Annex 9:

13 Posters

List of posters in Annex 1

Bioethanol potentials from macroalgaes

Fazio Coppalo[1], Zsófia Kádár[2] and Anne Belinda Thomsen[2]
[1]Department of Chemical and Biosystems Sciences, Siena University
Via della Diana, 2A, 53100 Siena, Italy
[2]Biosystems Department, Risø National Laboratory for Sustainable Energy
Technical University of Denmark, P.O. Box 49, DK-4000 Roskilde, Denmark

INTRODUCTION

Marine biomasses such as macroalgaes are interesting for 2nd generation bioethanol production due to their natural occurrence, fast growing rates, sugar contents and the fact that they grow in seawater, thus with no demand for agriculture land. In the present investigation three macroalgaes were studied for their sugar recovery and bioethanol potentials after thermal pretreatment and enzymatic hydrolysis.

Methods and materials

Tree species of macro-algae were selected and tested for bioethanol potentials: C. *linum (C)*, and G. *longissima (G)*, from Orbetello lagoon, located in southern Tuscany, Italy, U. *lactuca (U)* from the sea near Aarhus, Jutland, Denmark. Dried and milled samples were treated hydrothermally using a stirred and heated reactor with 6,0% DM/L water at conditions seen in Table 1. Raw and pretreated materials were analysed for their contents of cellulose, hemicellulose, starch and Klason lignin. Enzymatic hydrolysis (Celluclast, Novozym 188, Spirizyme Plus Tech) was carried out on raw and pretreated materials, to test the convertibility of cellulose and starch.

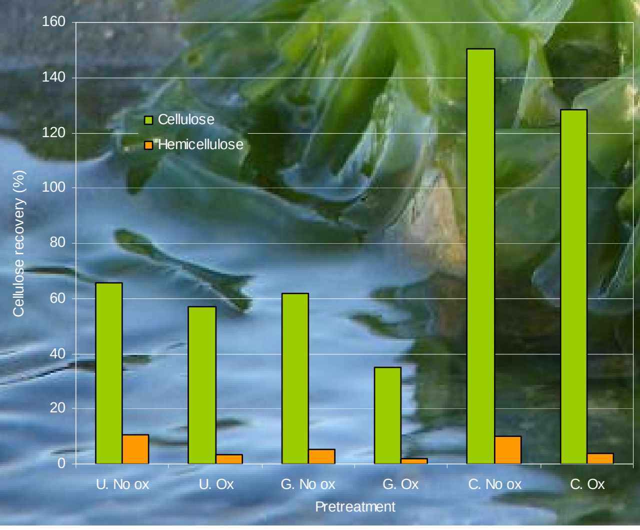


Figure 1. Sugar recovery after pretreatment.

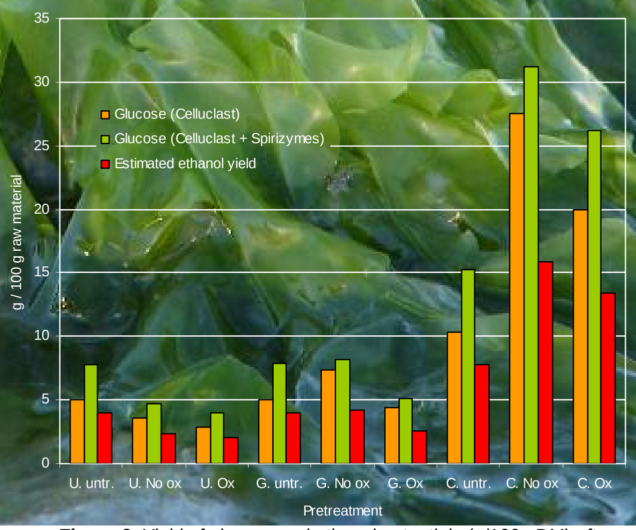


Figure 2. Yield of glucose and ethanol potentials (g/100g DM) after pretreatment and enzymatic hydrolysis.

RESULTS AND DISCUSSION

- Pretreatment of fibres resulted in enriched cellulose content and show very good effect on hemicellulose removal (Table 1).
- The maximal recovery of carbohydrates is an important point of an optimal pre-treatment. Both cellulose and hemicellulose recovery was low (< 60% and 10% respectively) at *U. lactuca* and *G. longissima*.
- The very high cellulose recovery for *C. linum* after pretreatment (Figure 1) can be partly explained by its starch content (8%).
- The enzymatic accessibility of the cellulose of *U. lactuca* and *G. longissima* was not increased by pretreatment (Figure 2).
- Even though final ethanol yields were rather low (Figure 2.) algaes may be interesting substrates in a biorefinery concept due to their high carbohydrate content.

Table 1. Effect of pretreatment on composition of algae.

Name	Temperature (ºC)	Time of treatment (min)	Gas/Pressure (bar)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Starch (%)
U. untr.	-	-	-	4.7	2.8	0	1.4
U. No ox	195	10	N_2 /4	8.6	1.1	0	0.8
U. Ox	195	10	O_2/12	9.9	0.4	0	0.1
G. untr.	-	-	-	12.9	21.2	24.3	7.2
G. No ox	195	10	N_2/4	32.2	4.0	29.8	0.5
G. ox	195	10	O_2/12	29.1	9.2	24.8	0.1
C. untr.	-	-	-	26.3	3.2	6.0	3.6
C. No ox	195	10	N_2/4	39.5	0.8	4,2	8
C. Ox	195	10	O_2/12	67.4	0.5	8.1	0.2

untr.: untreated samples; No ox.: pretreated with nitrogen; Ox.: pretreated with oxygen

FUTURE PLANS

Future studies need to address:

- pretreatment optimization in order to reach high recoveries of cellulose, starch and other carbohydrates (rhamnose)
- ethanol fermentation experiments on pretreated algae, focusing on C6 fermentation

ACKNOWLEDGEMENT

The study was financially supported by the PSO Project (Energy Production from Marine Biomass (*Ulva lactuca*): 2008-1-0050). We thank Mr. Tomas Fernqvist for technical assistance.

VP 1.3.44

Farming of aquatic biomass for *energy*

– growth potential of the marine seaweed *Ulva lactuca*

Annette Bruhn[1] (anbr@dmu.dk), Michael Bo Rasmussen[1] (mir@dmu.dk) & Birgit Olesen[2] (Birgit.Olesen@biology.au.dk)

[1]National Environmental Research Institute, Aarhus University • [2]Biological Institute, Aarhus University

Phase 1

Cultivation of biomass

Phase 2

Harvest technology – see poster VP1.3. 45

Phase 3

Characterisation of biomass – see poster VP1.3 46

Phase 4

Conversion to bioenergy – see poster VP1.3. 45

Aim – phase 1

We want to determine the biomass production potential of the green macroalgae *Ulva lactuca*

Motivation

- Algae produce lipids and carbohydrates that can be converted into 2nd generation biofuels
- Algae have higher growth rates than conventional energy crops
- Algae cultivation is not in conflict with agricultural interests
- Algae may serve as “flue gas and waste water treatment plants”
- Algae produce high value chemical compounds for food, feed and pharmaceutical industries

The ulva project

We aim at describing production and utilization of marine biomass for energy production.

The green macroalgae sea lettuce (*Ulva lactuca*) is our model organism.

The production system is land based and integrates the possibility of using CO_2 and excess heat from power plants as well as waste water for increasing the biomass production. The partners address multiple utilization of the biomass as feedstock for solid and liquid biofuel e.g. bioethanol, biogas and syngas and combustion.

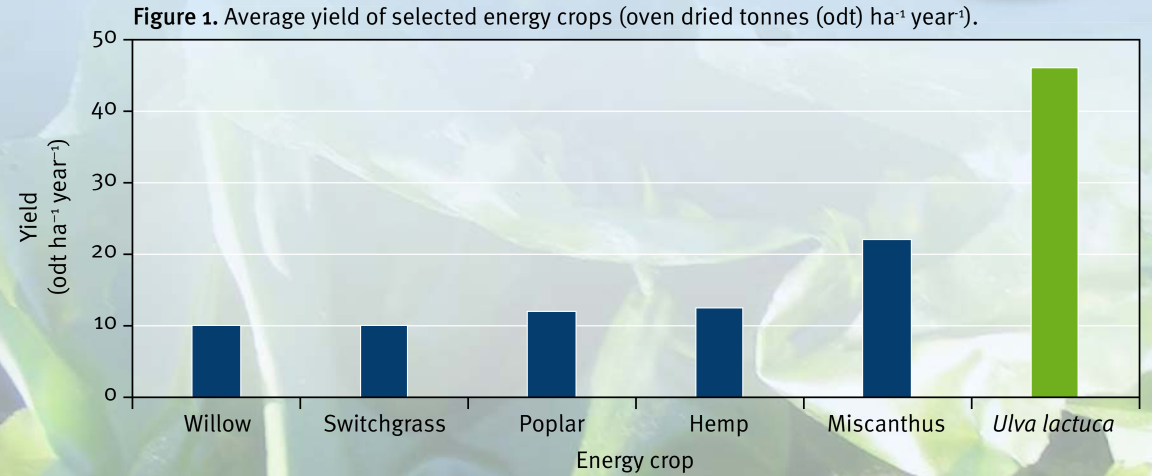
Results

- A conservative production estimate is 45 T DW ha^{-1} $year^{-1}$
- Growth rates ranged from negative to 43% per day with an average of 15% per day
- 4 kg's of biomass per m^2 was found to be the optimal carrying capacity in a landbased aerated system
- Addition of CO_2 or flue gas is expected to further increase the production potential

Conclusion

- **45 T DW ha^{-1} $year^{-1}$** is a realistic production estimate in a landbased aerated system.
- Production of Ulva is three to five times the production of conventional energy crops in temperate climate zones (figure 1).
- The future focus of NERI is on optimising growth and energy potential by addition of CO_2/flue gas and nutrient rich waste water.

Figure 1. Average yield of selected energy crops (oven dried tonnes (odt) ha^{-1} $year^{-1}$).



Ongoing projects are carried out in cooperation between the National Environmental Research Institute (NERI), the Danish Technological Institute (TI), Research Centre Risoe, Danish Technological University (DTU), Biological Institute at Aarhus University (AU) and DONG Energy A/S.

Projects are financed by energinet.dk (the PSO foundation), the Villum Kann Rasmussen Foundation and the Research Foundation of Aarhus University.

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VP 1.3.45

Energy production from marine biomass

Karin Svane Bech (karin.svane.bech@teknologisk.dk) and Peter Daugbjerg Jensen (peter.daugbjerg.jensen@teknologisk.dk)
Danish Technological Institute, Centre for SustainableEnergy and Transport, Kongsvangs Allé 29, DK-8000 Aarhus C, Denmark

Aim

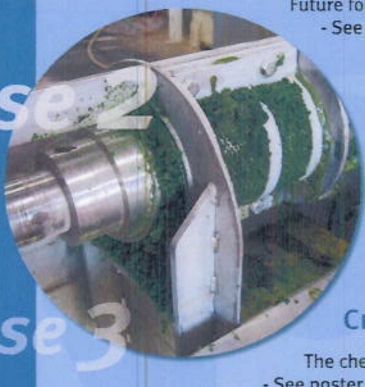
The project runs from 2008 to 2011 and the involved partners aims at describing production and utilization of marine biomass for energy production using the macroalgae sea lettuce *(Ulva lactuca)* as a model organism.
The production system is land based and integrates the possibility of using CO_2, excess heat from power plants and wastewater management to increase the biomass productivity.
The partners address multiple utilization of the biomass as feedstock for solid and liquid biofuel e.g. bioethanol, biogas and thermal conversion.

Phase 1

Farming of *Ulva lactuca* in land based basins

By using *Ulva lactuca* as a model organism this project will increase the knowledge of growing macroalgae in basins.
Future focus will be on utilizing flue gas to optimise growth.
- See poster VP 1.3.44

Phase 2



Harvest and pressing of *Ulva lactuca* and subsequent drying

Existiting technology and machinery for harvesting aquatic biomass will be identified and described, resulting in one or more *Ulva lactuca* harvester prototype.
Initial results indicates that it is possible to remove substantial amounts of water by pressing the biomass prior to drying.

Phase 3

Characterisation of biomass

The chemical and physical parameters of *Ulva lactuca* biomass will be analyzed.
- See poster VP 1.3.46

Phase 4

Utilization of macroalgae for energy production

- Bioethanol: The potential for producing ethanol from fermentation of soluble C6 sugars, hemicellulose and cellulose from *Ulva lactuca* will be evaluated. The effects of a hydro-thermal pre-treatment of the biomass will likewise be evaluated.
- Biogas: The conversion of *Ulva lactuca* biomass to methan will be evaluated. Pre-treatment of the biomass includes removal of excess water, removal of excess nutrients and salts, grinding and hydro-thermal treatment at 120-195°C to improve the degradation of lignocellulose.
- Combustion: A complete fuel analysis for combustion purposes will be carried out including heating value, moisture, ash, sulphure, chloride and major and minor components in the ash. A batch of 5-10 kg pelletized *Ulva lactuca* biomass will be burned and slagger formation will be catagorized.

Perspective

Aquatic biomass has a vast potential as feedstock for various applications including solid and liquid biofuel, fodder and pharmaceuticals providing substantial environmental benefits. To optimize the economy various products shall be exploited consecutively from high value products to conversion of biomass and energy rich residuals to bioenergy.

The present project runs from 2008 to 2011 and will give recommendations for methods of CO_2 transfer from flue gases into aquatic biomass, mass production of algae biomass and transformation of algae biomass into bioethanol, biogas and solid biofuel. These recommendations will be used for establishing a pilot plant production facility coupled to a CHP plant.

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VP 1.3.46

Energy Production by Thermal Conversion of Seaweed - characterisation

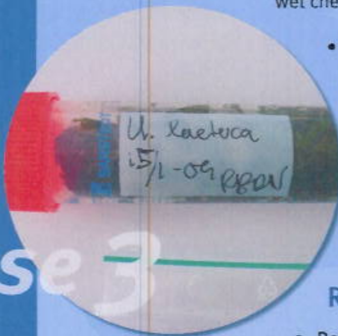
Jonas Dahl (Jonas.Dahl@teknologisk.dk), Peter Daugbjerg Jensen, Lars Nikolaisen and Karin Svane Bech, Danish Technological Institute
Centre for Sustainable Energy and Transport, Kongsvangs Allé 29. DK-8000 Aarhus C, Denmark

Aim - Phase 3

Investigating the potential of using the green seaweed *Ulva lactuca* as a feedstock for production of heat and electricity through combustion or other thermal conversion.

Phase 1

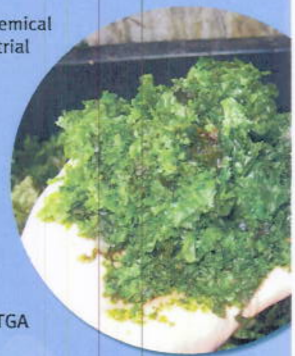
Phase 2



Phase 3

Phase 4

Methodology



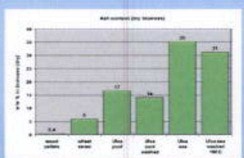
- The investigation and characterisation is performed by analyzing contents and chemical composition of the ash of the *Ulva lactuca* algae and compare it with typical terrestrial biomass used in biomass combustion plants such as wood and straw.
- Chemical analyses of the bioash is performed by a mixture of WD-XRF analyses, wet chemical and elemental analyses.
- Influence of harvesting and production technique by comparing harvest from sea and from growing pools
- Effects of simple pre treatment such as washing in cold and hot pressurized fresh water
- Investigation of combustion, gasification and pyrolysis behavior by TGA analyses and by combustion of pelletised makro algeas in a specially designed slag anlyser.

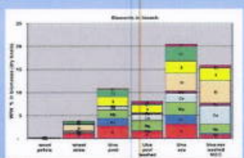
Results

- Results reveals seaweed to be a fuel with considerably high ash content and high Nitrogen content compared to typical terrestrial biomass fuels such as straw and wood
- The ash from Ulva consist of salts (chlorine- and sulphur- salts) .
- Washing seaweed in fresh water removes NaCl and parts of K. Main part of K and S do however remains in the biomass.
- The ash content remains high after washing in water, also at elevated washing temperatures.

Conclusions

- Considering the ash chemistry and what is known from combustion of straw, seaweed reveals to be very challenging for combustion or gasification in conventional units. It would most likely cause significant problems with molten ash, fouling, corrosion and particle emissions.
- Consequently, pre–processing of the seaweed which separates ash and salts has to be conducted or alternative conversion technologies such as pyrolysis or low temperature gasification, which are better suited for fuels with this type of ash content and quality have to be utilized.





Perspective

- Analyses with seaweed in slag analyser will reveal if the ashes without Cl (washed in water) will form low temperature melting ashes
- Termo gravimetric analyses (TGA) will reveal reactitivty of the Ulva during thermal conversion and thereby give important data for evaluating pyrolyses or gasfication potentials further on in the project

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Aquatic Biomass for Biofuels

Motivation

- Algae produce lipids and carbohydrates that can be converted into 2nd generation biofuels.
- Algae have higher growth rates than conventional energy crops.
- Algae cultivation is not in conflict with agricultural interests.
- Algae may serve as “flue gas and waste water treatment plants”.
- Algae produce high value chemical compounds for food, feed and pharmaceutical industries.

Aims

We want to determine the potential of the macroalgae sea lettuce (*Ulva lactuca*) as feedstock for 2nd generation biofuel and bioenergy production.

We focus on:

- Biomass production potential
- Energy potential
  - *Biogas*
  - *Bioethanol*
  - *Combustion/gasification*

Results

Production potential of U. lactuca

The annual production per hectare has been estimated to a minimum of 45 metric tonnes dry weight. This is more than three times the yield of conventional food or energy crops.

The potential production however, is expected to be significantly higher when further optimising growth conditions.

Energy potential

Regarding the potential for bioethanol and biogas production, as well as for combustion, we only have preliminary results.

Future

The ongoing research includes additional growth experiments exploiting flue gas and return heat of power plants for growth optimisation.

Figure 1. The green macroalgae sea lettuce (*Ulva lactuca*) is a promising candidate for production of biofuels. It has demonstrated growth rates of up to 40 % per day[1] and has a content of carbohydrates of up to 60 % of dry weight[2].

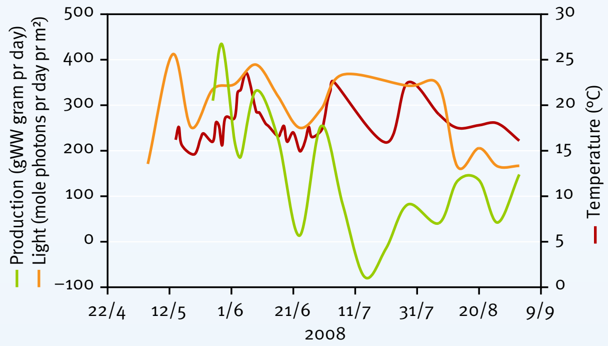

Figure 3. We reached an average growth rate of 15% per day, ranging from negative rates to a maximum of 43% per day. Large seasonal fluctuations in production were due to sporulation, where reproductive cells were released followed by disintegration of tissue.

Figure 2. A land based cultivation facility for documenting growth and energy potential of *U. lactuca* under various growth conditions throughout the season from April to September.

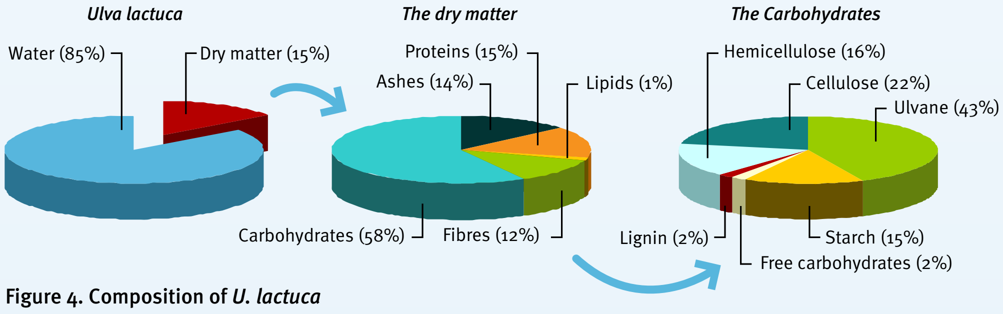

Figure 4. Composition of *U. lactuca*

Perspective

The ambition of the Danish government is to make Denmark independent of fossil fuels. To achieve this ambitious goal, vast amounts of biomass will need to be introduced to the energy sector as feedstock for heat, electricity, transport fuel etc.

The substantial environmental benefits and ethical advantages of cultivation of aquatic biomass combined with the potential for full-scale utilisation of the feedstock - from high value products to conversion of biomass and energy rich residuals to bioenergy – strongly emphasise the potential of utilising aquatic biomass as a sustainable way of achieving renewable energy in the future.

Conclusion

- Aquatic biomass has a high production potential – more than three times the potential of conventional energy crops.
- Aquatic biomass has a promising potential for bioenergy production.
- Further Reseach and Developement is needed to optimise biomass production, carbohydrate composition and fermentation processes.

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Risø DTU
National Laboratory for Sustainable Energy

DONG energy

Michael Bo Rasmussen, National Environmental Research Insititute, Aarhus University, Vejlsøvej 25, DK-8600 Silkeborg, Denmark (mir@dmu.dk)

Peter Daugbjerg Jensen, Technological Institute, Centre for Sustainable Energy and Transport, Kongsvangs Allé 29, DK-8000 Aarhus C, Denmark (peter.daugbjerg.jensen@teknologisk.dk)

Annette Bruhn, National Environmental Research Insititute, Aarhus University, Vejlsøvej 25, DK-8600 Silkeborg, Denmark (anbr@dmu.dk)

Lone Thybo Mouritsen, Havets Hus, Færgevej 4, DK-8500 Grenå, Denmark (lm@havetshus.dk)

Birgit Olesen, Aarhus University, Biological Institute, DK-8000 Aarhus C, Denmark (Birgit.Olesen@biology.au.dk)

Stiig Markager, National Environmental Research Institute, Aarhus University, Frederiksborgvej 399, DK-4000, Roskilde, Denmark (ssm@dmu.dk)

Anne Belinda Thomsen, Per Ambus & Jesper Bangsøe Nielsen, Biosystems Department, Risø National Laboratory for Sustainable Energy, Technical University of Denmark – DTU, Building 330, P.O. Box 49, DK-4000 Roskilde, Denmark (anne.belinda.thomsen@risoe.dk, per.ambus@risoe.dk, jesper.bangsoe.nielsen@risoe.dk)

Jonas Dahl, Technological Institute, Centre for Sustainable Energy and Transport, Kongsvangs Allé 29, DK-8000 Aarhus C, Denmark (Jonas.Dahl@teknologisk.dk)

Bo Sander & Erik Ravn, DONG Energy A/S, Kraftsværksvej 53, Skærbæk, DK-7000, Fredericia, Denmark (bosan@dongenergy.dk, erirs@dongenergy.dk)

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Literature cited

[1] Malta, E.J. & Verschuure, J.M. (1997).

[2] Márcia, P., Fontoura, A. & Mathias, A. (2004).

Conversion of macroalgae to bioethanol and biogas

Henrik Bangsø Nielsen[1], Fazio Coppola[2], Zsófia Kádár[1], Jens Ejbye Schmidt[1] and Anne Belinda Thomsen[1]

[1]Biosystems Division, Risø DTU, National Laboratory for Sustainable Energy, Technical University of Denmark, P.O., Frederiksborgvej 399, P.O. Box 49, DK-4000 Roskilde, Denmark

[2]Department of Chemical and Biosystems Sciences, Siena University, Vial della Diane, 2A, 53100 Siena, Italy

INTRODUCTION

At coastal areas, subject to eutrophication, macroalgae are often washed to the shores causing bad odours and other nuisances. These algae makes up a large waste bioenergy resource but are rarely utilized. Due to their fast growth rates, macoralgae also holds a large potential for biomass production and has a prospective of being used for CO_2-capturing at industries such as heat and power plants. Reuse of CO_2 for production of algae for bioenergy purposes is an appealing scenario because such systems can reduce the use of fossil fuels without the demand of arable land. In the present study we estimate the bioethanol and biogas potential of three macroalgae (*Ulva lactuca*, *Chaetomorpha linum* and *Gracilaria longissima*).

BIOETHANOL POTENTIALS

Dried and milled samples were treated hydrothermally using a stirred and heated reactor with 6,0% DM/L water at 195ºC/10 min. with oxygen at 12 bar and without oxygen at 4 bar. Raw and pretreated materials were analysed for their contents of cellulose, hemicellulose, starch and Klason lignin. Enzymatic hydrolysis (Celluclast, Novozym 188, Spirizyme Plus Tech) was carried out on raw and pretreated materials, to test the convertibility of cellulose and starch.

Table 1. Effect of pretreatment on composition of algae

Name	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Starch (%)
Ulva dried	4.7	2.8	0	1.4
Ulva without oxygen	8.6	1.1	0	0.8
Ulva with oxygen	9.9	0.4	0	0.1
Gracilaria dried	12.9	21.2	24.3	7.2
Gracilaria without oxygen	32.2	4.0	29.8	0.5
Gracilaria with oxygen	29.1	9.2	24.8	0.1
Chaetomorpha dried	26.3	3.2	6.0	3.6
Chaetomorpha without oxygen	39.5	0.8	4,2	8
Chaetomorpha with oxygen	67.4	0.5	8.1	0.2

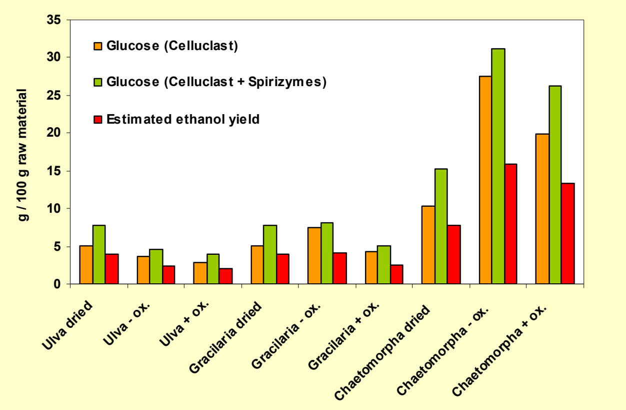

Figure 1. Glucose yield and ethanol potentials (g/100g DM) after pretreatment and enzymatic hydrolysis.

BIOGAS POTENTIALS

Substrate 1: Macroalgae were prepared as previously described (bioethanol potentials).

Substrate 2. Samples of *Ulva lactuca* that had been frozen immediately after harvest were thawed. Half of the material were washed for 24 hours by mixing 60 gram of algae with 10 liter of water. Half of the washes/unwashed fraction were chopped in pieces of app. 1 cm while the other fractions were blended.

All samples were mixed with effluent from an anaerobic thermophilic lab-scale reactor treating cattle manure and incubated in batch vials for 29 days at 55ºC.

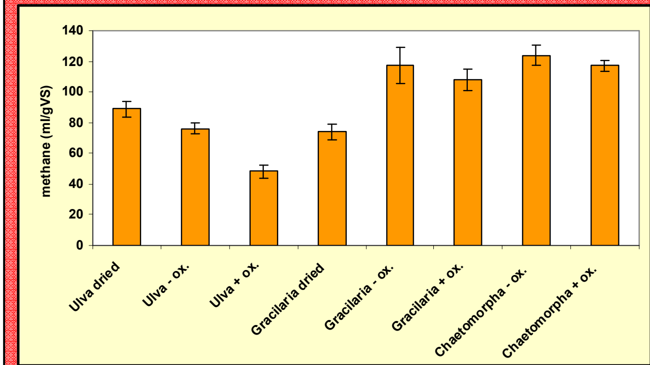

Figure 2. Biogas potential of dried and hydrothermally treated algae.

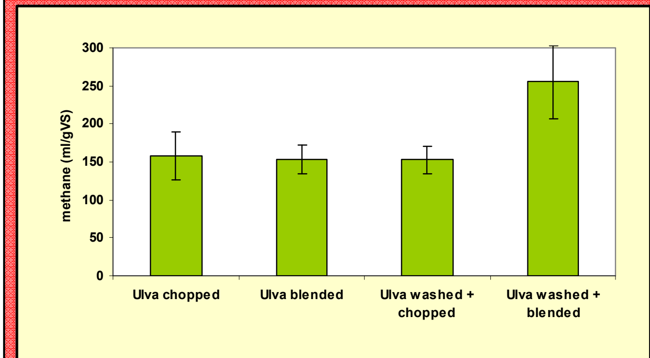

Figure 3. Biogas potential of *Ulva lactuca* that was frozen immediately after harvest.

Results

- Pretreatment resulted in an enriched cellulose content and showed a very good effect on hemicellulose removal (tab. 1).
-The enzymatic accessibility of cellulose were not increased by the pretreatment of *Ulva and Gracillaria* (fig. 1).
- Even though final ethanol yields were rather low (fig 1.) algae may be interesting substrates in a biorefinery concept due to their high carbohydrate content.
- Hydrothermal treatment had a negative effect on the methane yield of *Ulva* but increased the methane yield of *Gracillaria*.
- A signifcant loss in biogas potential was observed when *Ulva* was dried instead of frozen (fig. 2 + 3), showing that the algae should be treated in its fresh form.
- The methane yield of *Ulva* (fig 3) was significantly increased by the washing and blending procedure.

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ALGAE AS A VALUABLE INGREDIENT IN FOOD, FEED AND FUEL

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WHAT ARE ALGAE?

Algae are a diverse group of organisms ranging from unicellular phytoplankton to macro algae measuring several meters all able to perform photosynthesis and produce a wide variety of chemical compounds.

WHY ALGAE?

- High production rates (up to 100 tons dw/ha/year).
- Contains large amounts of valuable compounds, i.e. proteins, carbohydrates, fatty acids, antioxidants and pigments.
- Algae production is not in conflict with production of agricultural crops.

ALGAE IN FOOD, FEED AND FUEL VALUE CHAIN

FOOD:

- Algae are currently used in food, but by applying a value chain, all high-value compounds can primarily be used in food (i.e. essential amino acids, antioxidants, polyunsaturated fatty acids, vitamins).

FEED:

- Residues from food production can subsequently be used in feed production (i.e. proteins, pigments).

FUEL:

- Finally, the residues from the previous steps have the potential to enter into energy production (biogas, bioethanol, gasification and combustion).

R&D PROJECTS

The Danish Technological Institute is currently working on both national and international R&D projects involving algae as a resource for energy production and biorefineries.

CURRENT PROJECTS INCLUDE:
Energy production from marine biomass (*Ulva lactuca*)
Nationally funded project.

BioWalk4BioFuel
EU-funded project on energy production from algae biomass.

FUTURE PROJECT:
The MacroAlgae Biorefinery (MAB)
National proposal to utilize algae biomass in a value chain of proteins, lipids, fibers for bioplastics, pharmaceuticals and energy.

BY
KARIN SVANE BECH, MSC
DANISH TECHNOLOGICAL INSTITUTE, CENTRE FOR RENEWABLE ENERGY AND TRANSPORT
KONGSVANG ALLÉ 29, DK-8000 AARHUS C
TEL.: +45 7220 1000, E-MAIL: KARIN.SVANE.BECH@TEKNOLOGISK.DK

ENERGY PRODUCTION FROM SEA LETTUCE

AB Bjerre[a] (ANBJ@DTI.dk), A Bruhn[b], P Daugbjerg Jensen[a] ,Z Kadar[c], J Dahl[a], KS Bech[a],
MB Rasmussen[b], HB Nielsen[c], B Sander[d], L Nikolaisen[a]
[a] Danish Technological Institute , DK-2630 Tåstrup; [b]Aarhus University, DK-8600 Silkeborg; [c]Risø DTU, DK-4000 Roskilde;
[d]DONG Energy, DK-6780 Skærbæk

Results *Ulva lactuca*

Growth experiments in large scale have confirmed an annual production pr. hectare of 45 tons dry matter (terrestic crops 10-12 tons dry matter/ha). Growth rate was increased by 43 % when the water was bubbled with flue gas (13 % CO_2, 6 % O_2 and 81 % N_2). SO_2 and NO_x in natural flue gas does not inhibit growth.

Carbohydrate contents in the untreated *Ulva lactuca* biomass was 58 % and 6.1 % was C6 sugars. The ash contents ranged from 14 % to 35 % depending on the origin and if the biomass had been washed.

The methane production was 158-174 ml/g volatile solids (VS). Shredding of the biomass as a pretreatment resulted in a significantly higher methane yield of up to 271 ml/g VS.

The ethanol yield from a simple yeast fermentation was approximately 3 g ethanol/100 g dry matter.

The total ABE (Acetone, Butanol and Ethanol) yield was 10 g/100g dry matter (both C5 and C6 fermentations by *Clostridium sp.*). No inhibition was found when pretreated and sterilized.

Combustion of washed and dried pellets was difficult, and in future experiments the possibility of co-firing algae pellets with other types of fuel will be examined.

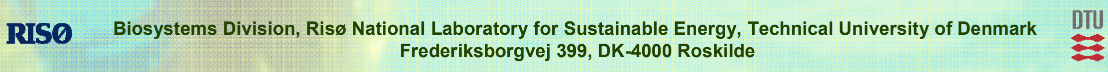
DANISH TECHNOLOGICAL INSTITUTE

The Fourth Annual Workshop of COST FP0602
Biotechnology for lignocellulose biorefineries
22-24. September 2010, Izmir, Turkey

Biofuel production from macroalgaes

- emphasize on butanol production

Zsófia Kádár, Annette Eva Jensen, Anne Belinda Thomsen

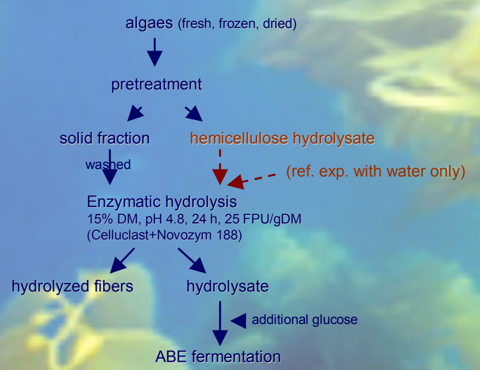


Biosystems Division, Risø National Laboratory for Sustainable Energy, Technical University of Denmark
Frederiksborgvej 399, DK-4000 Roskilde

ABSTRACT

Macroalgaes can grow on non-agricultural land, without increasing food prices, using fresh water, meanwhile consuming CO2 for growing. Due to all these advantages and moreover due to very high biomass yield with high carbohydrate content, macroalgae can be the well suited candidates as feedstock for biofuel production in the future.
In our study, two different macroalgaes (*Chaetomorpha linum* and *Ulva lactuca*) were investigated. The high carbohydrate content makes algaes an interesting substrate for Acetone-Butanol-Ethanol (ABE) fermentation by Clostridium strains (*C. acetobutylicum* or *C. beijerinckii*). The advantages of this fermentation process are (i) that all products (acetone, butanol, ethanol) formed during the process can be used as biofuel (ii) clostridia are able to use many different carbohydrates (both C6 and C5 sugars) (iii) due to cellulolytic and xylanolytic activities of these clostridia strains, direct production of ABE from the algae polysaccharides is possible **without** enzyme addition.

THE PROCESS



SUBSTRATES

Chaetomorpha linum and *Ulva lactuca* (both harvested in Denmark) were used in our experiments. Samples were pretreated under different conditions (Table 1).

Table 1. Pretreatment conditions of macroalgaes

Algae	ID	Pretreatment	t (min)	T (°C)
	S2		10	180°C
	S3*	hydrothermal	10	180°C
	S4		10	190°C
C. linum	S5		10	200°C
	S11		10	180°C
	S12	wet oxidation	10	190°C
	S13		10	200°C
	LB	hydrothermal	10	195°C
U. lactuca	UB	hydrothermal	10	195°C

* before pretreatment was dried (all other samples were pretreated fresh)

ABE (Acetone Butanol Ethanol) FERMENTATION

ABE fermentations were performed on the hydrolysates coming after enzymatic hydrolysis. To reach the initial 30 g/l glucose content additional glucose was added to the hydrolysates. Experiments were carried out in 100 ml serum flasks in a working volume of 30 ml at 35°C under anaerobic conditions by *C. beijerinckii*. Flasks were inoculated with fresh pre-culture grown overnight. Samples were taken daily for pH, OD (540 nm) measurements, glucose, intermediate acids (acetic and butyric acid) and solvents (acetone, butanol and ethanol) were analyzed by HPLC. Control experiment (B) was performed on synthetic medium containing 30 g/l initial glucose.

RESULTS

Acetone-Butanol-Ethanol fermentation are summarized in Figure 1.

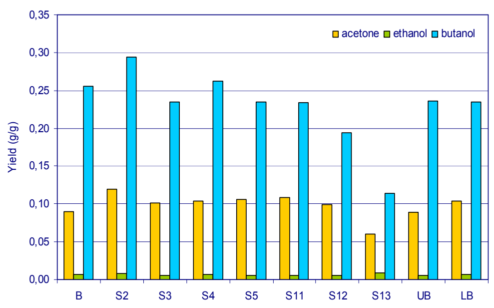


Figure 1. ABE production on macroalgaes by *C. beijerinckii*

– *C. linum* and *U. lactuca* was found to be suitable substrates for ABE fermentation
– Total ABE production of 0.35 g/g glucose was achieved.
– Hydrothermal pretreatment seems more favorable than wet oxidation.
  • No significant difference was observed in ABE fermentation between hydrothermal pretreated samples (S2-S5; UB, LB) at the different pretreatment temperatures.
  • ABE fermentation was significantly affected negatively by increasing the temperature of wet oxidation pretreatment (S11-13).
– ABE fermentation was negatively not influenced when hemicellulose hydrolysate was used (S5) instead of water (LB) on *C. linum*, proving the absence of inhibitors´effect on the process.

Acknowledgement

The work was financially supported by the PSO Project (Energy Production from Marine Biomass: 2008-1-0050). Sune Tjalfe Thomsen is thanked for providing hydrolysates, Ingelis Larsen and Tomas Fernqvist are thanked for technical assistance.

As shown in Figure 2. inhibitor concentrations of hemicellulose hydrolysate did not effect ABE fermentation at hydrothermal pretreated samples, while wet oxidezed algaes were strongly influenced. Effect of inhibitors was also evident on the growth.

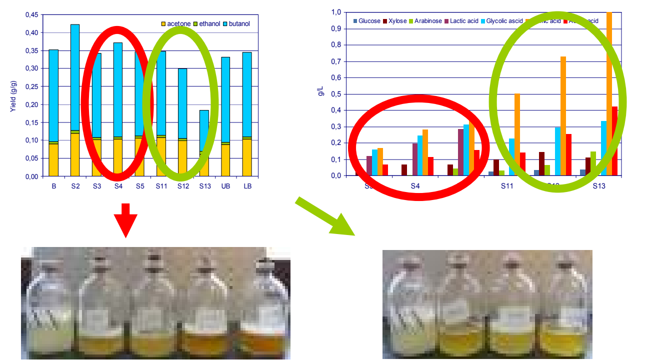


Figure 2. Connection between ABE production, inhibitor content of hemicellulose hydrolysates and growth.

FUTURE PLANS

Future studies need to address:
- Investigation of additional enzymatic hydrolyzis of macroalgaes.
- Examination of saccharolytic activity of *C. beijerinckii* on macroalages.
- Performing simultaneous hydrolyzis and ABE fermentation.

Cultivation of *Ulva lactuca*
on pig manure for simultaneous bioremediation and biomass production

M.M. Nielsen[1] (meni@dmu.dk), A. Bruhn[1], M.B. Rasmussen[1] and B. Olesen[2]

[1]National Environmental Research Institute, Aarhus University, Silkeborg, Denmark
[2]Biological Institute, Aarhus University, Aarhus, Denmark

Aim

With the binary perspective of **simultaneous bioremediation of agricultural wastewaters and biomass production**, we wanted to assess the potential of liquid pig manure as nutrient source for cultivation of the green macroalgae *Ulva lactuca*. We focus on optimizing the potential of the algae for growth and bioremediation.

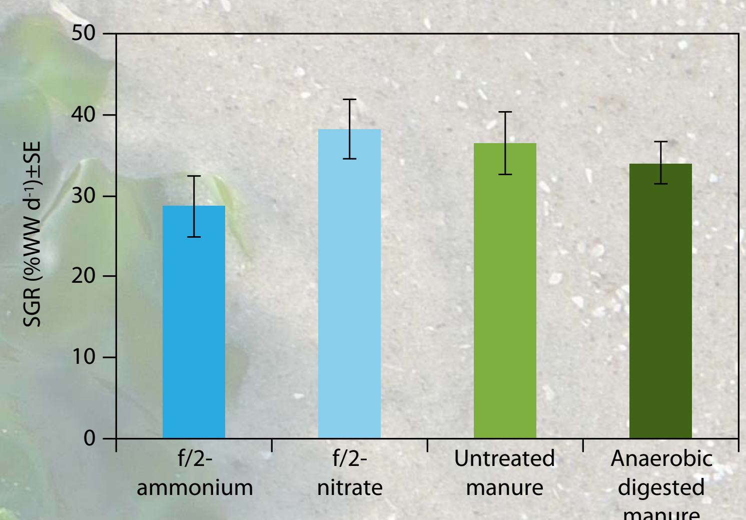
Results

We tested two types of liquid pig manure; untreated and anaerobic digested, against standard f/2 media [a] with either ammonium or nitrate as nitrogen (N) source. ***U. lactuca* had as high growth rates with both manure types as with f/2 medias (figure 1).**

Algae grown with anaerobic digested manure had significant higher bioremediation efficiency than algae grown with untreated manure (ANOVA; p<0.001). Bioremediation efficiency was defined as the amount of nutrients (N and P) incorporated in the algae tissue.

From these results we picked out the anaerobic digested manure for further research, since this product has already been exploited for energy purposes once, which gives a surplus in the overall energy balance, compared to the untreated manure.

The relation between specific growth rate (SGR) and ammonium concentration was tested with anaerobic digested manure as the only nutrient supply for the algae. **An ammonium concentration of 50 µM, was found to be sufficient for reaching the maximum growth rate of 44.8 % (figure 2)** (this corresponds to an approximately 8000 times dilution of the manure).



Figur 1. Average growth rates (%WW d^{-1}±SE) for the entire experimental period of 15 days shown for the four treatments. *U. lactuca* grown with f/2-media with ammonium as N source showed significant lower growth rate than the rest (p<0.01). WW= wet weight.

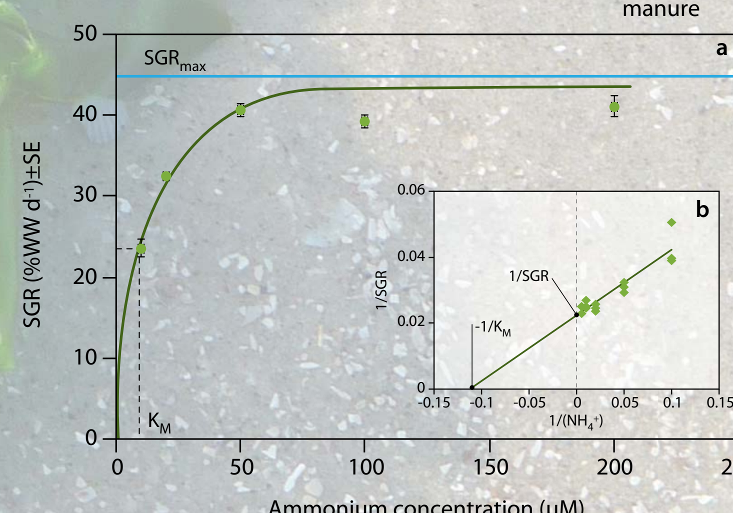


Figure 2. a) Plot of the specific growth rate (SGR)±SE as a function of the ammonium concentration. Maximum specific growth rate (SGR_{max}) is approached asymptotically and found to be 44.8%. The ammonium concentration where half SGR_{max} is reached (K_M) is 8.9 µM.
b) A double-reciprocal or Lineweaver-Burk plot generated by plotting 1/SGR as a function of 1/[NH_4^+]. From this SGR_{max} and KM is estimated. Each point represents independent replica. Statistics of the fitted line is performed with SAS (p<0.0001; R^2=0.86). [NH_4^+] = ammonium concentration.

Conclusion

- Use of manure as a future nutrient supply for commercial algae cultivation holds a promising potential.
- There is no immediate evidence that the biochemical composition of the algae changes as a response to the manure exposure.
- The bioremediation efficiency of *U.lactuca* is high, which makes it interesting to look at the possibilities for incorporating algae cultivation in modern agriculture on a local scale.
- Further research is needed on the issue of ammonia volatilization to reduce the negative environmental impact this might lead to.

Perspective of bioremediation

Tissue of *U. lactuca* grown with anaerobic digested manure contained 5.70% nitrogen (N) and 0.44% phosphor (P), which makes the opportunities for exploiting the algae for bioremediation purposes very promising. In Denmark an achievable yearly production of *U. lactuca* is estimated to be 45 T DW ha^{-1} [b]. Combining these numbers makes it possible to remove 2500 kg N ha^{-1} $year^{-1}$, corresponding to 550 tons of anaerobic digested manure, and 200 kg P ha^{-1} $year^{-1}$. For continuously cultivation with manure as only nutrient supply, extra P addition might be needed.

Ammonia volatilization

Since not all of the removed ammonium could be recognized in the algal biomass, we investigated the role of ammonia volatilization and found it to account for 30% of the total loss of ammonium from the media. An emission of ammonia this high might be a problem, due to its pollution potential and therefore we recommend this to be further investigated before exploiting manure in large scale cultivation of *U. lactuca*.

Acknowledgement

The project is supported by Region Midtjylland and EU in cooperation with Havets Hus, Kattegatcentret, Danish Technological Institute and DONG Energy.

Literature cited

[a] Guillard, R.L.L & J.H Ryther (1962). Studies of marine planctonic diatoms. I. Cyclostella nana Hustedt, and Detonula con fervacae Cleve. Canadian Journal of Microbiology. 8:229-239.

[b] Bruhn A, Dahl J, Nielsen HB, Nikolaisen LS, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD. (2010). Bioenergy potential of *Ulva lactuca*: growth yield, methane production and combustion. Bioresource Technology. Doi: 10.1016/j.biortech.2010.10.010.

33rd Symposium on Biotechnology for Fuels and Chemicals
2-5 May, 2011, Seattle, WA, USA

Combined Acetone-Butanol-Ethanol (ABE) and biogas production from macroalgaes

Zsófia Kádár, Martin Malthe Borch, Henrik Bangsø Nielsen, Jens Ejbye Schmidt

NRG program, Biosystems Division,Risø National Laboratory for Sustainable Energy,
Technical University of Denmark, Frederiksborgvej 399, DK-4000 Roskilde

ABSTRACT

Butanol as a liquid biofuel can provide more benefits than ethanol, due to its gasoline-like properties. It can be produced from the same feedstocks as ethanol (starch and cellulosic sugars) but the butanol producing *Clostridia sp.* is able to ferment different kind of carbohydrates including C6 and C5 sugars.
Macroalgaes can grow on non-agricultural land, without increasing food prices, using fresh water, meanwhile consuming CO_2 for growing. In addition, it has very high biomass yield with high carbohydrate content and represent a huge unexploited bioresource with potential for production of biofuel in the near future.
The aim of our studies was to examine a combined biorefinery concept with butanol and biogas production. The effluent as a substrate was further studied in batch experiments by anaerobic digestion for biogas production.

THE CONCEPT

Algae → Pretreatment (hydrothermal/sterilization) → Solid → Enzymatic hydrolysis → ABE Fermentation → Biogas

What about the liquid…

SUBSTRATES

Chaetomorpha linum and *Ulva lactuca* (both harvested in Denmark) were used in our experiments.

RESULTS – ABE fermentation

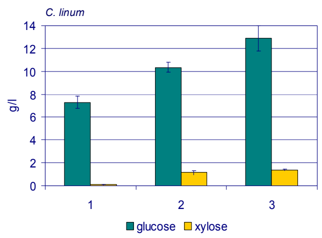
Enzymatic hydrolysis (EH) experiments

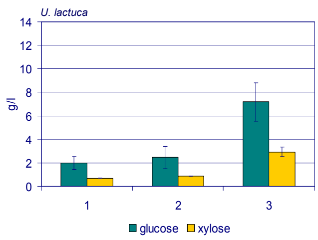
EH was performed on hydrothermal pretreated (195°C, 10 min, without oxygen) *U. lactuca* and *C. linum* at 5% DM content to find the best enzyme mixtures:

1, Cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM
(Hydrolysis at 50°C pH4.8)

2, Cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM and Spirizyme
(Hydrolysis at 50°C pH4.8)

3, Liquozyme and cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM and Spirizyme
(Hydrolysis at 85°C for 1h at pH5.7 followed by additional cellulases and Spirizyme at 50°C, pH 4.8

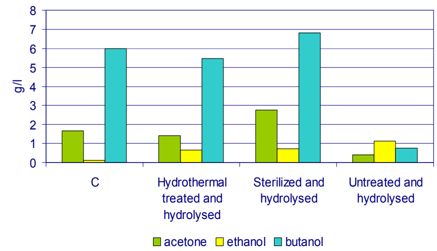




The highest final glucose content (13 and 7 g/l, respectively) was achieved when pretreated macroalgaes were hydrolyzed by Liquozyme at 85°C for 1h at pH 5.7 followed by hydrolyzis at 50°C, pH 4.8 applying Celluclast, Novozym 188 and Spirizyme.

Pretreatment

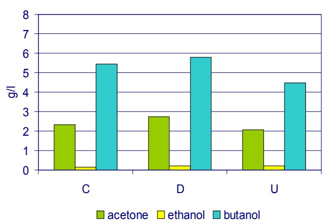
Further studies aimed to test sterilization (121°C, 20 min) as a pretreatment method on dried *U. lactuca*. Enzymatic hydrolysis was performed with enzyme mixtures, according to our earlier studies described above. The hydrolysate was further used for ABE fermentation (*C. beijerinckii* under anaerobic conditions at 35°C) with additional glucose to reach the initial 30 g/l glucose content.

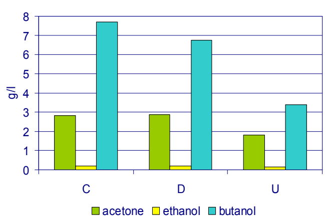


Final total solvents production results was significantly higher (40%) compare to hydrothermal pretreated algae.

Inhibitory studies

Liquid fractions of pretreated macroalgaes were also tested to check any inhibitory effect. The liquid fraction was supplemented with additional glucose (30 g/l), salts and nutrients. Fermentations were performed on diluted (D, 50%) and undiluted (U) liquid fractions with *C. beijerinckii* under anaerobic conditions at 35°C.





According to our results compare to control synthetic medium (C), undiluted samples (U) showed some inhibitory effect on ABE fermentation, however detailed investigation was not performed to identify inhibitors.

Biogas production

The biogas trials with the effluent from ABE fermentation will be carried out in batch wise in 500mL flasks with cattle manure as inoculum. The anaerobic digestion will take place in thermophilic conditions (52°C) for approximately a month. The total methane production will measured with Automatic Methane Potential Test System (AMPTS), from Bioprocess Control AB, Lund, Sweden. In the AMPTS, CO_2 and H_2S are stripped in a NaOH bath, and the volume of the remaining pure methane is measured continuously by liquid displacement in individual flow cell units for e…

CONCLUSIONS

- Macroalgaes are certainly interesting substrates in a biorefinery concept due to their high carbohydrate content.

FUTURE PLANS

Future studies need to address:
- Finalize biogas experiments.
- Examination of saccharolytic activity of *C. beijerinckii* on macroalages.
- Performing simultaneous hydrolyzis and ABE fermentation.

Acknowledgement

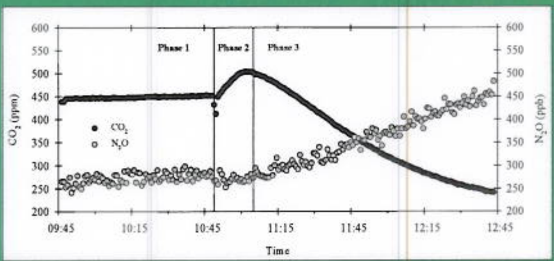
The work was financially supported by the PSO Project (Energy Production from Marine Biomass: 2008-1-0050). Annette Eva Jensen, Ingelis Larsen and Tomas Fernqvist are thanked for technical assistance.

GHG balance of *Ulva lactuca*

- *CO_2 uptake vs. N_2O emission*

- Macroalge uptake of CO_2 and biomass yields are high
- Sustainability assessments investigates total GHG balance
- Are there any counterbalancing GHG associated with growing algae?
- Release of 1 N_2O equals the uptake of 296 CO_2
- This study investigated CO_2 uptake and N_2O emission in *Ulva*

- Online laboratory measurements of N_2O and CO_2 in a closed system
- Traditional gaschromatography validated the measured N_2O values
- Significant N_2O emission occured along with CO_2 uptake
- High substrate availability and light was needed to drive N_2O emission



- Phase 1: Online CO_2 and N_2O measured on seawater and light only
- Phase 2: Algae is added and system is open
- Phase 3: Photosynthetic CO_2 uptake (black) and N_2O release (grey)
- Light induction suggest algae to mediate the N_2O emission

- N_2O emissions could not be detected in large scale growing systems
- Deployment of transparent GHG chamber at Alge Center Denmark, a large scale research and algae growing facility hosted by Aarhus University

- Living vital algae may contribute with N_2O emissions under certain circumstances
- The exact mechanism is pending further research
- Sustainability assessments must take into account N_2O emissions

Contact: Risø-DTU; Biosystems; Kristian Rost Albert kria@risoe.dtu.dk and Per Ambus peam@risoe.dtu.dk
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Dyrkning af søsalat på gylle

Ulva lactuca

Mette Nielsen[1], Annette Bruhn[1], Birgit Olesen[2], Michael Bo Rasmussen[1], Kitte Gerlich[1], Tanja Quottrup[1], Egon Frandsen[1] og Henrik B. Møller[3]

[1]Danmarks Miljøundersøgelser, Aarhus Universitet
[2]Biologisk Institut, Aarhus Universitet
[3]Det Jordbrugsvidenskabelige Fakultet, Aarhus Universitet

Baggrund

Traditionel dyrkning af alger på kunstige næringsmedier er omkostningsfuldt, og der er behov for alternative næringsmedier. I denne sammenhæng er gylle et oplagt valg, grundet dets høje næringsværdi. Derudover er tilgængeligheden stor, og der savnes alternative anvendelsesmuligheder for produktet.

Formål

I dette studie ønsker vi at afklare, hvorvidt gylle er en brugbar næringskilde i konventionel algedyrkning med fokus rettet mod den hurtigvoksende grønalge *Ulva lactuca*, der er en lovende kandidat til produktion af bioenergi.

Vækst, struktur og evne til at fjerne kvælstof blev sammenlignet hos alger dyrket på gylle og alger dyrket på kunstigt næringsmedie (f/2 (Guillard & Ryther 1962)) med henholdsvis nitrat og ammonium som kvælstofkilde. Der blev arbejdet med to gylle-typer: ubehandlet svinegylle og afgasset gylle, dvs. en gylle, der har passeret et biogasanlæg.

Perspektivering

En realistisk produktion på 45 tons alger (tørvægt) pr. ha pr. år (Bruhn, et al., 2010) og et N-indhold på 5 % gør det muligt at fjerne 2.250 kg N (svarende til 500 tons gylle) pr. ha pr. år. Et større landbrug med en årlig gylleproduktion på f.eks. 8.000 tons vil dermed med 8 ha algedyrkningsbassiner i teorien kunne rense 50 % af dets gylle for N og samtidig opnå en årlig biomasseproduktion på ca. 360 tons (tørvægt). Denne biomasse vil evt. kunne erstatte mindre produktive energiafgrøder som majs og græs i et biogasanlæg.

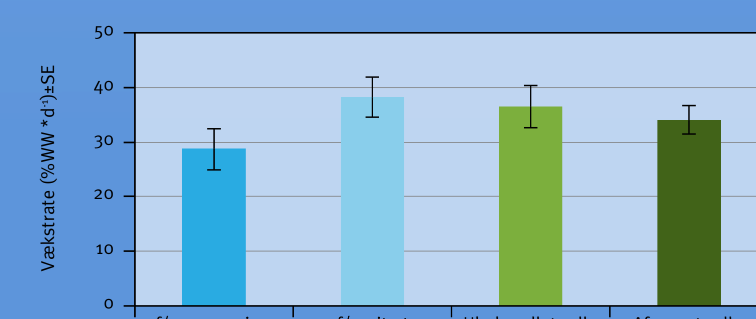
Konklusion

- Brugen af gylle som fremtidig næringskilde til kommerciel algedyrkning har bestemt potentiale, da der hos alger dyrket på gylle er påvist vækstrater på fuld højde med alger dyrket på kunstige næringsmedier.
- Brug af gylle som næringsmedie påvirker ikke umiddelbar algernes strukturelle sammensætning. Igangværende undersøgelser søger yderligere at afklare, hvorvidt algernes indhold af tungmetaller, proteiner og kulhydrater påvirkes ved brug af gylle som næringsmedie.
- Fremadrettede studier vil koncentreres omkring den afgassede gylle, hvor det søges at afklare den optimale gyllekoncentration i forhold til algernes vækst og biokemiske sammensætning.

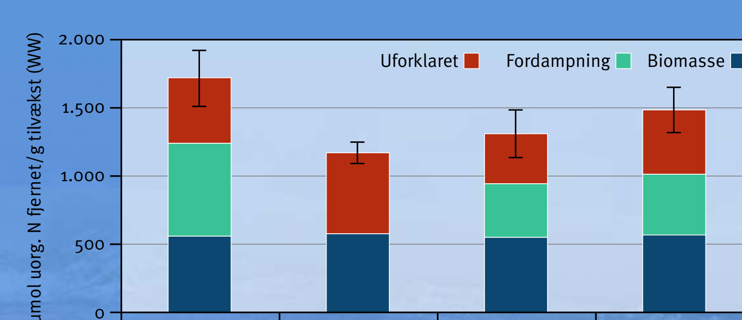
Resultater

- Gyllen i sig selv kunne fungere som næringsmedie uden tilsætning af hverken vitaminer eller sporstoffer.
- Algerne dyrket med gylle som næringskilde opnåede mindst ligeså store vækstrater som algerne dyrket på det kunstige næringsmedie. Vækstraterne varierede fra negative til 66 % per dag, men over hele perioden sås en gennemsnitlig vækstrate på omkring 30-35 % per dag (figur 2).
- Algerne i de fire behandlinger inkorporerede lige store mængder N i deres væv. Derimod var der en lille forskel mellem behandlingerne på den totale mængde uorganiske kvælstof, der forsvandt. Disse forskelle kan øjensynligt tilskrives forskelle i ammoniakfordampning (figur 3).
- Strukturelle parametre som vandindhold og N-indhold adskilte sig ikke mellem behandlingerne. De gennemsnitlige værdier var på henholdsvis 85 % og 5,3 %.

Figur 1. Udsnit af forsøgsopstillingen (n=4). Gyllebehandlingerne er ca. 400 gange fortyndet svarende til en ammoniumkoncentration på omkring 300 μM. Vandet blev udskiftet hver anden dag for at undgå næringsbegrænsning.



Figur 2. Den gennemsnitlige vækstrate (%WW*d^{-1} ± SE) over hele perioden (15 dage) for de fire behandlinger. Alger dyrket på f/2-medie med ammonium som kvælstofskilde havde signifikant mindre vækstrate end de øvrige ($p < 0,01$). WW = wet weight.



Figur 3. Den fjernede mængde uorg. N pr. g tilvækst (WW) fra de fire behandlinger fordelt på de tre poster: biomasse = den del, der via CN-analyser kan dokumenteres inkorporeret i biomasen, fordampning = den estimerede fordampning ud fra et særskilt forsøg, uforklaret = den del, der forsvinder, men ikke kan påskrives de øvrige poster. Denne del skal sandsynligvis påskrives aktivitet af mikroalger og bakterier. SE-barene er beregnet på den totale mængde fjernet uorg. N.

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Referencer

Guillard, R.L.L. & Ryther, J.H. (1962). Studies of marine planctonic diatoms. I. Cyclostella nana Hustedt, and Detonula con fervacae Cleve. Canadian Journal of Microbiology. 8:229-239.

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