Pretreatment methods for the production of fermentable sugars from algal dried biomass

E. Koutsaftis, P. F. Chatzimaliakas, E.M. Barampouti, S. Mai, D. Malamis

National Technical University of Athens, School of Chemical Engineering, Unit of Environmental Science & Technology, 9 Iroon Polytechniou Str., Zographou Campus, GR-15780 Athens, Greece
Keywords: Algae, Cellulose, Enzymatic hydrolysis, Saccharification yield, Starch.
Presenting author email: fotischatzimaliakas@gmail.com

Introduction

The rapid growth of the world population and emerging economies has led to a sharp increase in global energy consumption (Harun et al. 2010). The total energy demand is approximately 200 million barrels per day of oil equivalent. This demand represents a fivefold surge compared to the demand in 1950, with over 80% of this increased demand being met with the use of fossil fuels. More specifically, 60% is covered by oil and gas, while biomass consumption accounts for only 11%, and nuclear energy contributes a share of 6.4% (Kusmiyati, Hadiyanto, and Fudholi 2023). Algae is an interesting source of biomass due to its characteristics i.e., high carbohydrate and low lignin contents. This biomass could be considered as a third-generation feedstock to produce biofuels, especially bioethanol (Kumar et al. 2020). The scope of this study is to investigate several pretreatment methods for maximizing the yield of the sugars produced via enzymatic hydrolysis in order to stand a suitable substrate for ethanolic fermentation.

Materials and Methods

The dried algal biomass utilized for this study was kindly provided by AlgEn (Slovenia). It was received at the Unit of Environmental Science and Technology (UEST), National Technical University of Athens, Greece where it underwent a series of pretreatment methods for maximizing the concentration of sugars produced via enzymatic hydrolysis. The moisture content of the material received was 8.04% determined by KERN DAB (moisture analyzer). The composition of the substrate was estimated in % w/w dry basis as: cellulose 9.22 ± 0.57, hemicellulose 17.69 ± 1.52, starch 1.78 ± 0.16, fats and oils 0.95, acid soluble lignin 1.07 ± 0.07, acid insoluble residue 26.72 ± 4.38, water soluble solids 12.25 ± 0.00, volatile solids 65.79 ± 0.66 (Chatzimaliakas et al. 2023).

All chemicals utilized were of analytical quality. Novozymes (Denmark) kindly supplied us with Spirizyme XL and CelliCtec3. The activities of Spirizyme XL and CelliCtec3 were quantified at 2337 U/mL and 171.7 FPU/mL (Zhong et al. 2022), respectively. The enzyme dose remained constant throughout the experiments at 45μL/g_{starch} and 500 μL/$g_{cellulose}$ based on preliminary experiments. In accordance with the NREL laboratory analytical procedures outlined by (Sluiter et al. 2008), structural carbohydrates and lignin content were assessed in both the raw and treated biomass. For the determination of sugars produced by the saccharification process, HPLC (HyperREZ™ XP Carbohydrate H^+) analysis was employed, and all analyses were conducted in duplicate.

Experimental procedure

Due to the lignin content of the biomass, a series of physicochemical pretreatments were carried out in 250mL boro-bottles with 100mL final volume. The solid loading was 10% w/w for all experiments to maintain comparability of results. Three different pretreatment techniques were employed in this research study.

(A) Hydrothermal pretreatment using an autoclave (ISOLAB Laborgerate GmbH) at 121°C for 30 minutes.
(B) The samples were treated in a water bath at 90°C for 75 minutes.
(C) The samples were ultrasonicated at 150W for 10 minutes using an ultrasonic probe (Branson Ultrasonics™ Sonifier™ SFX550 Cell Disruptor).

For each pretreatment three different cases were examined as solvents:

(1) Distilled water
(2) Alkaline solution using NaOH (0,2M)
(3) Acid solution using H_2SO_4 (1% v/v).

An experiment using just distilled water was conducted as blank.

After the pretreatment, the pH of each sample was set to approximately 5.5 and the enzymes were added to the mixture for the saccharification process. The enzymatic hydrolysis time was set to 72 hours. In addition, the glucose concentration was monitored throughout the 72-hour period of each experiment and the maximum glucose concentration was recorded.

Results

The saccharification yields along with the degradation of solid and the main carbohydrates are presented in the following (Table 1). The results of the saccharification process appeared to be very encouraging compared with other similar studies of Ho et al. 2013 and Kusmiyati et al. 2023, with a maximum yield of 85.73% and 9.8 g/L glucose concentration using 90ºC water bath and 0.2M NaOH, in comparison with 7.78 g/L according to the study of Mr. Ho.

Table 1. Saccharification yields and degradations of selected pretreatment methods (each letter refers to the pretreatment method mentioned above and each number to the solvent respectively).

Experiment	A.1	A.2	A.3	B.1	B.2	B.3	C.1	C.2	C.3
Saccharification Yield (%)	31.15	79.98	38.75	0.55	85.73	23.44	1.22	1.17	25.64
Degradation of Solid (%)	22.71	38.17	16.13	20.52	11.58	36.73	20.52	11.58	12.33
Degradation of Starch (%)	77.00	96.35	72.79	70.21	92.48	84.11	90.18	89.52	80.64
Degradation of Cellulose (%)	72.98	92.31	39.17	77.04	92.72	24.07	91.13	80.12	59.30

Regarding the degradation of solid, the maximum value recorded was 38% as shown in the table above. This result was achieved with the use of NaOH and autoclave at 121ºC. Furthermore, the breakdown of polysaccharides i.e., cellulose and starch proved to be quite high in most cases, over 80%. However, a high saccharification yield was only achieved in a few experiments (A.2 and B.2).

Conclusions

To sum up, the overall performance of the pretreatment methods on the saccharification process is considered to be quite satisfactory, especially for the experiments A.2 and B.2 with the use of NaOH. However, considering the scalability of the future fermentation process, the use of 90ºC water bath would favor the overall process.

Acknowledgements

This project has received co-funding from the European Union's Horizon 2022 research and innovation programme under grant agreement No 101084405 (CRONUS).

References

Chatzimaliakas, Fotis et al. 2023. “Piloting Bioethanol Production from Source-Separated Food Waste Boosts Technology Readiness.” *Sustainability* 15(23): 16349.

Harun, Razif, Michael K. Danquah, and Gareth M. Forde. 2010. “Microalgal Biomass as a Fermentation Feedstock for Bioethanol Production.” *Journal of Chemical Technology and Biotechnology* 85(2): 199–203.

Ho, Shih Hsin et al. 2013. “Bioethanol Production Using Carbohydrate-Rich Microalgae Biomass as Feedstock.” *Bioresource Technology* 135: 191–98.

Kumar, Manish et al. 2020. “Algae as Potential Feedstock for the Production of Biofuels and Value-Added Products: Opportunities and Challenges.” *Science of the Total Environment* 716.

Kusmiyati, Kusmiyati, Hadiyanto Hadiyanto, and Ahmad Fudholi. 2023. “Treatment Updates of Microalgae Biomass for Bioethanol Production: A Comparative Study.” *Journal of Cleaner Production* 383.

Sluiter, A et al. 2008. “Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP) (Revised July 2011).”

Zhong, Na et al. 2022. “Sulphite Addition during Steam Pretreatment Enhanced Both Enzyme-Mediated Cellulose Hydrolysis and Ethanol Production.” *Bioresources and Bioprocessing* 9(1): 1–9.