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*Publication date:*  
2011

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Kádár, Z., Borch, M. M., Bangsø Nielsen, H., & Schmidt, J. E. (2011). Combined Acetone-Butanol-Ethanol (ABE) and biogas production from macroalgae. Poster session presented at 33rd Symposium on Biotechnology for Fuels and Chemicals, Seattle, WA, United States.

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

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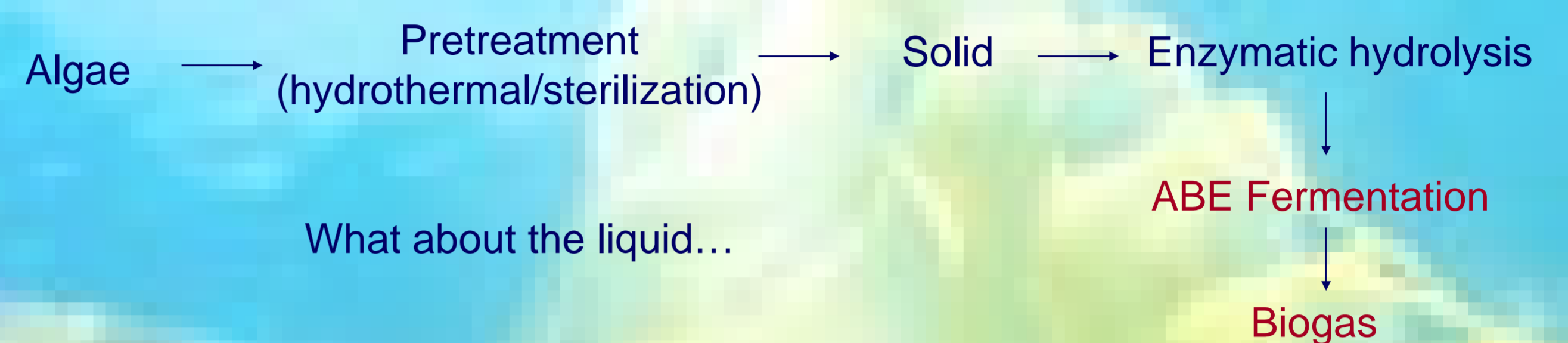
## ABSTRACT

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.

Macroalgae can grow on non-agricultural land, without increasing food prices, using fresh water, meanwhile consuming CO<sub>2</sub> 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



## SUBSTRATES

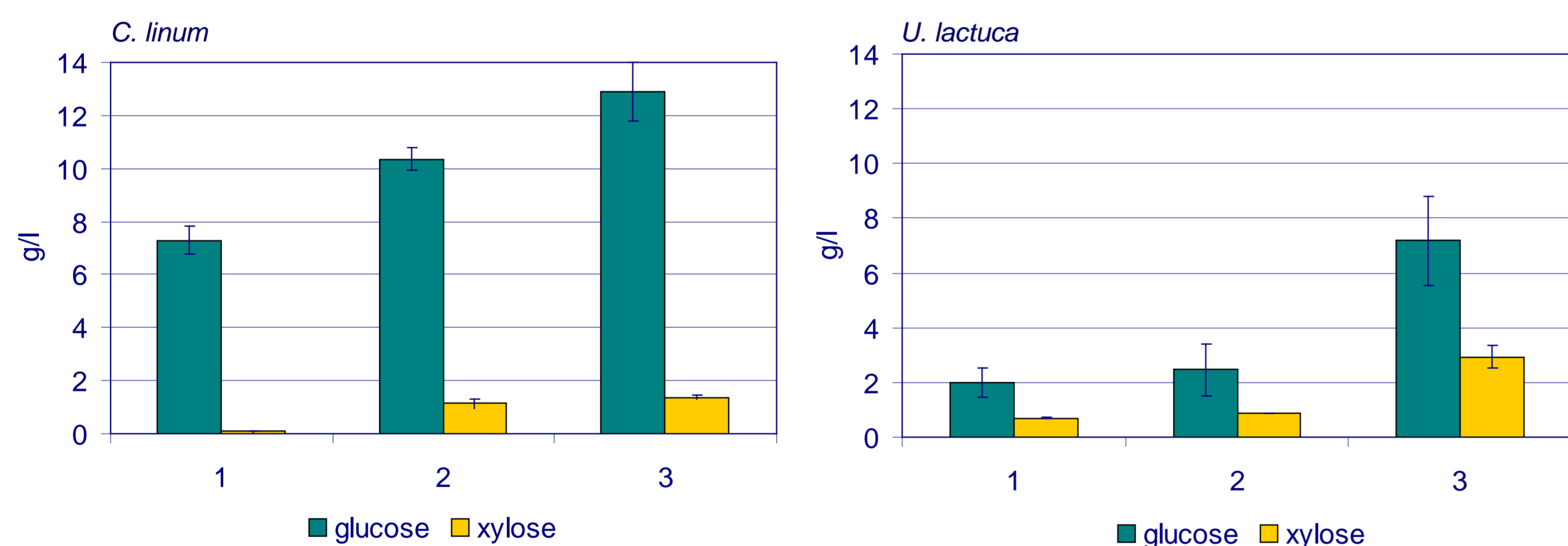
*Chaetomorpha linum* and *Ulva lactuca* (both harvested in Denmark) were used in our experiments.

## 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:

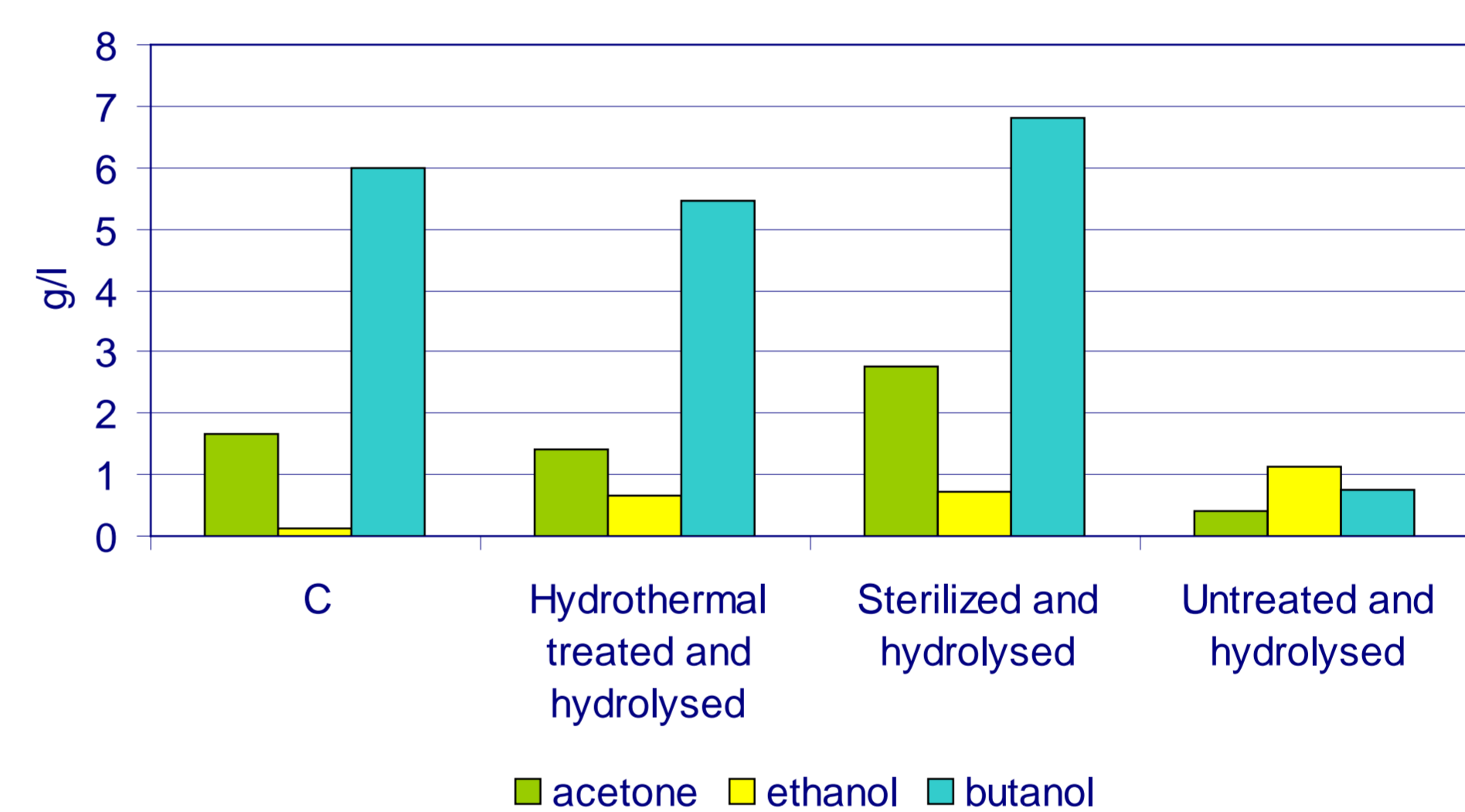
- 1, Cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM (Hydrolysis at 50°C pH4.8)
- 2, Cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM and Spirizyme (Hydrolysis at 50°C pH4.8)
- 3, Ligozyme and cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM and Spirizyme (Hydrolysis at 85°C for 1h at 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 macroalgae were hydrolyzed by Ligozyme at 85°C for 1h at pH 5.7 followed by hydrolysis at 50°C, pH 4.8 applying Celluclast, Novozyme 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.

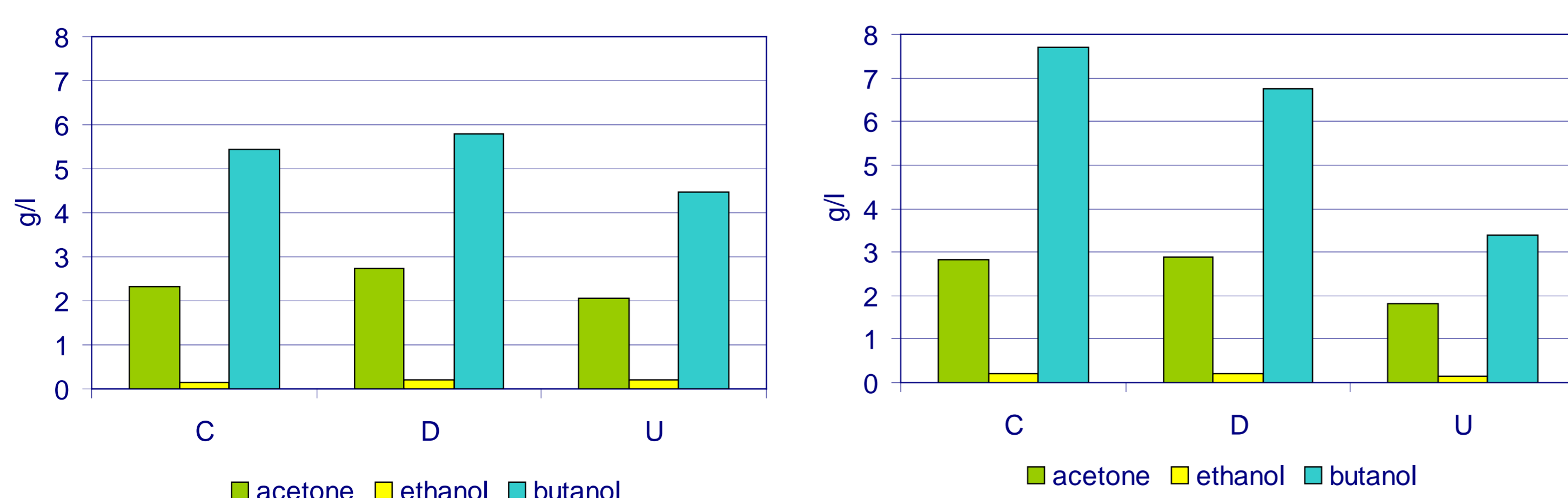
### Biogas production

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<sub>2</sub> and H<sub>2</sub>S 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 each batch.



### Inhibitory studies

Liquid fractions of pretreated macroalgae 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.

## CONCLUSIONS

- Macroalgae 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 macroalgae.
- Performing simultaneous hydrolysis 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.