Enzymatic hydrolysis of *Chlorella pyrenoidosa* biomass with high solids loadings

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Introduction

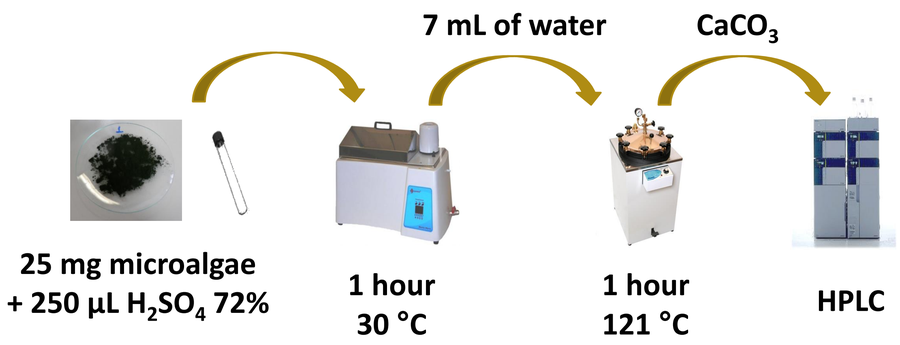
Microalgae has been considered a promising biomass for the production of biofuels and chemicals due to its high biomass productivity and its content of carbohydrates, lipids, proteins and carotenoids. Usual extraction methods use toxic solvents that do not fit in the principles of green chemistry. Enzymatic hydrolysis is shown as a good method for producing reducing sugars and might be used as a preliminary step in the fractioning of microalgae biomass.

Our goal

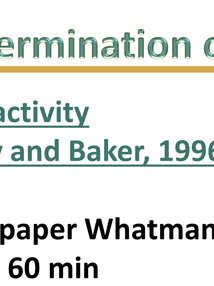
To evaluate and optimize the enzymatic hydrolysis of the biomass of a commercial lyophilized *C. pyrenoidosa* using enzymes produced by *Trichoderma reesei* RUT C30 and/or *Aspergillus awamori.*

Materials and Methods

C. pyrenoidosa total sugar content (Van Wychen & Laurens, 2013)



Ball mill treatment



- Vibratory ball mill (Fritsch, Germany)
- 90 minutes
- Amplitude: 1.5 mm

Enzymatic hydrolysis of algae biomass

- 5% biomass consistency
- 10 FPU/g biomass; 20 or 30 BGU/g biomass*
- Glucose determination: Biochemical Analyzer (YSI 2700)

* Chosen based on Rodrigues and Bon (2011)

Optimization of enzymatic hydrolysis

Coded and real levels of independents variables					
	-1.68	-1	0	+1	+1.68
% solids	1.23	6	13	20	24.77
FPU	1.23	6	13	20	24.77
BGU	1.00	16	60	38	75.00

Determination of enzyme activity

FPase activity (Adney and Baker, 1996)

- Filter paper Whatman n°1
- 50 °C, 60 min
- Reducing sugars by DNS method (Sumner, 1924)

β-glucosidade activity (Ghose, 1987)

- Cellobiose solution (15mM)
- 50 °C, 30 min
- Glucose determination on Biochemical Analyzer (YSI 2700)

Results

Composition of *Chlorella pyrenoidosa* cell wall

Table 1 – Carbohydrate composition of *Chlorella pyrenoidosa* determined by acid hydrolysis

	glucose	xylose	galactose	arabinose	mannose	Total
(%) of total biomass	9.30	0.34	3.62	1.11	0.56	14.92%
(%) of total sugars	62.32	2.27	24.27	7.40	3.74	100%

Standard deviation lower than 5%

Enzymatic Hydrolysis Time Course

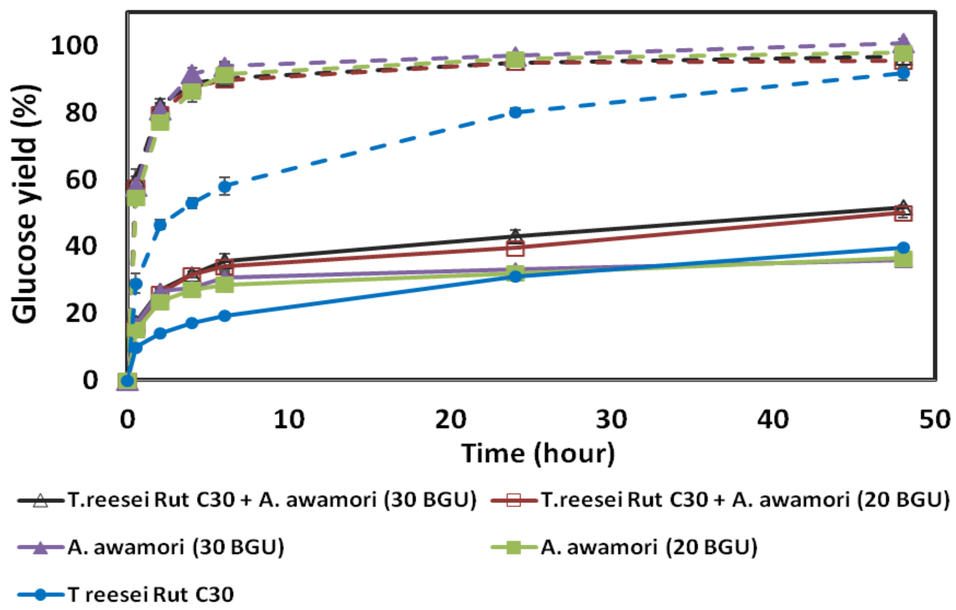


Fig. 1 – Glucose and reducing sugars yields of enzymatic hydrolysis of whole microalgae biomass (full lines) and after ball mill pretreatment (dotted lines) with enzymes from *T. reesei* RUT C30, *A. awamori* and blends of both enzymatic preparations. Figure shows glucose yield in relation to total theoretical glucose.

Optimization of enzymatic hydrolysis

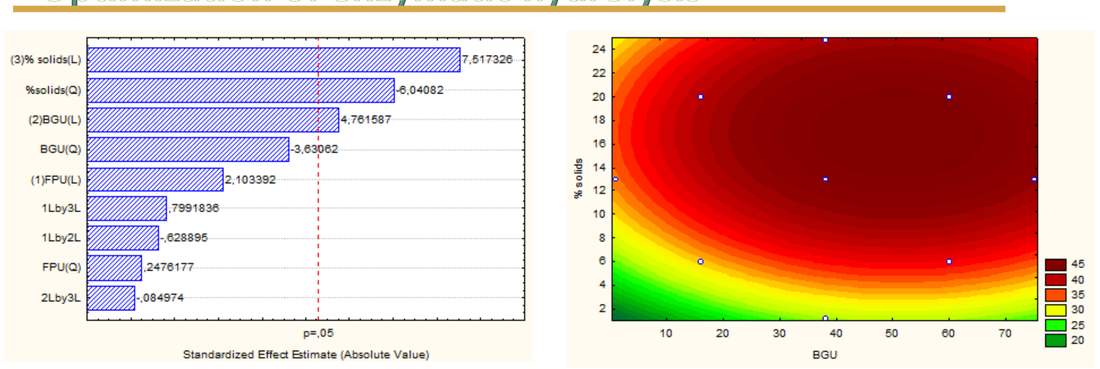


Fig. 2 – Pareto chart and fitted surface generated by Statistica 7.0 for DCCR regarding influence of % of solids, FPU and BGU loadings on glucose yields after a 24-hour enzymatic hydrolysis of whole *C. pyrenoidosa* biomass

	Critical values
% solids	15.2
FPU	-8.6
BGU	58

	Glucose yield (%)
Predicted	44.2
Observed	47.2

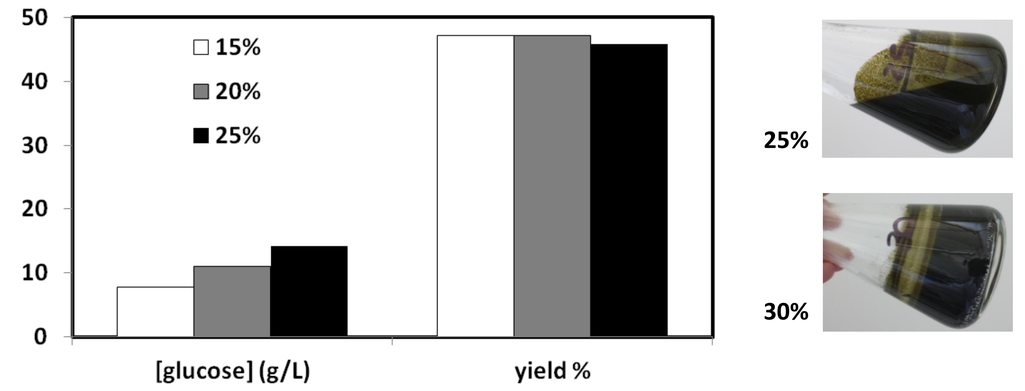


Fig. 3 – Glucose concentration and yield after a 24-hour enzymatic hydrolysis of *C. pyrenoidosa* with increasing solids percentage (15-25%) using 58 BGU/g glucan and 13 FPU/g glucan. Photos on the right show that solids percentage higher than 25% are not liquefied within 24 hours of hydrolysis.

Table 2 – Summary of conditions and results before and after optimization of enzymatic hydrolysis of whole *C. pyrenoidosa* biomass

	% solids	glucose yield (%)	glucose (g/L)	UI/ glucose produced
Initial	5	39.59	1.94	19.30
Optimized	25	45.87	14.22	2.99
Difference	400%	15.87%	631.9%	- 84.5%

Conclusions

- Ball milling was an effective pretreatment for the hydrolysis of the cellulosic content of the algal biomass, greatly increasing hydrolysis yields;
- Enzymatic hydrolysis was succesfully performed by *A. awamori* enzymes (EG's and β-glucosidase), showing that exoglucanases are not necessary for *Chlorella pyrenoidosa* hydrolysis;
- Optimization allowed for an increase of 600% in final glucose concentrations with a reduction of more than 80% in the amount of enzyme used per gram of glucose produced.

References:

- Adney, B, Baker, J. 1996. Measurement of cellulase activities. National Renewable Energy Laboratory
- Ghose, T.K. 1987. Measurement of cellulase activities. Pure & Appl. Chem. 59(2), 257-268
- Rodrigues, M.A. and Bon, E.P.S. 2011. Evaluation of Chlorella (Chlorophyta) as Source of Fermentable Sugars via Cell Wall Enzymatic Hydrolysis. Enzyme Research
- Sumner, J.B. 1924. The estimation of sugar in diabetic urine using dinitrosalicylic acid. J. Biol. Chem. 62(2), 287-290
- Van Wychens, S., Laurens, L.M.L. 2013. Determination of Total Carbohydrates in Algal Biomass. Laboratory Analitical Procedure. NREL

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