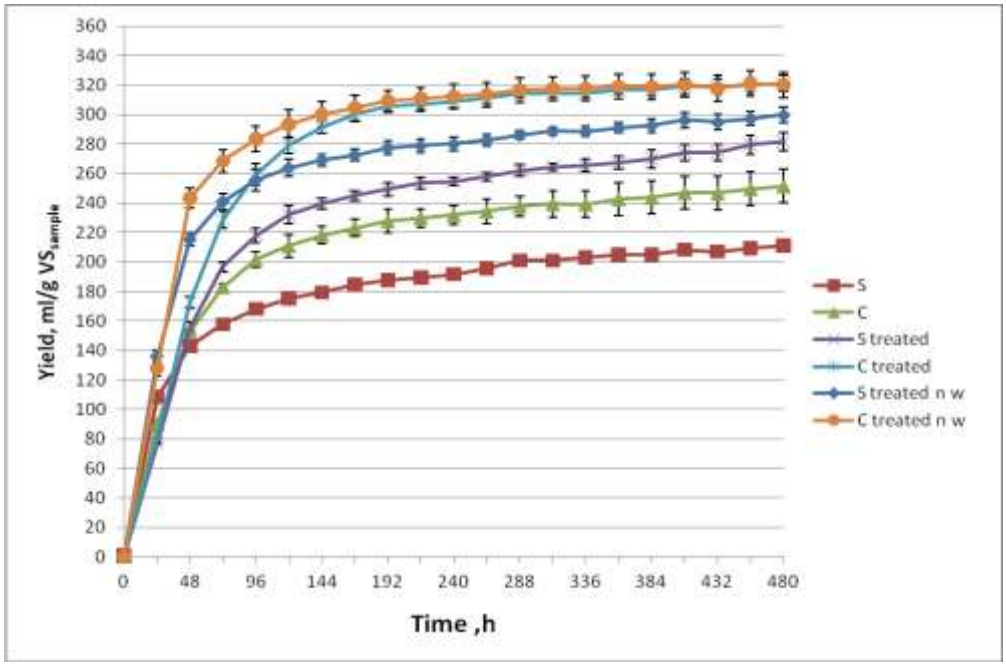

Figure 22 Third batch test's average methane yields with standard deviation bars (S being *Scenedesmus* and C being *Chlorella*)

7.4.3 Summary

The average methane yields from all three batch tests have been combined in table 16. The methane yields of the alkali treated algae include the second and third batch test but not the first one because of high standard deviation in the results, which suggests that the alkali treated samples of the first test were not homogenous enough. For the alkali treated algae from which lipids have not been washed out, table includes only results from the third batch test where they were included.

Table 16 Average methane yields of all samples tested in this work

	CH_4 ml/g VS		CH_4 ml/g TS	
Substrate	Average	St dev	Average	St dev
Chlorella	236	16	202	18
Alkali treated Chlorella, n w	314	16	277	14
Alkali treated Chlorella	301	23	284	22
Scenedesmus	217	12	188	10
Alkali treated Scenedesmus, n w	290	15	250	13
Alkali treated Scenedesmus	283	16	254	16

Figure 23 visualises the methane yield results per gram volatile solids as shown in table 16. The results of the original algae and the not washed alkali treated algae can be compared directly to each other since both substrates have the same amount of organic matter. The alkali and heat treatment im-

proved methane yields significantly. Approximately the improvement was one third of the yield, about 33 % for *Chlorella* and 34 % for *Scenedesmus*. Even for the alkali treated algae where lipids and other material had been extracted, figure 24 shows that they still produced more methane per organic matter (i.e. specific methane production) than the organic matter in original algae.

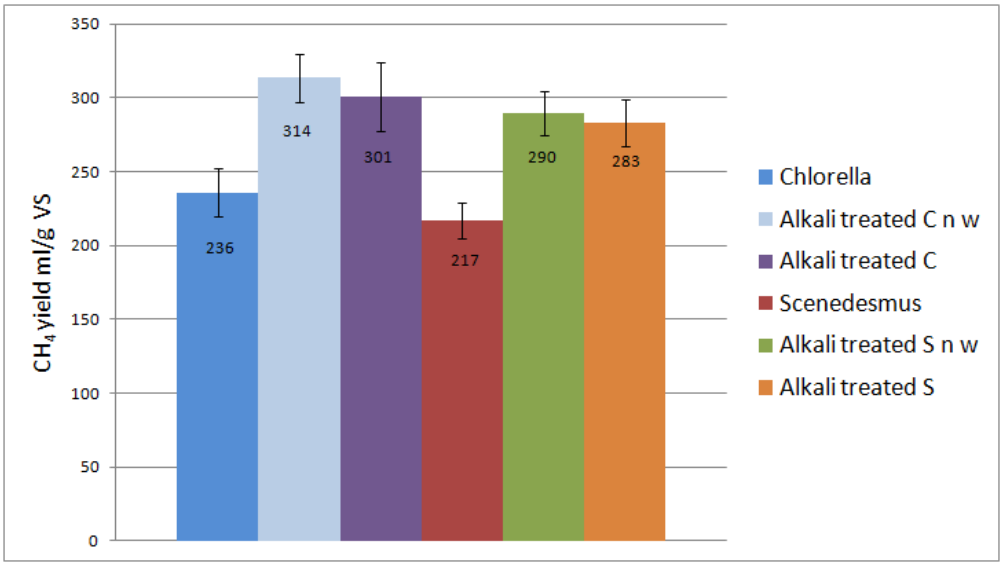

Figure 23 Combined average methane yields with standard deviation bars (S being *Scenedesmus* and C being *Chlorella*)

Yet when taking into account the recovery of the biomass from the alkali treatment and washes, the alkali treated *Chlorella*'s methane yield is about 63 % of the original. This is illustrated in figure 24. The VS yield of 49 % was used in the calculation.

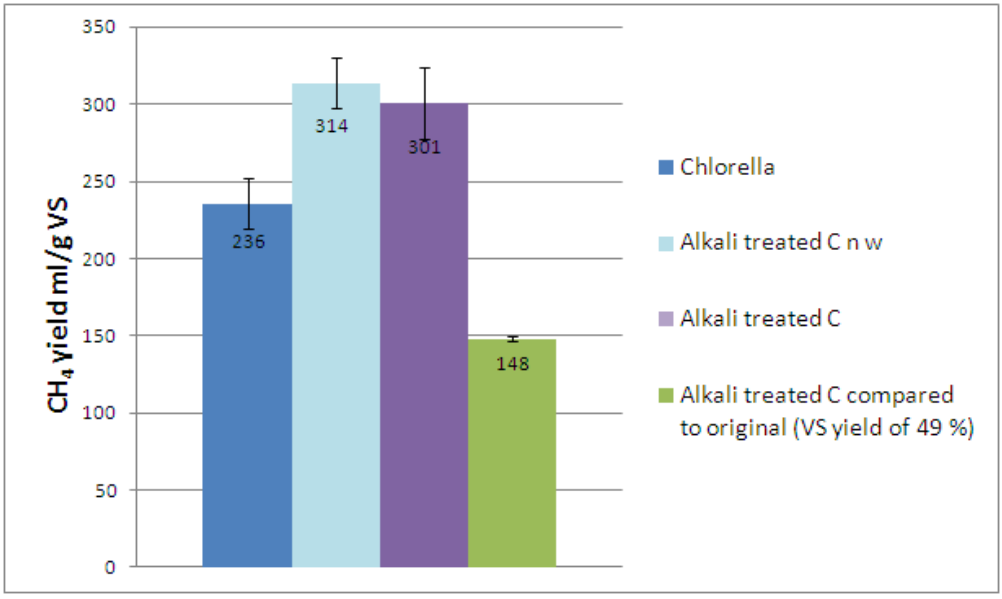

Figure 24 Alkali treated *Chlorella*'s average methane yield compared to original with deviation bars (C being *Chlorella*)

Similarly when adapting the yield for *Scenedesmus* according to the biomass recovery from the alkali treatment, the methane yield falls to about 64 % of the original. This can be seen in figure 25. The VS yield of 49 % was used in the calculation.

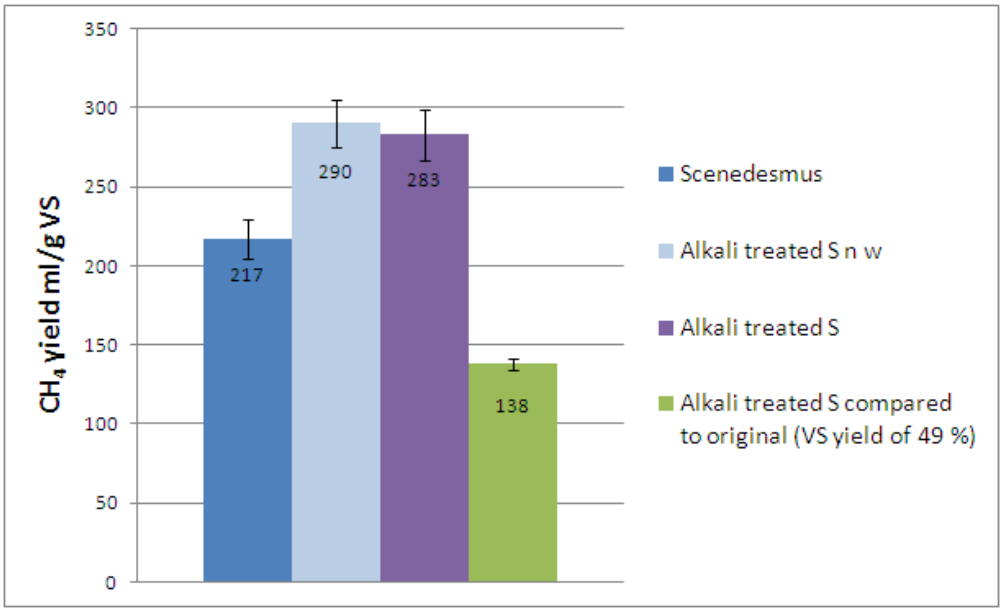

Figure 25 Combined average methane yields with standard deviation bars (S being *Scenedesmus*)

The average yields of *Chlorella* and *Scenedesmus* were very similar to previously published results of anaerobic digestion of algae. Mussgnug et al. reported in their study the biogas yield of *Scenedesmus obliquus* to be 287

ml/gVS of which 62 % was methane. This means that methane yield was on average 178 ml/gVS. The biogas yield of *Chlorella kessleri* was 335 ml/gVS, of which 65 % was methane, accounting 218 ml/gVS. In their research, best productivity of the biogas was with *Chlamydomonas reinhardtii*, topping 587 ml/gVS with the methane content of 66 % meaning methane yield of 387 ml/gVS. (Mussgnug et al. 2010.)

7.5 Ammonia formation

Ammonia (NH_3) and ammonium (NH_4^+) concentrations can be considered inhibitory indicators of the degradation process. When nitrogenous biomass is decomposed, the outcome is ammonia and ammonium. High ammonia concentration can inhibit the production of methane and have even toxic effects on the process. Ammonium, on the other hand, is rather harmless. (Deublein & Steinhauser 2008, 123.) Then again, mineralised form of nitrogen is easier for plants to utilise when digestate is used as a fertiliser.

The ammonium analysis was carried out for some sample bottles after each batch test. Proportionally there is more inoculum than sample nitrogen in bottles and ammonium content of the bottles is skewed by it. To get an idea of which level the ammonium contents originated from the sample, results were calculated by subtracting the average ammonium results of the inoculum.

Before the anaerobic digestion, both *Chlorella* and *Scenedesmus* had ammonium content of 7 % on average of the total nitrogen. At the end of the batch test the ammonium content of the total nitrogen reached about 44-48 % with *Chlorella* and 43-70 % with *Scenedesmus*. The precise results are attached (appendix 2).

7.6 Reduction of solids

To find out how successful the batch process had been, the reduction of the total solids (TS) and the volatile solids (VS) was calculated. The whole content of the batch sample bottle was first dried at 60 °C (Figure 26). The TS and VS analysis was performed to the evaporated samples.

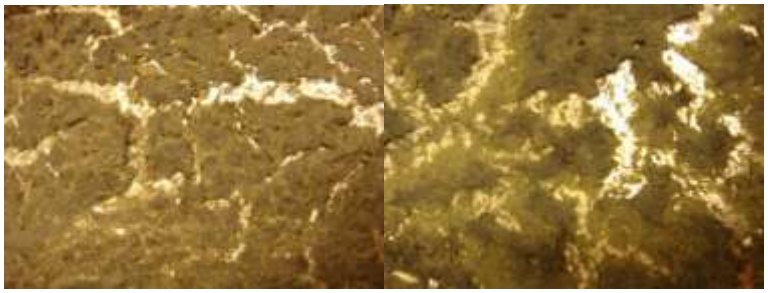

Figure 26 Dried algae biomass after the batch test

The average TS reduction for both *Chlorella* and *Scenedesmus* was around 40 %, the VS reduction reaching fairly similar percentage. The TS reduction of the alkali treated *Chlorella* was on average 45 % and the VS reduction 48 %. The same results for the alkali treated *Scenedesmus* were 39% and 45 %, respectively (Figure 27).

Those alkali treated samples that had not been washed had the highest TS and VS reduction. The alkali treated *Chlorella*, in which lipids were not washed out reached reduction of the total solids approximately 55 % and reduction of the volatile solids 62 % while the alkali treated *Scenedesmus* in which lipids were not washed had the TS reduction of 47 % and the VS reduction of almost 59 %.

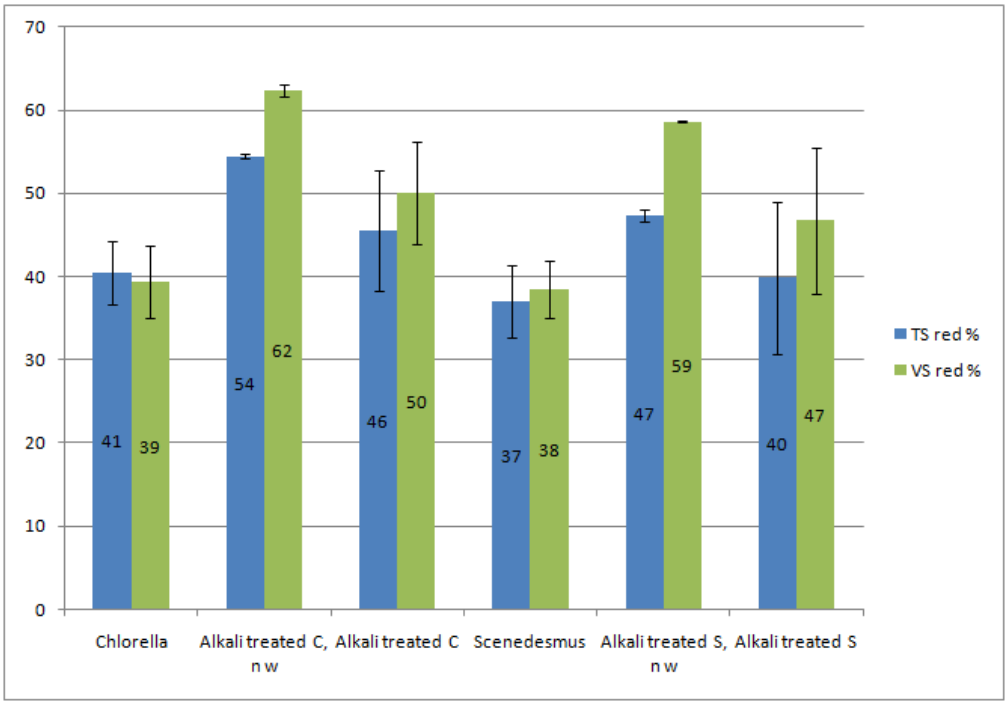

Figure 27 Average TS reduction (%) and VS reduction (%) during batch tests

Nonetheless, these results are indicative and should be treated with caution. For example theoretical calculations suggest that the TS reduction of *Chlorella* has been more than that of the VS reduction. However, results are in line in regard to the methane formation. The alkali treated samples in which lipids were not washed out produced more methane and the VS reduction was also the highest.

8 CONCLUSIONS

The methane yields with both tested algae species, *Chlorella* and *Scenedesmus*, are relatively high and they correspond well to those presented in the literature. They are almost equal to the methane yields of other cultivated crops and about half of the biowaste methane yields.

For the original algae, the TS and VS reductions in biogasification tests showed that about half of the organic matter degrades in the batch test, which means that comparatively high amount of organic matter is still available after batch test. To improve the reduction of organic matter of these species, some kind of a pre-treatment of the original algae could be used. One option is to use alkali and heat as the pre-treatment method to improve the methane yields significantly, which was experimentally found in this thesis.

The original *Chlorella*, the alkali treated *Chlorella*, and the alkali treated *Chlorella* after washing all produced more methane on the average compared to those of *Scenedesmus*. Both species studied in this thesis, have, according to the literature a strong and sturdy cell wall structure that does not degrade easily in the anaearobic digestion. The alkali treatment presumably has weakened the cell wall structure so that more organic material became bioavailable in the biogasification.

In larger or commercial scale the alkali heat treatment as a lipid extraction method is probably not the best practise available. About half of the total organic matter of algae was found to be extracted with lipids.

The ammonium conversion in the batch tests showed that a high proportion of the nitrogen converts to the ammonium during the biogasification, approximately 40-70 %. If the digestate is to be used as a fertiliser, mineralised form of nitrogen is preferable form as it is easier for plants to utilise.

If there will be enough ALDIGA's focus strains available it would be interesting in the near future test the methane potential with those strains. The research can be further continued with semi-continuous or continuous bioreactors in order to find out optimal parameters for the biogasification. If the biogas is to be used as a biofuel, the quality of the biogas could be measured to find out distribution of the methane and carbon dioxide, and whether the biogas should contain any impurities. In addition, the composition and the quality of the digestate is of interest.

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Appendix 1

Plans for batch tests

PLAN	18.1.2011	START	19.1.2011	PLAN	8.2.2011	START	16.2.2011	PLAN	8.4.2011	START	11.4.2011
ALDIGA Thesis								Inoculum from Envor Group Oy			
Inoculum from Envor Biotech				Inoculum from Envor Biotech				brought from Forssa 8.4.2011			
brough from Forssa 17.1.2011				brought from Forssa 15.2.2011				A: Scenedesmus			
								B: Alkali treated S. freeze-dried			
A: Scenedesmus				A: Scenedesmus				C: Alkali treated S n w. freeze-dried			
B: Scenedesmus (treated)				B: Alkali treated S. freeze-dried				D: Chlorella			
C: Chlorella				C: Chlorella				E: Alkali treated C. freeze-dried			
D: Chlorella (treated)				D: Alkali treated C. freeze-dried				F: Alkali treated C n w. freeze-dried			
Inoculum				Inoculum				Inoculum			
- inoculum starved overnight at 36 °C				- inoculum starved overnight at 36 °C				- inoculum starved over weekend at 36 °C			
METHANE POTENTIAL				METHANE POTENTIAL				METHANE POTENTIAL			

Bottle		Inoculum g	Sample g	Bottle		Inoculum g	Sample g	Bottle		Inoculum g	Sample g
Water bath 1				Water bath 1				Water bath 1			
1	Inoculum	230		1	Inoculum	250		1	Inoculum	250	
2		230		2		250		2		250	
3		230		3		250		3		250	
4		230		4	Scenedesmus	250	4.1	4	Scenedesmus (A)	250	4.1
5	Scenedesmus	230	4.3	5		250	4.1	5		250	4.1
6		230	4.3	6		250	4.1	6		250	4.1
7		230	4.3	7	Alkali treated S	250	4.0	7	Alkali treated S (B)	250	3.8
8		230	4.3	8		250	4.0	8		250	3.8
9	Scenedesmus	230	6	9		250	4.0	9		250	3.8
10		230	6	10		250	4.0	10		250	3.8
11		230	6	11		250	4.0	11		250	3.8
12	Alkali treated S	230	13.1	12	Chlorella	250	4.3	12	Alkali treated S. n w (C)	250	4.1
13		230	13.1	13		250	4.3	13		250	4.1
14		230	13.1	14		250	4.3	14		250	4.1
15		230	13.1	15	Alkali treated C	250	3.9	15		250	4.1
Water bath 2				Water bath 2				Water bath 2			
1	Alkali treated S	230	18.4	1	Alkali treated C	250	3.9	1	Alkali treated S. n w (C)	250	4.1
2		230	18.4	2		250	3.9	2	Chlorella (D)	250	4.3
3		230	18.4	3		250	3.9	3		250	4.3
4	Chlorella	230	4.3	4		250	3.9	4		250	4.3
5		230	4.3					5		250	4.3
6		230	4.3					6	Alkali treated C (E)	250	3.7
7		230	4.3					7		250	3.7
8	Chlorella	230	6					8		250	3.7
9		230	6					9		250	3.7
10	Alkali treated C	230	11.7					10		250	3.7
11		230	11.7					11	Alkali treated C. n w (F)	250	4.2
12		230	11.7					12		250	4.2
13		230	11.7					13		250	4.2
14	Alkali treated C	230	16.4					14		250	4.2
15		230	16.4					15		250	4.2

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Analysis results

TS yields of *Chlorella* (%) from alkali treatment
44.3
47.6
46.8
46.7
48.0
44.1

TS yields of *Scenedesmus* (%) from alkali treatment
42.4
49.1

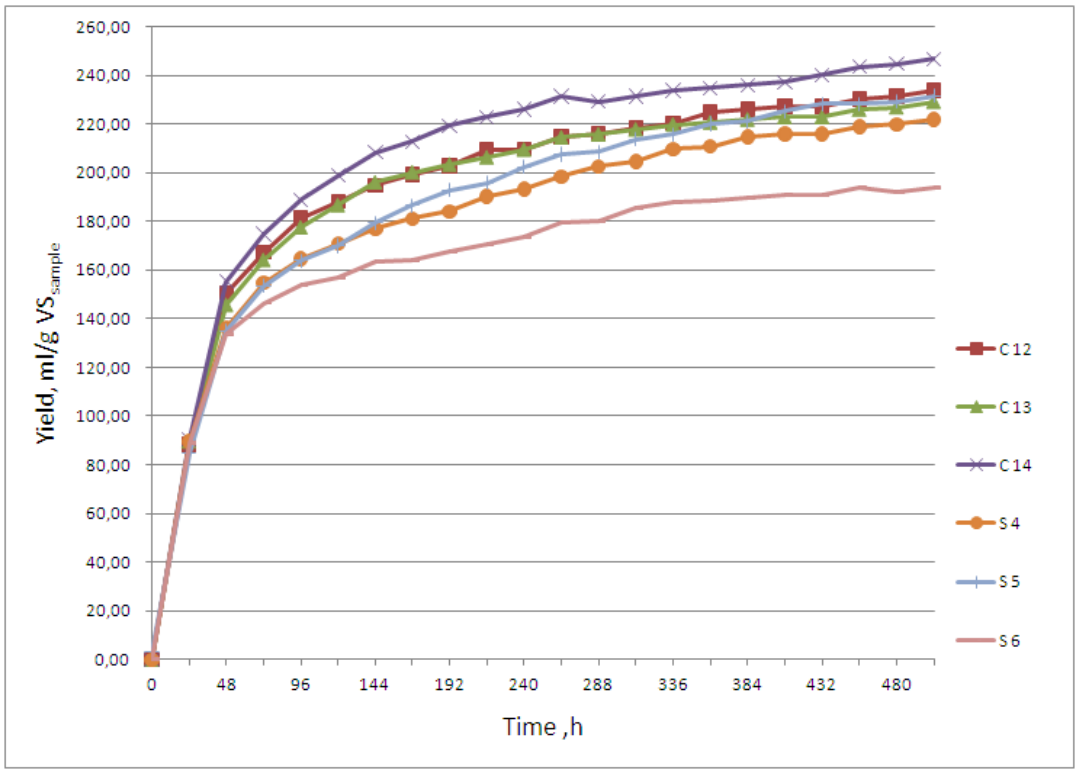
TKN	gN/kgTS	Compared to original (TS yield 54.22 %) gN/kgTS	Yield of N (%)	Loss of N (%)
Scenedesmus	67.6			
Alkali treated *Scenedesmus*	62.5	28.6	42.3	57.7
Scenedesmus	61.7			
Alkali treated *Scenedesmus*	54.4	24.9	40.4	59.6

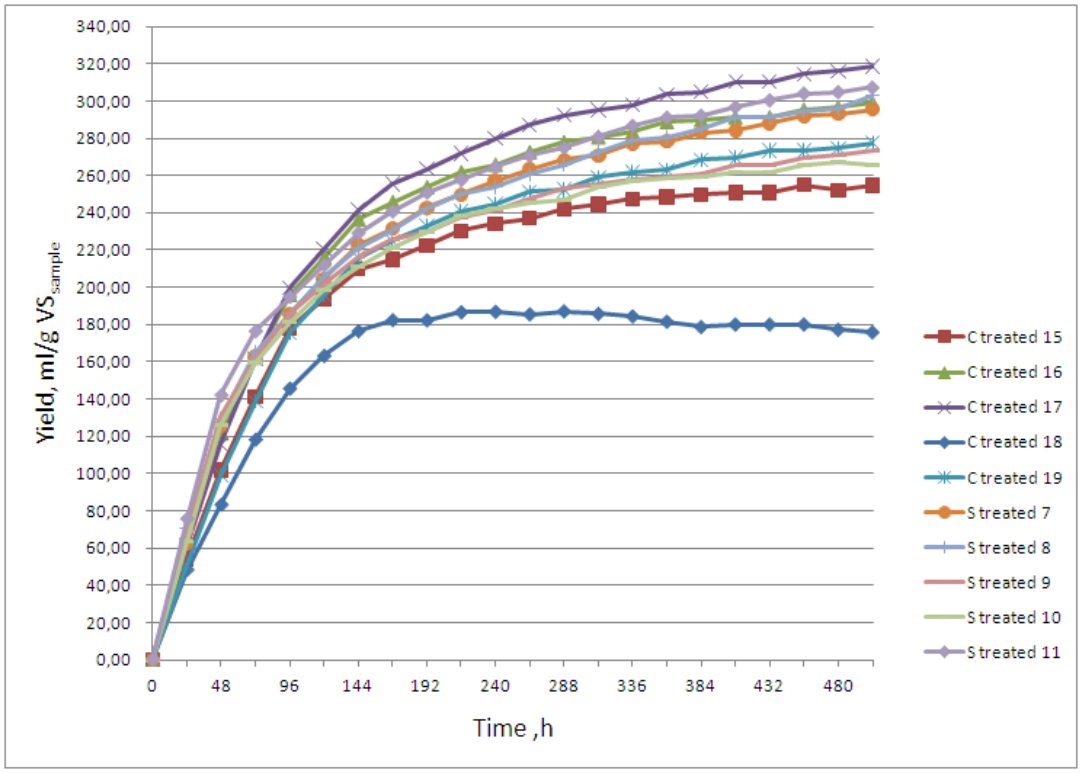
TKN	gN/kgTS	Compared to original (TS yield 54.22 %) gN/kgTS	Yield of N (%)	Loss of N (%)
Scenedesmus	61.7			
Scenedesmus supernatant (pooled)	83.1	45.1	73.1	26.9

TKN Analysis Sample bottle	mgNH_4-N/g	gN/bottle	In the end NH_4-N/TKN %
Scenedesmus 9	1,912	0,35932778	51,34034482
Scenedesmus 10	1,908	0,25751722	69,97542904
Alkali treated S 16	1,568	0,18275294	25,81148824
Alkali treated S 17	1,586	0,18275294	30,16220911
Chlorella 21	1,897	0,39739237	44,30642047
Chlorella 23	2,072	0,55443512	43,69822148
Alkali treated C 27	1,639	0,144291	51,02456476
Alkali treated C 29	1,716	0,20226054	52,20051368
Scenedesmus 5	1,8980892	0,24475235	42,79496505
Alkali treated S 10	1,8535032	0,17775197	48,97207792
Alkali treated S 11	1,8407643	0,17775197	47,77763699
Chlorella 14	2,0838641	0,39563443	44,39821732
Alkali treated C 15	2,007431	0,23980995	60,85262283
Alkali treated C 16	2,0084926	0,23980995	59,96727914
Scenedesmus 6	2,0132743	0,246479	54,96288743
Alkali treated S 10	2,0829646	0,20471373	80,6219711
Alkali treated S 11	2,1073009	0,20471373	83,21570744
Alkali treated S nw 15	2,1747788	0,2283492	87,58515543
Alkali treated S nw 16	2,1814159	0,2283492	89,03844894
Chlorella 20	2,1471239	0,39885733	48,42372962
Alkali treated C 21	2,2433628	0,30832708	74,76831912
Alkali treated C 25	2,2300885	0,30832708	72,68743499
Alkali treated C nw 26	2,3495575	0,38586809	70,00649911
Alkali treated C nw 30	2,3617257	0,38586809	70,80919443

Appendix 3

Results from batch test 2





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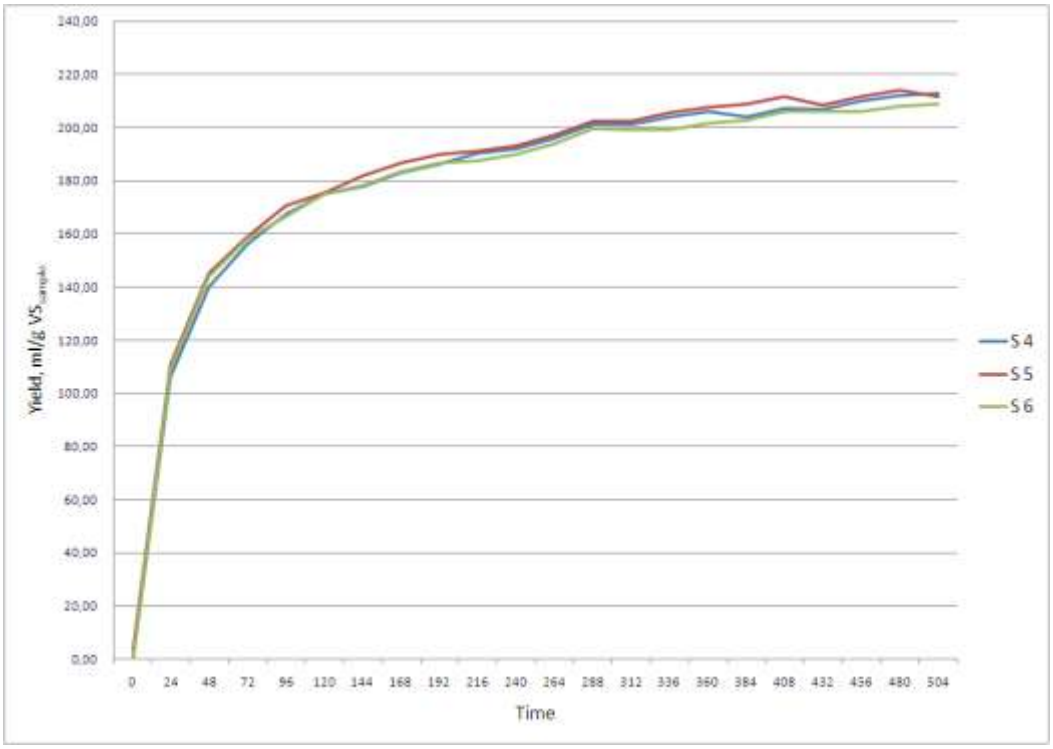
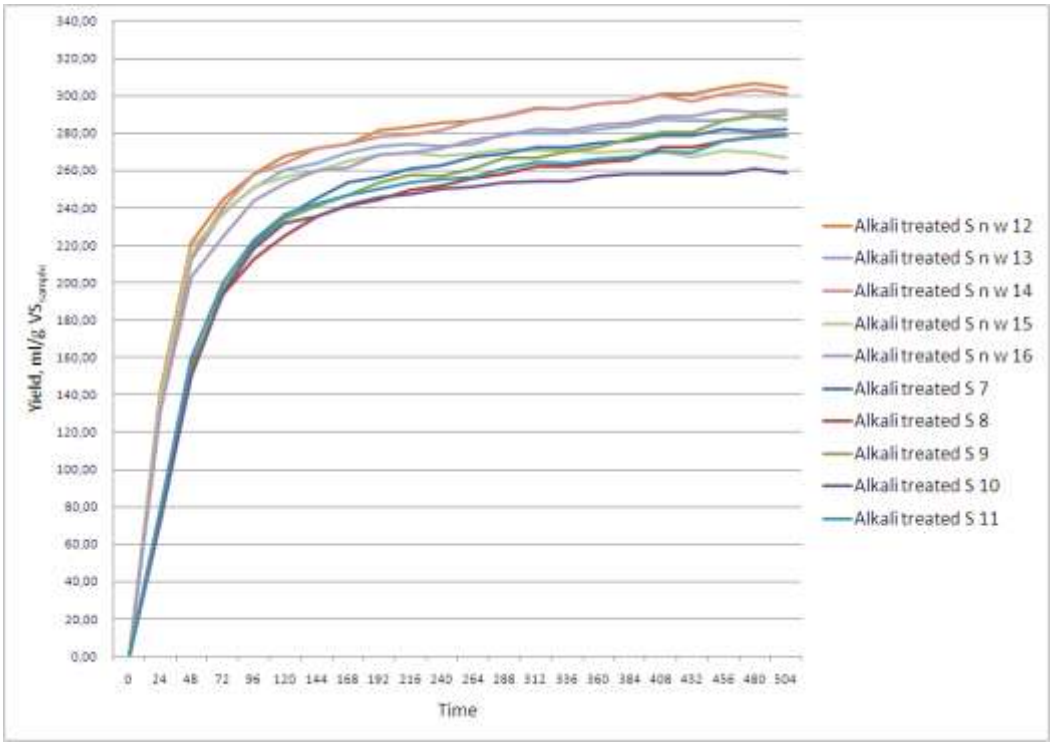
Results from batch test 3





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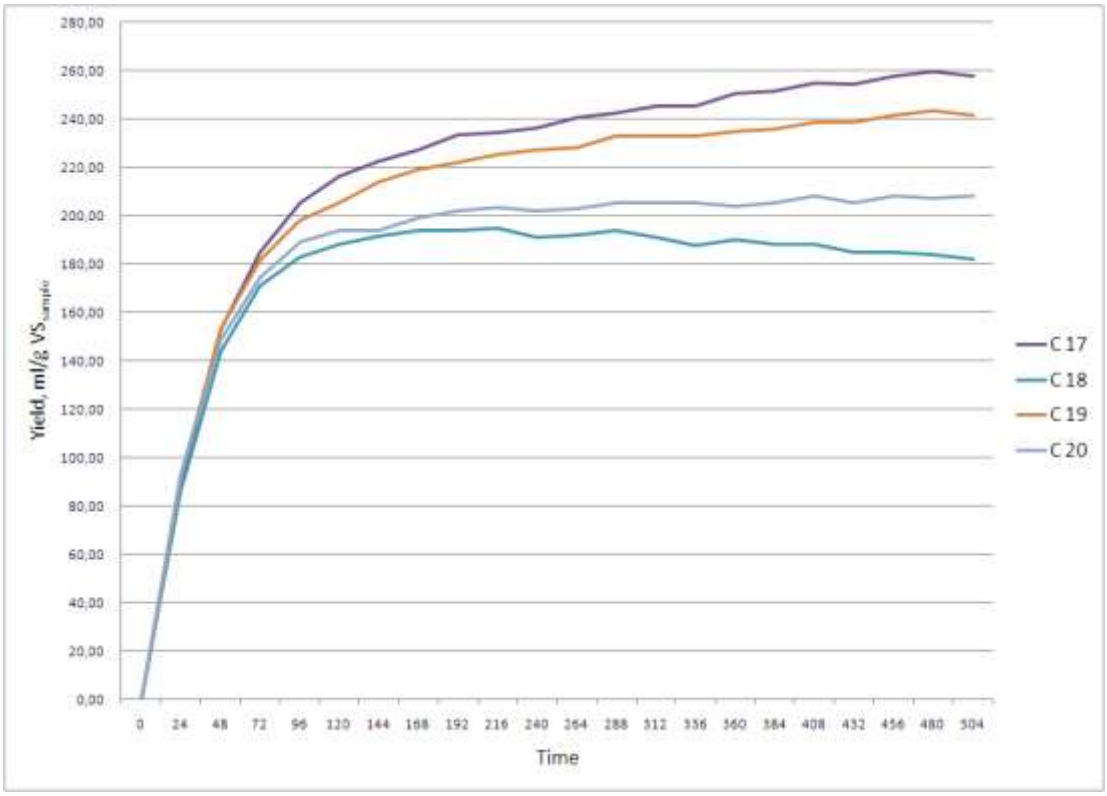
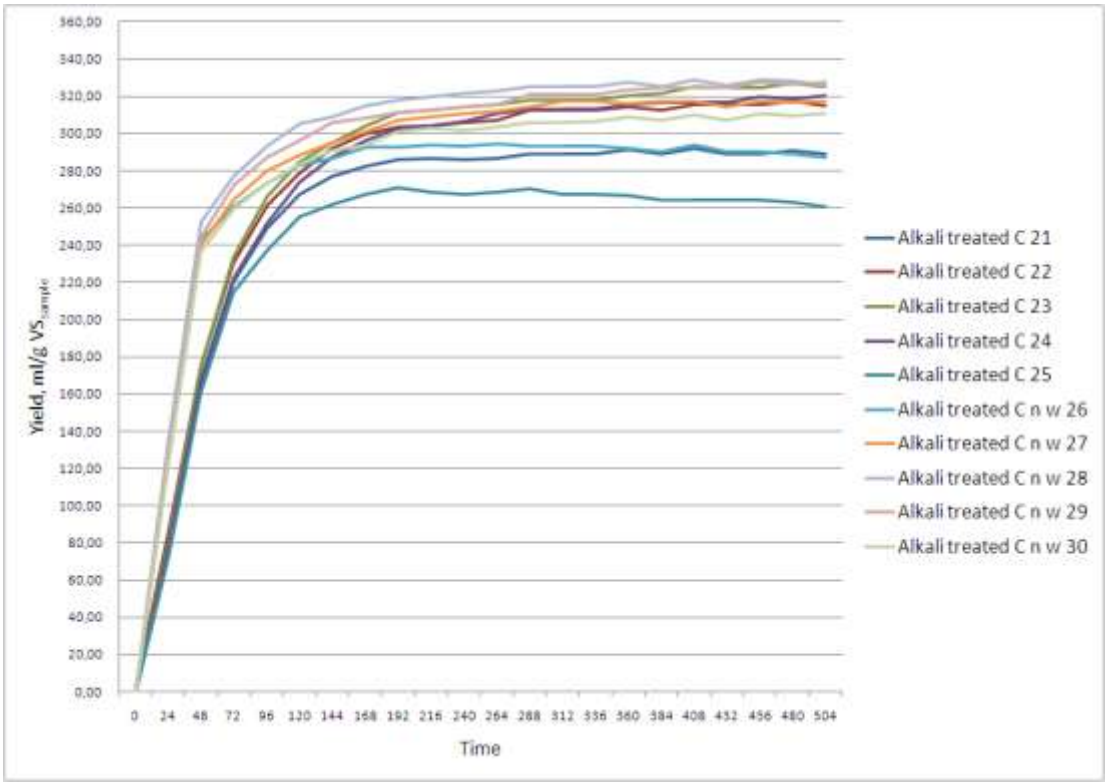

Yield, ml/g VS sample
Time
C 17
C 18
C 19
C 20


Yield, ml/g VS sample
Time
Alkali treated C 21
Alkali treated C 22
Alkali treated C 23
Alkali treated C 24
Alkali treated C 25
Alkali treated C n w 26
Alkali treated C n w 27
Alkali treated C n w 28
Alkali treated C n w 29
Alkali treated C n w 30

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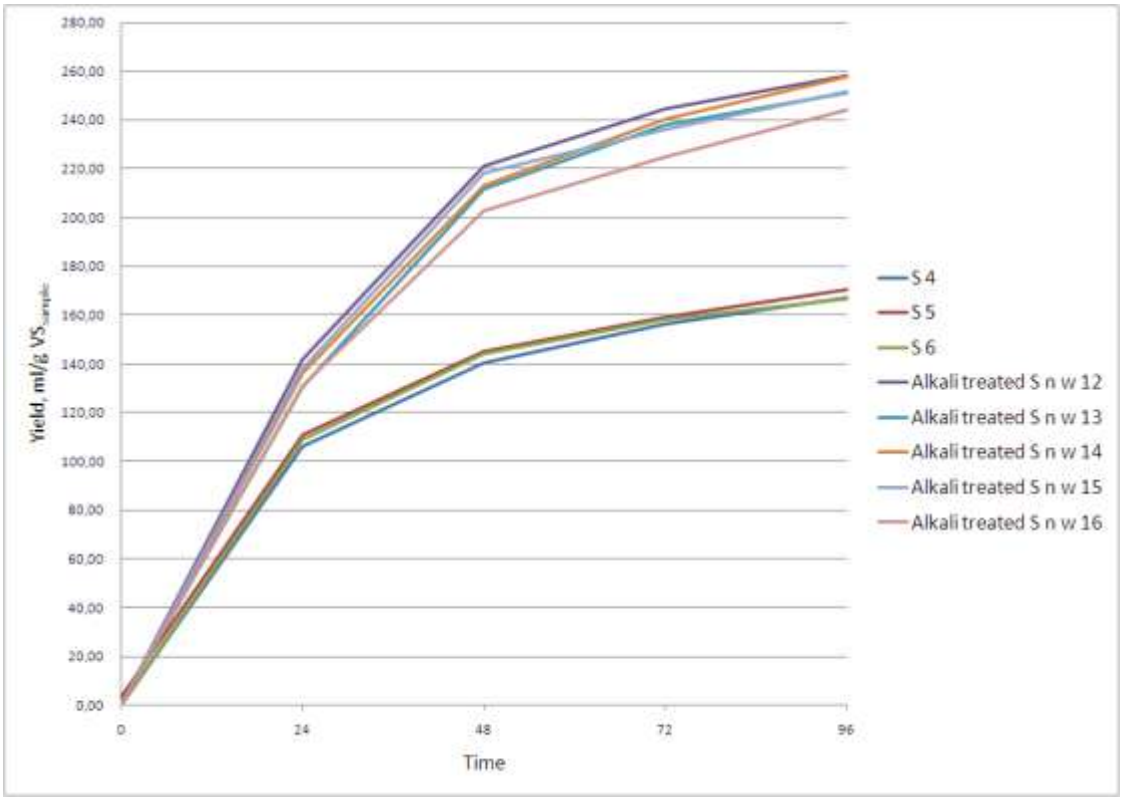
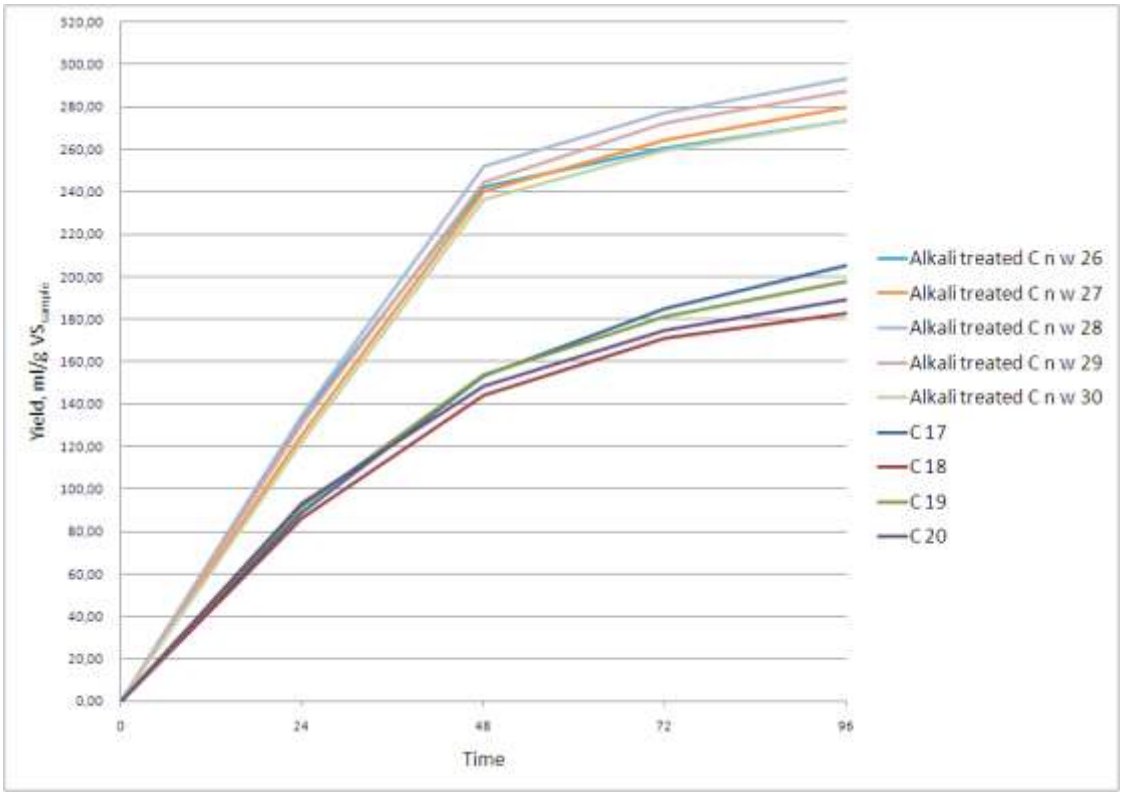

Yield, ml/g. VS
Time
S 4
S 5
S 6
Alkali treated S n w 12
Alkali treated S n w 13
Alkali treated S n w 14
Alkali treated S n w 15
Alkali treated S n w 16


Yield, ml/g. VS
Time
Alkali treated C n w 26
Alkali treated C n w 27
Alkali treated C n w 28
Alkali treated C n w 29
Alkali treated C n w 30
C 17
C 18
C 19
C 20