SGC Rapport 2013:278

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(Utvärdering av förbehandlingsmetoder för ökad biogasproduktion från makroalger)

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"Catalyzing energygas development
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Denna studie har finansierats av:
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Kemiteknik/LTH
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SGC Rapport 2013:278

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Malmö 2013

Martin Ragnar
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Malmö, Sweden 2013

Martin Ragnar
Chief Executive Officer

Preface

The project was performed by Water and Environmental Engineering, Department of Chemical Engineering at Lund University (Sweden), the department of sustainable development at Trelleborg municipality (Sweden) and TK Energi A/S (Denmark).

Algae collection and species identification was performed by Water and Environmental Engineering at Lund University together with the department of sustainable development at Trelleborg municipality. Mechanical pretreatment was performed by Water and Environmental Engineering at Lund University together with TK Energi A/S. All analyses and experiments were performed by guest researcher Huili Li at Water and Environmental Engineering at Lund University.

The project was run between September 2012 and January 2013 and had a reference group consisting of the following persons.

Tobias Persson, SGC
Henriette Draborg, Novozymes
Ellinor Tjernström, Avdelningen för Hållbar utveckling, Trelleborgs kommun
Matilda Gradin, Avdelningen för Hållbar utveckling, Trelleborgs kommun
Jes la Cour Jansen (Lund University)
Thomas Koch, TK Energi A/S (Denmark)
Irene Bohn, NSR

Summary

The eutrophication of the Baltic Sea causes increased production of fast growing algae species which has direct and indirect negative impacts on the marine ecosystem causing depletion of habitats and anoxic sea bottoms. Furthermore the beach cast of algae and sea grass has increased which has negative effect on tourism industry and recreational values along the coast line. It has been suggested that anaerobic digestion of beach cast algae would be a way of mitigating the negative impacts of algae decomposing along the coast and producing a high value fuel, methane, simultaneously. However, the physical structure of algae and sea grass makes conventional anaerobic digestion difficult and pretreatment is needed to aid mechanical handling and microbiological digestion.

This study investigated the effect of mechanical and enzymatic pretreatment on three different kinds of algae (*Fucus vesiculosus, Furcellaria lumbricalis* and filamentous red algae) and one sea grass (*Zostera marina*). The algae with the most promising results from the pretreatment were selected for batch methane potential tests where both pretreated and raw algae were tested to see the effect on biogas production. In addition an energetic analysis was made to compare the energy used for pretreatment to the methane produced.

The results showed that the concentration of soluble COD could be greatly increased by mechanical or enzymatic treatment compared to that of raw algae. However during enzymatic treatment the algae was heated to 50 °C during 6.5h which for several algae had an almost equal effect on increasing the COD concentration even without the addition of enzyme. The batch methane potential tests showed that mechanical pretreatment increased the rate of production of methane for the two types of algae investigated. Enzymatic pretreatment increased the methane production for most samples, however to a much smaller extent than mechanical pretreatment. For algae with more rigid surface structure (*Fucus vesiculosus*) the final methane potential was also increased by 100% however for filamentous red algae the final methane potential was the same as for raw algae. The energetic analysis showed that the energy use for pretreatment (for mechanical pressing or for heating of sample during enzymatic pretreatment) lowered the net energy gain so much that the best option, out of an energy perspective, would be to digest raw algae.

Sammanfattning på svenska

Övergödningen av Östersjön orsakar ökad produktion av snabbväxande tång vilket ger direkta och indirekta negativa effekter på det marina ekosystemet. Två exempel är att habitat med bottenväxande tång trängs undan och att friflytande snabbväxande tång ansamlas i stora sjok som sjunker till botten där syrekonsumtionen under nedbrytning skapar syrefria bottnar med påföljande negativa effekter för djurliv. Vidare har tång när det spolas upp på stränderna kring Östersjön negativ inverkan på turistindustri och rekreationsvärden då tången ruttnar och avger illaluktande vätesulfid. Rötning av uppspolad eller strandnära tång skulle kunna vara ett sätt att minska de negativa effekterna och samtidigt producera energirik biogas. Dock finns det praktiska problem med att göra detta då tångens struktur gör den svårhanterad med konventionell rötkammarteknik som baseras på hantering av en lättpumpad vätskefas. Många tångarter har seg ytstruktur som är svår att bearbeta mekaniskt till en sådan västkefas och därför skulle förbehandling av tången kunna underlätta både hantering vid transport och pumpning men även eventuellt öka hastigheten på mikrobiell nedbrytning vid rötning för biogasproduktion.

Inom denna studie undersöktes effekten av mekanisk och enzymatisk förbehandling på tre olika arter av tång; *Fucus vesiculosus* (blåstång)*, Furcellaria lumbricalis* (kräkel) och fintrådiga rödalger samt på sjögräset *Zostera marina* (ålgräs). Vidare analyserades arterna för torrsubstanshalt (TS), glödförlust (VS) samt innehåll av kväve och kol varav det senare användes för att beräkna COD-innehåll. Alla prover hämtades in i strandnära vatten vid Trelleborg under oktober 2012 och artbestämdes med expertis från Trelleborgs stad. Proverna sköljdes sedan i vatten för att rensa ut sand och övrigt material och förvarades i kyl vid 5 °C inför analys. Som mekanisk förbehandling utvärderades effekten av att hantera en blanding av tång och vatten i en trycksatt kammare varefter den pressades genom en spaltöppning med en hastighet av omkring 300 m/s genom kombination av tryckskillnad och press med pistong. Detta gav en tillsynes lätthanterlig tjock vätska för samtliga undersökta arter. Enzymatisk förbehandling utfördes genom att blanda tångprover med vatten och tillsätta enskilda enzym eller kombinationer av cellulas, hemicellulas, pectinas och proteas. Temperatur och pH justerades för att hamna inom ett optimalt intervall för de undersökta enzymerna. För samtliga prover hölls en konstant temperatur vid 50 °C under försöken medan pH initiellt justerades till 5.0 för cellulas, hemicellulas, pectinas samt blandning av de tre. pH vid behandling med protease justerades initiellt till 8.0. Samtliga försök utom de initiella experimenten utfördes genom att kombinera behandling med en blanding av cellulas, hemicellulas, pectinas under 5 h varefter blandingarna behandlades med proteas under 1.5 h. Att bedriva försöken vid så hög temperatur som 50 °C motiverades av syftet att undersöka den maximala potentialen av enzymatisk förbehandling. Vid enzymatisk förbehandling testades både obehandlad tång samt den mekanisk förbehandlad tången. Effekten av respektive förbehandlingsmetod utvärderades genom att mäta halten löst COD (sCOD) i de behandlade proverna efter filtrering genom filterpapper med 2-3 µm pordiameter. För mekanisk förbehandlade prover mättes denna enbart vid bladning med vatten i start av de enzymatiska försöken medan förändringen av sCOD mättes vid flera tillfällen under den 6.5 h långa förbehandlingen med enzym. Vidare mättes förändringen av sCOD även på tångprover till vilka en-

zym inte tillsatts men som under samma behandlingstid hettades upp till 50 °C. Baserat på halterna av sCOD och arternas förekomst vid uppsamlingsplatsen valdes de tångarter som visade de mest lovande resultaten ut och metanpotentialen för både förbehandlad tång och obehandlad tång fastställdes för dessa arter. Vid metanpotentialsförsöken användes ymp från fullskalerötkammare vid Sjölunda avloppreningsverk i Malmö och försöken bedrevs vid 35 °C. Vidare utfördes en grov beräkning av energiåtgång för förbehandling kontra energiinnehållet i producerad metan från rötning av tång för respektive kombination av förbehandling respektive obehandlad tång. Energiberäkningen inkluderade den kinetiska energin som krävdes för att förflytta den blandning av tång och vatten som behandlades genom den mekaniska förbehandlingen samt energi för drift av den prototypmaskin som användes vid försöken med en antagen verkningsgrad på 50%. Vidare beräknades energiåtgången för att hetta upp blandningarna av tång och vatten till 50 °C under den enzymatiska förbehandlingen. Som ett alternativt scenario beräknades även nettoenergivinsten ut för mekanisk förbehandlingen med mindre andel vatten samt då värmen för uppvärmning av enzymatisk behandling kunde tillgodoses utan kostnad.

Resultaten visade att koncentrationen av sCOD kan ökas kraftigt genom mekanisk eller enzymatisk förbehandling jämfört med den från obehandlad tång i vatten. Bäst resultat erhölls för de tångarter med tillsynes mest svårbehandlad ytstruktur (*Fucus vesiculosus* och *Furcellaria lumbricalis*) men goda resultat erhölls även för den vanligt förekommande fintrådiga rödalgen. För sjögräset ålgräs gav vare sig mekanisk eller enzymatisk förbehandling någon fastställbar effekt på sCOD jämfört med obehandlade prover. Det bör dock hållas i åtanke att proverna vid enzymatisk förbehandling hettades upp till 50 °C under 6.5 h vilket på blankproverna utan enzym visades ge en en nästan lika stor effekt på ökning av COD-koncentrationen som i proverna med enzym. Baserat på resultaten och det faktum att röd filamentbildande tång var en vid provområdet mycket vanligt förekommande problematisk snabbväxande tång valdes *Fucus vesiculosus* och rödfilamentbildande tång ut för utrötningsförsök för fastställande av metanpotential. Resultaten från de satsvisa utrötningsförsöken visade att mekanisk förbehandling ökade metanbildningshastigheten för båda de undersökta tångarterna (*Fucus vesiculosus* och fintrådiga rödalger). Enzymatisk förbehandling ökade metanproduktionen för de flesta proverna, men i mycket mindre utsträckning än mekanisk förbehandling. För tång med styvare ytstruktur (*Fucus vesiculosus*) kunde den slutliga metanpotentialen ökas med 100% genom förbehandling gentemot metanpotentialen för icke-förbehandlad tång (131 NL CH_4/kg VS respektive 67 NL CH_4/kg VS) men för fintrådiga rödalger var den slutliga metanpotentialen densamma i förbehandlade och icke-förbehandlade prover (omkring 220 NL CH_4/kg VS). De erhållna metanpotentialerna för icke-förbehandlade tångprover låg omkring tidigare rapporterade värden från liknande försök och var låga i förhållande till substrat som avloppsslam och matavfall. Vidare var metanpotentialerna uttryckt i enheterna NL CH_4/kg COD låga i förhållande till det teoretiska maximumet på 350 NL CH_4/kg COD. Resultaten för energianalysen visade att energianvändningen för förbehandling (mekanisk pressning eller för uppvärmning av prov under enzymatisk behandling) sänkte nettoenergivinsten så mycket att det bästa alternativet, ur ett energiperspektiv, var att röta tång utan förbehandling. För det alternativa scenario där en mer fördelaktig blandning av vatten och tång förbehandlats mekaniskt samt värmen för enzymatisk förbehandling antogs tillgodoses utan kostnad gav endast kobinationen av mekanisk och enzymatisk förbehandling på arten *Fucus vesiculosus* högre nettoenergivinst än rötning av obehandlad rå tång. Detta visar att den ekonomiska nyttan med förbehandling av *Fucus vesiculosus* eller fintrådiga rödalger är väldigt låg, särskilt då energiberäkningen inte inkluderade uppsamling eller transport av dessa tångarter. Den positiva effekten av arbeta med ett substrat som är enkelt att transportera och pumpa vilket erhålls genom mekanisk förbehandling är dock inte inkluderad i den ekonomiska analysen.

Content

1. Background

Eutrophication of the world oceans and fresh water systems is one of the major environmental problems on our planet today. The over nourishing of the seas have both primary and secondary negative effects on the ecosystem as well as on the physical and chemical conditions on the seabed (Leppäranta & Myrberg, 2009).

The primary symptoms of eutrophication are extensively increased production of phytoplankton and annual fast growing filamentous algae species (Bosdorff et. al., 1997). Over production of algae, whether it is micro or macro, increases the turbidity in the water which shadows the perennial macro algae growing at the bottom. As an indirect effect of the decrease in sunlight reaching the bottom the perennial macro algae, e. g. bladder wrack, withdraws from their original depth range and thereby the habitats, where bladder wrack is a key species, decreases in area. (Bosdorff et al.,1997).

The overproduction of algae also affects the deeper parts of the sea as the dead algae sinks to the sea bed and decomposes. The process of decomposition consumes oxygen and when large amounts of algae sink to the bottom this causes anoxic bottom sediment and water. This disrupts the bottom fauna ecosystem which effects the whole food web above (Conley et al., 2009). In the Baltic Sea the anoxia of the sea floor is an especially difficult environmental problem. The physiology of the brackish sea makes the sea bed more sensitive to high loads of organic material (Conley et al., 2009).

The combination of an enclosed, brackish sea and overproduction of algae due to eutrophication has led to the situation in the Baltic Sea today with the largest area of anoxic bottoms since measurements began in the 1960´s (Havet, 2011).

The symptoms of eutrophication in marine coastal areas are not only negative for the marine ecosystem but also for residents living close to the sea, recreational values and tourism industry. In Trelleborg municipality, Sweden, algae often appear in great masses along the beaches during peak tourism season, June to August. When the algae decompose at the beach, hydrogen sulfide is produced which smells terrible and makes the shallow waters by the beach unpleasant to swim in. The algae masses that comes to shore mostly consists of the red and brown filamentous algae *Polysiphonia fucoides*, *Ectocarpus siliculosus*, and *Pylaiella littoralis*, but also some of perennial algae species like *Fucus vesiculosus* and *Furcellaria lumbricalis* and the sea grass *Zostera marina* (Tjernström, 2012). The coast along Trelleborg municipality is very shallow and consists mainly of a combination of sandy and rocky bottoms. At about five meters depth and below, the rocky substrate covers almost 100% of the bottom (Tjernström, 2010). *F. vesiculosus* and *Fucus serratus* should be the dominating algae species at hard substrates at five meters depth, although in Trelleborg *F. lumbricalis* dominates instead. The filamentous algae species grow on top of the perennial species which probably have caused the brown algae *F. vesiculosus* and *F. serratus* to withdraw from their normal depth range. *F. lumbricalis* contains the pigment phycobilin that makes it possible for red algae to photosynthesize in deeper waters than green and brown algae and also in waters with less light available e.g. because of eutrophication (Haxo & Blinks, 1950).

Measurements and modulations approximate the beach cast of algae to about 70 000 m^3 per year along the coastline of Trelleborg municipality. The composition of the algae masses varies over the season but also with location. Generally the filamentous algae species is about 50-90% (Gröndal, 2010). Within the filamentous algae species composition, *P. fucoides* is the single one dominating species, although during May and June the beach cast portion of filamentous algae can consist of up to about 50% of the brown filamentous species *E. siliculosus* and *P. littoralis* (Tjernström, 2012).

Today, the beach cast algae is transported away during tourist season and returned to the sea in fall (Tjernström, 2012). Biogas production from macro algae collected in this way could potentially give several beneficial effects including production of climate neutral energy as well as mitigating formation of anoxic sea beds. Anaerobic digestion of as little as 10 000 m^3 of the 70 000 m^3 of the annual beach cast algae in Trelleborg has been calculated to potentially generate 14,5 GWh (Davidsson & Turesson-Ulfsdotter, 2008; Tjernström, 2012). However it is currently difficult to access this methane potential since macro algae is not suitable for digestion by conventional methods (Haghighatafshar, 2012). Pretreatment of the algae is thus needed to aid both mechanical transport (pumping) as well as microbiological anaerobic digestion. One option is mechanical pretreatment of the algae, however a method which can handle the long fibrous material in some algae species is needed. Another method, which is relatively untested but promising, is enzymatic pretreatment which during recent years has been tested on many substrates to investigate effect on biogas potential (Davidsson et al, 2007; Ziemiński et al, 2012; Jensen, 2012). Experiments with enzymatic treatment using cellulase have previously been shown to increase the hydrolysis and the biogas potential from macro algae (Haghighatafshar, 2012). However it was also shown that cellulase was not suitable to hydrolyze all types of macro algae which most likely is an effect of the different surface composition of the algae species.

An investigation of the effect from pretreatment on hydrolyzation and biogas production from marine macro algae is thus necessary to establish if these methods can increase the production of biogas from this substrate.

1.1 Aim:

The aim of the project was to evaluate if the commonly found types of marine macro algae and sea grass could be pretreated mechanically enzymatically to increase the methane production from subsequent anaerobic digestion of the algae and sea grass. The combination of both pretreatment methods was also to be evaluated. Furthermore an economical estimation of energy use for pretreating algae versus the increase in methane production was to be made.

2. Experimental

2.1 Collection of algae

A mixture of beach cast algae was collected on the coast line of Trelleborg, Sweden, in October 2012. This was subsequently sorted by species and rinsed with water to remove sand. In the sorting the following species were found in significant amounts in the algae mix; *Furcellaria lumbricalis*, *Fucus vesiculosus*, *Zostera marina* and Filamentous red algae according to Figure 1. The sorted algae were stored in a fridge at 5 °C until use. For the initial testing of enzymes *Zostera marina* (common eelgrass) was also collected from the coast line of Malmö, Sweden. It is notable that *Zostera marina* is in fact a sea grass and not an algae species.

Figure 1. Photos of algae normally found along the Trelleborg Baltic sea coast line. A: Furcellaria lumbricalis (red seaweed), B: Fucus vesiculosus (Bladderwrack), C: Filamentous red algae, E: Zostera marina (common eelgrass). Photo by Salar HaghighatAfshar at Water and Environmental Engineering, Lund University.

2.2 Mechanical pretreatment

Mechanical pretreatment was performed in a prototype machine owned by TK Energi A/S which can be seen in Figure 2 below. The machine consists of a pressure chamber and a feeding chamber with a rectangular clearance measuring 1x10 mm in the end. After the clearance an external outlet pipe is placed in order to prevent scattering of the treated sample. The sample is placed in the feed chamber together with tap water in a ratio of 2:1 water versus algae in order to recover maximum amount of substrate after treatment. When the machine is started the pressure is increased to 1 000 bar in the pressure chamber which is in turn opened to the feed chamber and the substrate is forced through the clearance at a velocity of 300 m/s leaving it shredded to a homogenous mush. Photos of a sample of *Zostera marina* eel grass before and after treatment can be seen in Figures 3 and 4 below. No particle size distribution analysis was performed on the treated algae due to economic limitations.

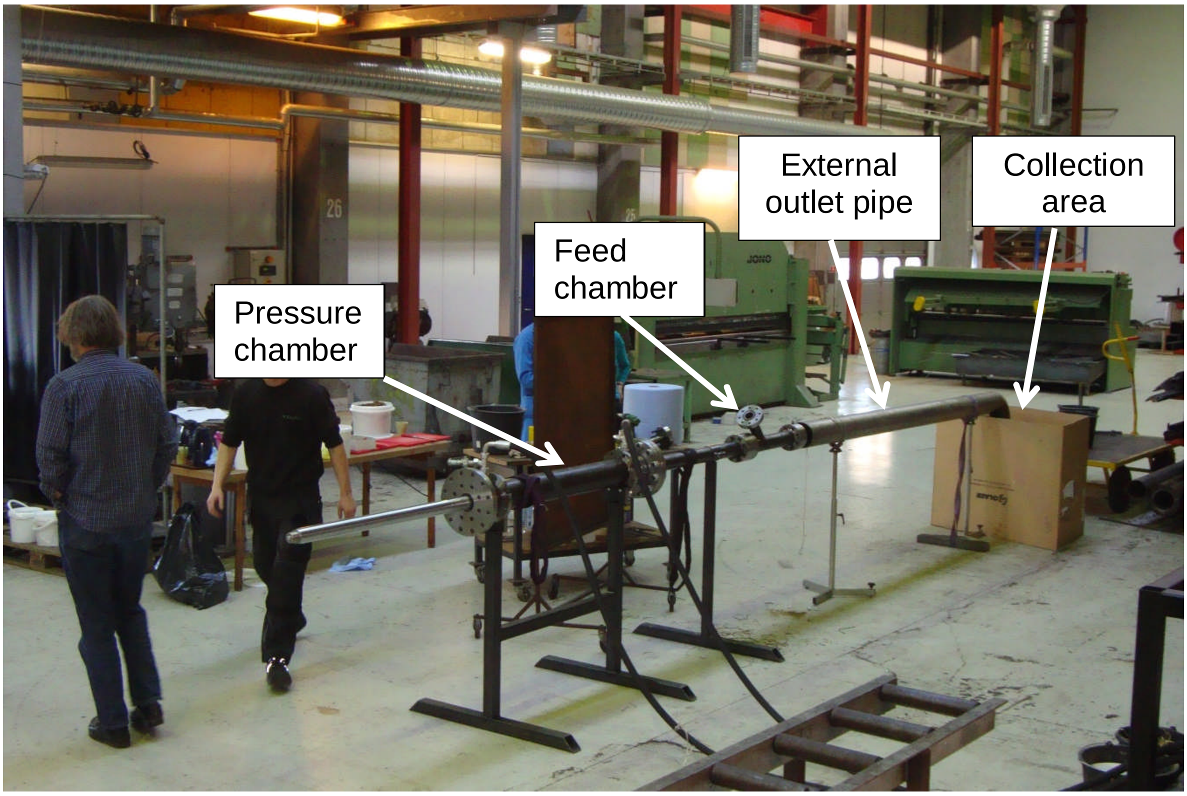

Figure 2. The prototype machine used to mechanically pretreat algae.

Figure 3 and 4. Sample of the Zostera marina (common eelgrass) grass being fed into the feed chamber and after mechanical pretreatment respectively.

2.3 Enzymatic pretreatment

The method of enzymatic pretreatment was suggested by Novozymes A/S who also supplied the enzymes used. A description of the enzymes is given in Table 1.

Table 1. Characteristics of the enzymes used in the project.

Enzyme product name	Type	pH range	Temperature range (°C)
NS81235	Cellulase complex	4.5-6	35-65
NS81236	Hemicellulase	5-6.5	35-65
NS81223	Pectinase	4.5-5.6	25-65
NS81303	Protease	7-9	40-70

Algae was cut into pieces of 1 cm in order to aid handling. At the beginning of the experiment (t=0h) the algae was mixed with tap water to reach a dry matter content (DM) of 3% in a reactor with 500 mL active volume. A photo of the reactors can be seen in Figure 5. Immediately after mixing the pH was adjusted to 5.0 using a 5.0 M HCl solution and a mixture of 1/3 each of cellulase complex, hemicellulase and pectinase in total was added corresponding to 1% of the DM-content of the algae. The sample was incubated under gentle manual agitation in a water bath at 50 °C for 5 hours. After this (t=5h) the pH was changed to 8.0 using a 19.1 M NaOH-solution and protease corresponding to 1% of DM-content of the algae was added. The sample was then incubated under gentle agitation in a water bath at 50 °C for 1.5 hours until time t=6.5. During the experiment samples for soluble COD were taken at t=0, 2, 4, 5, 5.5, 6 and 6.5h. Blank samples containing only algae and water were prepared as well, in addition to the samples with added enzyme. These were also treated in the water bath with gentle agitation but were not pH adjusted. Liquid samples for COD-analysis were prepared by centrifugation at 10 000 rpm for 5 minutes followed by filtration through filter paper with 2-3 µm pore diameter and filter velocity of 10 mL/100 s. COD analysis was performed spectrophotometrically using Hach Lange Gmbh LCK 114 cuvettes. A photo showing the corresponding color change in a sample at the different intervals for not mechanically pretreated filamentous red algae dosed with enzyme can be seen in Figure 6, the color change is caused by increase of soluble COD at the addition of protease.

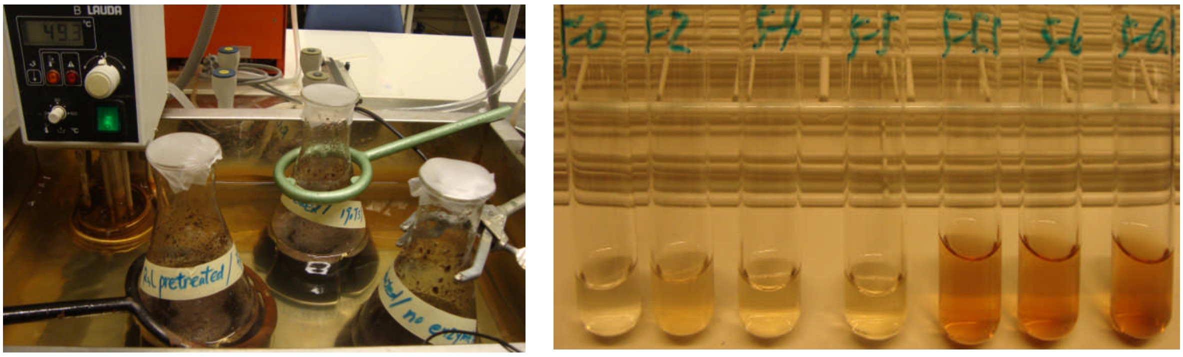

Figure 5 and 6. Water bath with reactors and centrifuged and filtered samples of liquid from enzymatically pretreated algae respectively. From left to right samples from t=0, 2, 4, 5, 5.5, 6 and 6.5 h.

In an initial trial the effect of individual enzymes as well as from a mixture was tested on *Zostera marina* (common eelgrass). In addition the effect from temperature was evaluated by adding a sample at room temperature (20 °C). Algae for this experiment were collected from a beach located in Malmö, Sweden. All samples containing enzyme were dosed with enzymes corresponding to 1% of the DM-content of the algae added and pH-regulated to 8.0 (for sample with protease) or 5.0 (all others) at the beginning of the experiment using a 19.1 M NaOH-solution or 5.0 M HCl solution respectively. The sample containing enzyme mixture used 1/3 each of cellulase, hemicellulase and pectinase.

2.4 Biomethane potential test

Theoretical methane production can be calculated from COD concentration but since the amount of biodegradable COD was not known a biomethane potential test had to be made to experimentally decide the methane potential. Due to economical and practical limitations only samples from treatment of two species could be selected for the test. These were *Fucus vesiculosus* and red filamentous algae based on the results from the enzymatic treatment

For each type of algae samples from mechanical pretreatment and enzymatic pretreatment (1% Enzyme) was selected for biomethane potential tests as well as samples for mechanical pretreatment that had subsequently been treated enzymatically. Samples treated with 3% Enzyme dose were excluded since the results for the enzymatic pretreatment showed that dosing with 3% enzyme only marginally increased the soluble COD for the chosen types of algae. Also samples of algae that had not been pretreated were included.

Biomethane potential was measured using batch digesters and the method described by Hansen et al. (2004). In short substrate is placed together with inoculum from an anaerobic digester in 2 L glass bottles with rubber stoppers in the lids. The DM content of the substrate was 3% for not mechanically pretreated samples and for mechanically pretreated samples of *Fucus vesiculosus* and 2 % for mechanically pretreated samples of filamentous red algae. The DM content of the inoculum was 2 % making the final DM concentration in the bottles low (less than 3% DM). A photo of the bottles is shown in Figure 7. The bottles were kept in heating cabinets at 37 °C and methane production was measured using a gas chromatograph. For these experiments inoculum from a full scale digester at Sjölunda municipal wastewater treatment plant was used and methane production was measured in an Agilent 6850A gas chromatograph equipped with flame ionization detector (FID) and a 25m (length), 0.32 µm (diameter and 0.5 µm (width) column.

Figure 7. Glass bottles used for BMP test.

2.5 Further analysis

Dry matter content (DM) was assumed to be equivalent to content of total solids (TS) which was determined together with volatile solids (VS) were determined using standard methods (SS-EN 12879, SS-EN12880). pH was measured using portable apparatus WTW pH 320. Analysis for content of carbon and nitrogen was performed in an elemental analyzer MAX CN from Elementar Analysensysteme GmbH.

3. Results and discussion

3.1 Characterization of algae

Collected algae was analyzed and the results can be seen in Table 2. COD was calculated from oxygen consumption according to eq.1 by using carbon content and assuming standard biomass composition ($CH_{1,8}O_{0,5}N_{0,2}$) from Villadsen et al. 2011.

$$C_nH_aO_bN_{0,2} + \left(n + \frac{a}{4} - \frac{b}{2} - \frac{3c}{4}\right) O_2 \rightarrow nCO_2 + \left(\frac{a}{2} - \frac{3c}{2}\right) H_2O + cNH_3 \qquad \text{eq.1}$$

Table 2. Analyzed characteristics of different species. COD calculated from C-content. Values for TS and VS are averages (n=3).

Species	**TS (%)**	**Stdev (%)**	**VS (%)**	**Stdev (%)**	**VS/ TS**	**C (mg/ g TS)**	**N (mg/ g TS)**	**COD (mg/g ww)**
Furcellaria lumbricalis (red seaweed)	15.07	0.34	13.59	0.77	90%	373	26.8	261
Fucus vesiculosus (Bladderwrack)	16.51	1.06	14.24	0.58	86%	382	11.4	195
Zostera marina (common eel-grass)	16.07	0.18	11.84	0.37	74%	363	12.2	154
Filamentous red algae	14.29	0.49	9.73	1.04	68%	400	29.1	95

3.2 Mechanical pretreatment

The mechanical pretreatment effectively broke up the structure of all four algae samples into a homogenous slurry. Photos of a sample of filamentous red algae before and after treatment can be seen in Figure 8.

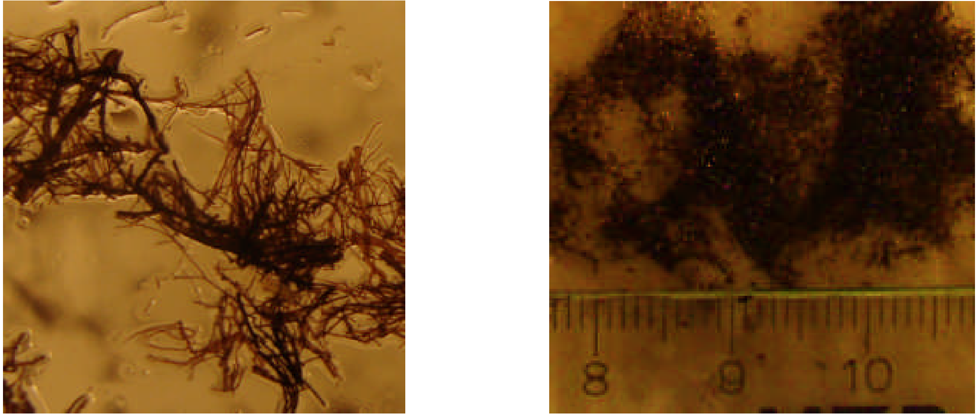

Figure 8. Photo of untreated raw filamentous red algae (left) and mechanically pre-treated raw filamentous algae (right).

3.2 Enzymatic pretreatment

The results from the initial trial are presented below in Figure 9. It is clear from the Figure that the enzyme mixture has roughly the same hydrolyzing effect as the individual enzymes and furthermore that the sample with no enzyme (but a temperature of 50°C) is almost as hydrolyzed as the samples treated with enzyme. Upon these results it was decided to include a stronger 3% enzyme dose in further enzyme experiments. However dosing with protease was done at 1% of DM content even in the 3% dose samples. Furthermore it is clear from Figure 7 that the hydrolyzing effect of treatment at 20 °C was much lower than for samples treated at 50 °C and samples of raw algae in water at room temperature was thus disregarded in future experiments.

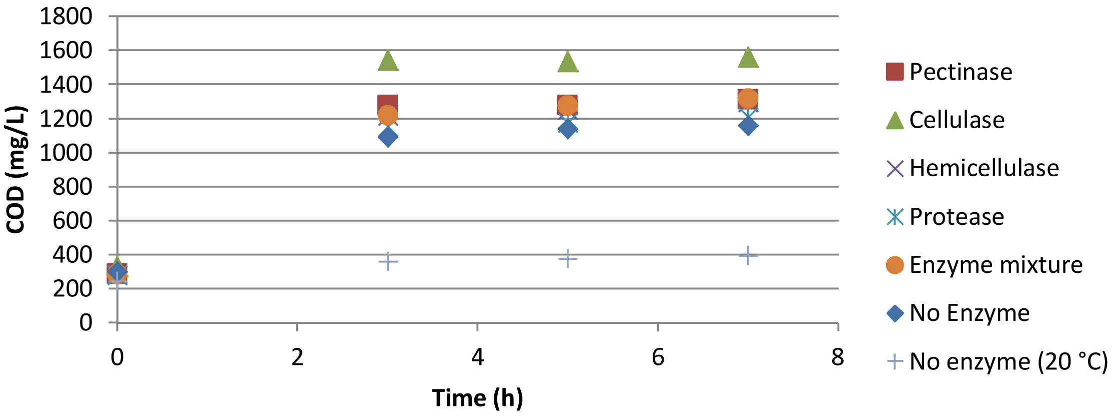

Figure 9. Results from initial trial treating Zostera marina (common eelgrass). Enzyme is dosed in a dose corresponding to 1% of the added algae DM-content.

The results of the main enzymatic pretreatment experiment of 4 different types of algae can be seen in Figure 10 to 14. No sample at room temperature was included in these experiments, instead all samples have been treated at 50 °C. The only exception is for the measurement at time 0 which was done directly after mixing of algae sample in the water and thus at room temperature (20 °C) after which the sample was put in the water bath and heated to 50 °C.

The COD-content of the unfiltered enzymes was also measured and their contribution to the COD-concentrations in the results were calculated to be 267 mg/L for 1% samples and 567 mg/L for 3% samples respectively. These numbers are not subtracted in the results. It was assumed that all enzyme COD was soluble.

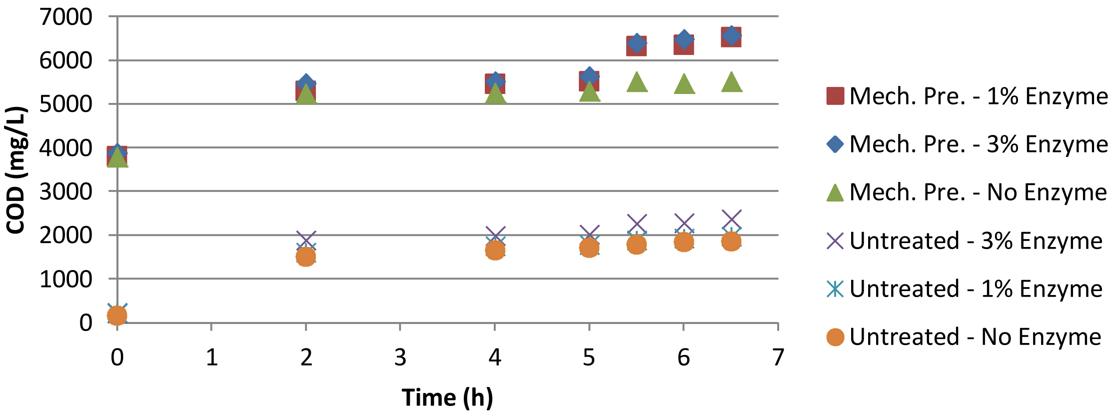

Figure 10. Soluble COD concentration from Fucus vesiculosus (Bladderwrack). Samples of mechanically pretreated (Mech. Pre.) and raw algae (untreated) were either dosed with enzyme (1 or 3% of DM content of algae respectively) or just heated to 50 °C without pH-adjustment (No Enzyme).

It is clear from Figure 10 that mechanical pretreatment of *Fucus vesiculosus* drastically increases the soluble COD concentration compared to raw algae (Untreated – No Enzyme). Enzymatic treatment at 1 or 3% gives no increase in soluble COD compared to only thermally treated algae (Untreated – No Enzyme) when treating with the enzyme mix (cellulose, hemicellulose and pectinase) up to t=5. An increase of roughly 1000 mg/L can be seen when treating mechanically pretreated algae with protease (t=5.5 to 6.5) compared to mechanically pretreated without enzyme (Mech.Pre. – No Enzyme). This increase is greater than the contribution to COD from the enzymes themselves. Also a smaller increase for raw algae treated with protease can be seen, however the increase corresponds well to increase due to the COD from the added enzymes.

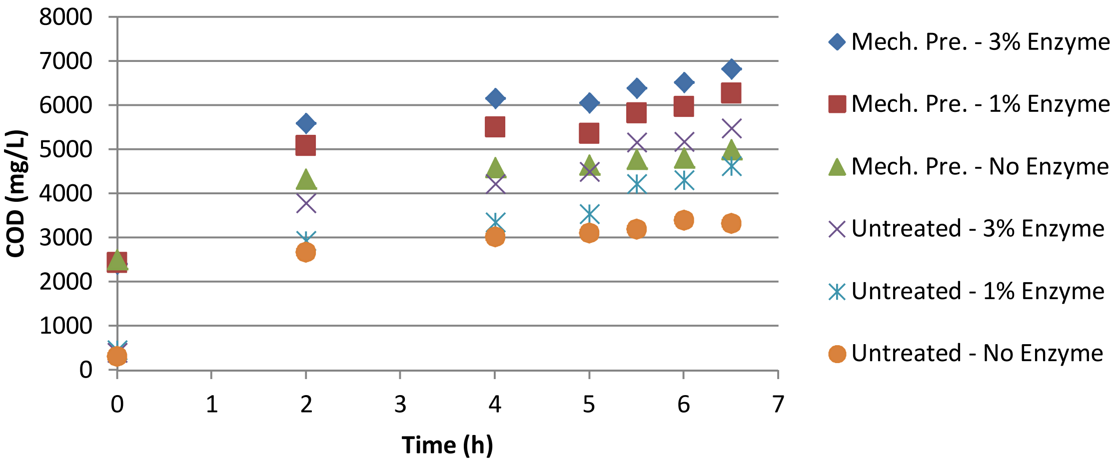

Figure 11. Soluble COD concentration from Filamentous red algae. Samples of mechanically pretreated (Mech. Pre.) and raw algae (untreated) were either dosed with enzyme (1 or 3% of DM content of algae respectively) or just heated to 50 °C without pH-adjustment (No Enzyme).

From Figure 11 it can be seen that all treatments of Filamentous red algae increase the concentration of soluble COD compared to raw algae heated to 50 °C (Untreated – No enzyme). The greatest increase, just above 100%, is seen for a combination of mechanical pretreatment together with a dose of 3% enzyme (Mech.Pre. – 3% Enzyme). The addition of enzymes increases the soluble COD even if corrections are made for the contribution to COD from the enzymes (267 mg/L for 1% samples and 567 mg/L for 3% samples respectively).

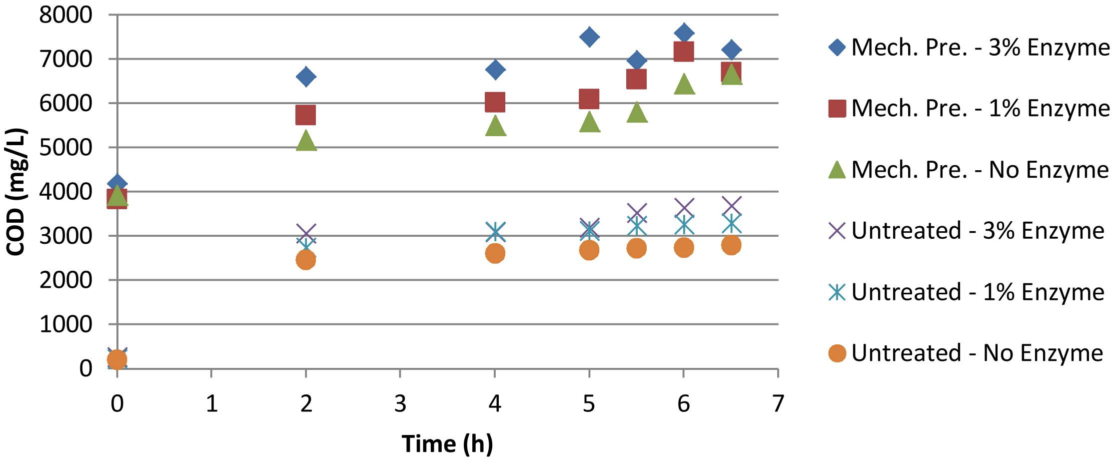

Figure 12. Soluble COD concentration from Furcellaria lumbricalis (red seaweed). Samples of mechanically pretreated (Mech. Pre.) and raw algae (untreated) were either dosed with enzyme (1 or 3% of DM content of algae respectively) or just heated to 50 °C without pH-adjustment (No Enzyme).

Results for *Furcellaria lumbricalis* is shown in Figure 12 and it can be seen that mechanical pretreatment greatly increase the concentration of soluble COD. Enzymatic treatment alone only slightly increases the concentration while enzymatic treatment combined with mechanical pretreatment gives the highest results. The final contribution from enzymes to soluble COD is the same as, or slightly higher, than the COD content of the enzymes themselves.

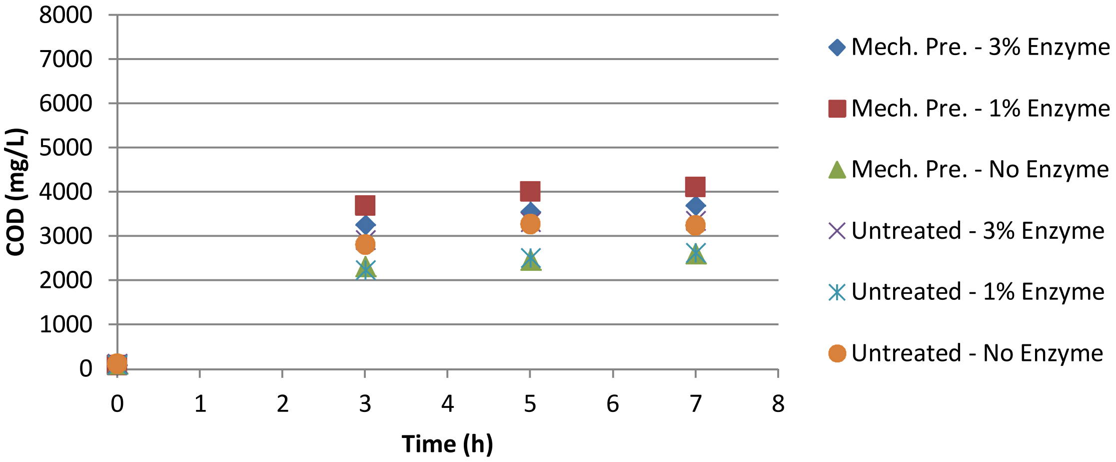

Figure 13. Soluble COD concentration from Zostera marina (common eelgrass). Samples of mechanically pretreated (Mech. Pre.) and raw eel grass (untreated) were either dosed with enzyme (1 or 3% of DM content of eel grass respectively) or just heated to 50 °C without pH-adjustment (No Enzyme).

Results for the sea grass *Zostera marina* (common eelgrass) can be seen in Figure 13 and it is clear that the results differ considerably from those of algae. The concentrations of untreated raw eel grass (No enzyme) are similar to those of treated samples and neither mechanical pretreatment nor enzymatic treatment increases the concentration to any great extent.

To simplify comparison of the results in Figures 11 to 14 the ratio between the final COD-concentrations of the samples to the final concentration of COD in untreated algae/sea grass has been presented in Table 3. It should be kept in mind that even the untreated samples were exposed to 50 °C for 6.5h as the other samples. From Table 3 it is clear that the greatest effect was achieved on algae (*Fucus vesiculosus*, *Furcellaria lumbricalis* and filamentous red algae) while treatment on sea grass (*Zostera marina*) was less successful. However, as shown during the initial trials in Figure 9, it should be remembered that the concentration of soluble COD in samples only exposed to 50 °C were much higher than for samples kept in water at room temperature (20 °C).

Table 3 – Ratio of final soluble COD concentration compared to soluble COD in untreated raw algae/sea grass (Untreated – No enzyme) for all treatments.

	Fucus vesiculosus (Bladderwrack)	Filamentous red algae	*Furcellaria lumbricalis* (red seaweed)	*Zostera marina* (common eelgrass)
Mech. Pre. - 3% Enzyme	3.5	2.1	2.6	1.1
Mech. Pre. - 1% Enzyme	3.5	1.9	2.4	1.3
Mech. Pre. - No Enzyme	3.0	1.5	2.4	0.8
Untreated - 3% Enzyme	1.3	1.7	1.3	1.0
Untreated - 1% Enzyme	1.1	1.4	1.2	0.8
Untreated - No Enzyme	1	1	1	1

In conclusion the enzymatic pretreatment showed promising results for all algae types (*Furcellaria lumbricalis, Fucus vesiculosus* and filamentous red algae) but not for sea grass (*Zostera marina*). Based on these results *Fucus vesiculosus* and red filamentous algae were selected for biomethane potential tests. *Fucus vesiculosus* was also chosen to represent algae with a more rigid surface structure and of the two algae with a softer surface structure, *Furcellaria lumbricalis* and filamentous red algae, the later was selected based on the fact that filamentous red algae is a dominating, environmentally problematic species in Trelleborg were the algae was collected.

3.3 Batch Methane Potential tests

The results from the batch methane potential tests of filamentous red algae and *Fucus vesiculosus* (Bladderwrack) are shown in Figures 14 and 15 respectively.

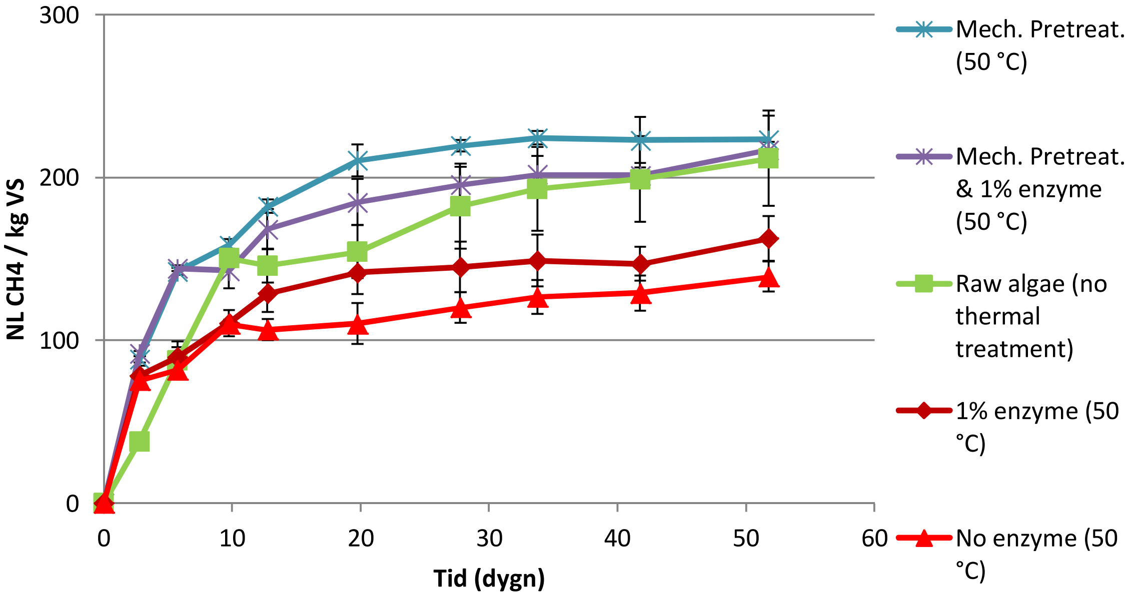

Figure 14. Batch methane potentials for filamentous red algae. Cellulose reference (not shown in graph) was at 364 NL CH_4/kg VS.

It can be seen from Figure 14 that mechanically pretreated algae and raw algae (i.e. not thermally pretreated) reach the same final value for batch methane potential at day 52. However mechanically pretreated algae show a trend to more quickly reach a high methane potential which of course is beneficial since a digestion time of 50 days or more is unrealistic in a full-scale digestion process. This also corresponds well to the results for soluble COD which showed high values for mechanically pretreated algae which would suggest a more rapid methanisation. The trend can however only be determined for one of the samples (Untreated filamentous – No enzyme) since the standard deviations overlap for Untreated filamentous – 1% Enzyme and for raw filamentous algae. Furthermore a decreased methane potential can be seen for not mechanically pretreated algae that has undergone thermal treatment in the water bath (Untreat. Filamentous – No enzyme and Untreat. Filamentous – 1% enzyme). This is somewhat in contrast to the results for soluble COD shown in Table 3 above which showed that the concentration of soluble COD increased in all thermally treated samples from which it could be expected that the methane potential should be higher than, or at least similar to, that of raw algae. A possible explanation for the lower methane production from the thermally, but not mechanically, pretreated algae could be that the batch methane potential tests were performed two weeks after the enzymatic treatment during which time some microbial degradation of the released soluble COD could have occurred even at the low storage temperature of 5 °C. This would of course have affected the mechanically pretreated algae as well which, if so, would have had an even higher batch methane potential than in these experiments. Another explanation could be that pretreatment of the algae released compounds inhibitory to the anaerobic digestion process, however this is only speculation since no analysis of common inhibitory compounds were made.

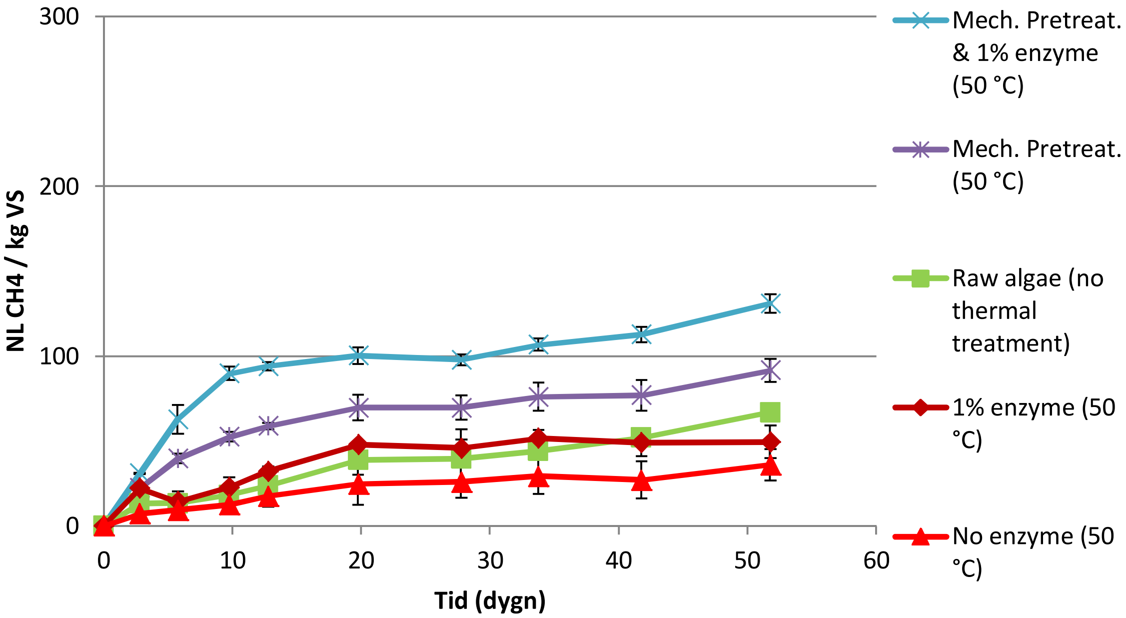

Figure 15. Batch methane potentials for Fucus vesiculosus (Bladderwrack). Cellulose reference (not shown in graph) was at 364 NL CH_4/kg VS.

Results from batch methane potential for *Fucus vesiculosus* indicates a higher potential for mechanically pretreated samples. However, like for filamentous algae, not mechanically pretreated samples (Untreat. Fucus – No enzyme & Untreat. Fucus – 1% enzyme) show a lower methane potential than for raw algae. This contradicts the results from the enzymatic treatment in Table 3 where soluble COD concentrations were higher in thermally pretreated samples why at least an equally high methane potential could be expected from these samples compared to raw algae. A possible explanation could be the long storage time of the samples which was also mentioned for filamentous red algae.

The final biomethane potentials (BMP) are shown in Table 4. For untreated algae the values are roughly the same as for previously published data by Nkemka (2012) were mixed algae from the same area was shown to have a BMP of 160 L CH_4/ kg VS. In general all the methane potentials for filamentous red algae and for *Fucus vesiculosus* are low compared to substrates like sewage sludge (~350 NL CH_4/kg VS) or food waste (~480 NL CH_4/kg VS) (Davidsson, 2007; Johansson & Kjerstadius, 2011) but . Also the measured BMP are low compared to the theoretical maximum of 350 NL CH_4/kg COD.

Table 4. Final biomethane potentials from the batch digestion experiments expressed as normal liters (at STP) of methane per mass of COD or VS.

Sample	**BMP (NL CH$_4$/kg COD)**	**BMP (NL CH$_4$/kg VS)**
Fucus vesiculosus		
Raw algae (no thermal treatment)	41.4	67
No enzyme (50 °C)	31.5	41
1% enzyme (50 °C)	38.0	49
Mech. Pretreat. (50 °C)	69.9	92
Mech. Pretreat. & 1% enzyme (50 °C)	99.1	131
Filamentous red algae		
Raw algae (no thermal treatment)	167	212
No enzyme (50 °C)	109	139
1% enzyme (50 °C)	126	163
Mech. Pretreat. (50 °C)	174	223
Mech. Pretreat. & 1% enzyme (50 °C)	164	217

It could also be noted that the methane potential between the two species differs quite a lot (212 and 67 NL methane/kg VS for *Fucus Vesiculus* and filamentous red algae respectively). It could be hypothesized that this is an effect from the more rigid cell walls of the *Fucus Vesiculus* which potentially could resist microbial hydrolysis better than the softer structure of filamentous red algae.

3.4 Energetic analysis

A simple energetic analysis was performed where energy consumption required to heat and mechanically pretreat the algae was compared to the energy content of the biogas formed. The total energy content in the methane produced from 50 days batch anaerobic digestion is presented below in Table 5. The energy content is calculated based on an energy content of 9,969 kWh for 1 Nm^3 CH_4 (SGC, 2012).

Table 5. Total energy content in methane produced in the batch methane potential tests.

Sample	**BMP (NL CH4/kg VS)**	**Methane production (NL CH4/kg ww algae)**	**Energy content in methane (Wh/kg ww algae)**
Fucus vesiculosus			
Raw algae (no thermal treatment)	67	8.1	80.3
No enzyme (50 °C)	41	5.0	49.6
1% enzyme (50 °C)	49	5.9	59.3
Mech. Pretreat. (50 °C)	92	11.0	109.8
Mech. Pretreat. & 1% enzyme (50 °C)	131	15.8	157.1
Filamentous red algae			
Raw algae (no thermal treatment)	212	15.1	150.7
No enzyme (50 °C)	139	9.9	99.0
1% enzyme (50 °C)	163	11.6	115.8
Mech. Pretreat. (50 °C)	223	15.9	158.9
Mech. Pretreat. & 1% enzyme (50 °C)	217	15.5	154.5

From Table 5 it can be deducted that the total energy content of the produced methane per kg wet weight algae is highest in the samples which has undergone both mechanical and enzymatic pretreatment.

The energy consumption for mechanical pretreatment is presented in Table 6 and is based on the kinetic energy required to deliver a sample at 300 m/s velocity to the outlet pipe of the prototype machine used. The kinetic energy was calculated according to eq.2 and the energy consumption by assuming 50% thermal efficiency.

$$E_k = \frac{1}{2} m * v^2 \tag{eq.2}$$

Table 6. Energy consumption for mechanical pretreatment of algae. Data is presented per kg of wet weight (ww) sample except for energy consumption which is also presented in kg of wet weight algae.

Velocity (m/s)	Kinetic Energy, K_E (kJ/kg ww)	Energy consumption (kJ/kg ww)	Energy consumption (Wh/kg ww)	Energy consumption (Wh/kg ww algae)
300	45	90	25	75

It is clear from the Table above that the energy consumption for mechanical pretreatment per kg of wet weight algae is quite high in relation the potential energy from methane production. However this could most likely be lowered by optimization of the mixing ratio between water and algae.

Finally in Table 7 the net energy gain from digestion of different samples of algae is calculated. Here also the theoretical energy required for heating to 50 °C of algae diluted with water to 3% DM is included, however presented as heat required per wet weight of algae. The calculation does not include heat losses over time.

Table 7. Calculation of net energy gain from anaerobic digestion of untreated algae and from mechanically and enzymatically treated algae.

Sample	Energy content in methane (Wh/kg ww algae)	Mechanical pretreatment (Wh/kg ww algae)	Heating for enzymatic treatment (Wh/kg ww algae)	Net energy gain (Wh/kg ww algae)
Fucus vesiculosus				
Raw algae (no thermal treatment)	80.3	0	0.0	80.3
No enzyme (50 °C)	49.6	0	140.0	-90.4
1% enzyme (50 °C)	59.3	0	140.0	-80.7
Mech. Pretreat. (50 °C)	109.8	75	140.0	-105.2
Mech. Pretr. & 1% enzyme (50 °C)	157.1	75	140.0	-57.9
Filamentous red algae				
Raw algae (no thermal treatment)	150.7	0	0.0	150.7
No enzyme (50 °C)	99.0	0	83.1	15.9
1% enzyme (50 °C)	115.8	0	83.1	32.7
Mech. Pretreat. (50 °C)	158.9	75	83.1	0.8
Mech. Pretr. & 1% enzyme (50 °C)	154.5	75	83.1	-3.6

It is clear from Table 7 that even if more methane is produced no pretreatment method gives a net energy yield higher than that from digesting raw untreated algae. However it must be stressed that the conditions used during pretreatment were highly favorable to achieve the best possible effect, especially in regards to the amounts of water used in mechanical pretreatment and enzymatic pretreatment. Both of these could most likely be performed using less diluted samples which would require less energy in pretreatment for acceleration and heating of the algae. A hypothetical scenario were the mechanical pretreatment was done more efficiently and only a 1:1 ratio of mixing water needed to be used and in addition no cost for heating was required (due to use of cheap low grade heat from district heating) was also calculated and can be seen in table 8. It is clear that even in this scenario only one combination of treatments, mechanical pretreatment and enzymatic treatment of *Fucus vesiculosus*, renders higher net energy yield than digestion of raw algae. Thus even using more effective pretreatment will not drastically increase the methane production. However the mechanically pretreated algae is much easily handled in transportation and pumping compared to raw algae which is an effect not used in the calculations shown here.

Table 8. Calculation of net energy gain from anaerobic digestion of untreated algae and from mechanically and enzymatically treated algae.

Sample	**Energy content in methane (Wh/kg ww algae)**	**Mechanical pretreatment (Wh/kg ww algae)**	**Heating for enzymatic treatment (Wh/kg ww algae)**	**Net energy gain (Wh/kg ww algae)**
Fucus vesiculosus				
Raw algae (no thermal treatment)	80.3	0	0	80.3
No enzyme (50 °C)	49.6	0	0	49.6
1% enzyme (50 °C)	59.3	0	0	59.3
Mech. Pretreat. (50 °C)	109.8	50	0	59.8
Mech. Pretr. & 1% enzyme (50 °C)	157.1	50	0	107.1
Filamentous red algae				
Raw algae (no thermal treatment)	150.7	0	0	150.7
No enzyme (50 °C)	99.0	0	0	99.0
1% enzyme (50 °C)	115.8	0	0	115.8
Mech. Pretreat. (50 °C)	158.9	50	0	108.9
Mech. Pretr. & 1% enzyme (50 °C)	154.5	50	0	104.5

4. Conclusions

- Mechanical pretreatment could increase the soluble COD-concentration of the tested algae (not sea grass) by 1.5 to 3 times compared to raw algae. Enzymatic treatment increased it by 1.3 to 1.7 times. The best results were achieved by combining mechanical and enzymatic treatment where the concentration could was increased 3.5 times compared to raw algae.

- The production of biogas was faster for mechanically pretreated samples of *Fucus vesiculosus* and filamentous red algae, however enzymatic treatment alone did not increase the rate of biogas production.

- The final methane potential (after 50 days of digestion) could not be increased by thermal or enzymatic pretreatment for filamentous red algae but could be increased by 100% for *Fucus vesiculosus*. However all final methane potentials for pretreated algae were low compared to common biogas substrates such as sewage sludge or food waste.

- The energy potential from anaerobic digestion of *Fucus vesiculosus* is 80 Wh/kg wet weight algae and 150 Wh/kg of wet weight for digestion of Filamentous red algae.

- The net energy gain per mass of wet weight algae is lower when pretreating algae compared to digestion of raw untreated algae alone.

5. Acknowledgements

The authors are thankful for the financial support provided by Swedish Gas Technology Centre (SGC), Water and Environmental engineering, Department of Chemical Engineering at Lund University, the department of sustainable development at Trelleborg municipality, Nordvästra Skånes Renhållnings AB (NSR AB) and TK Energy A/S (Denmark). Further appreciation is given to Novozymes A/S (Denmark) for providing the enzymes used in the experiments.

In addition the authors would like to acknowledge the contribution of Henriette Draborg (Novozymes) for providing a method for enzymatic treatment, Thomas Koch (TK Energy A/S) for providing experimental equipment and Matilda Gradin and Ellinor Tjernström (Trelleborg municipality, department for sustainable development) for participating in algae collection and species determination.

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