

ENERGETIC VALORIZATION OF A HETEROTROPHIC MICROALGA RESIDUE FROM BIODIESEL PRODUCTION THROUGH ANAEROBIC DIGESTION

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ABSTRACT

The production of biodiesel from the oil fraction of the heterotrophically grown microalga *Chlorella protothecoides* generated two waste streams: defatted microalga residues and crude glycerol. These wastes were anaerobically digested separately and in admixture to quantify their energy potential.

The defatted microalga residues (from the oil extraction process) were shown to be a suitable substrate for anaerobic digestion since they provided a methane yield of 498.4 mL CH₄/g volatile solids (VS). On the other hand, because glycerol acted as an inhibitory agent for methanogenesis, anaerobic co-digestion of defatted microalga residues and crude glycerol resulted in the diversification of the produced energy carrier. By using glycerol at a ratio of 1.24 g glycerol/g volatile solids of inoculum in admixture with microalga residues, hydrogen was obtained instead of methane. The resulting yield was 39.9 mL H₂/g VS of microalga residues. Based on these results, both effluents can be valorized through simple and sustainable methods in order to recover their energy potential in the form of methane or hydrogen.

Keywords: heterotrophic microalga residues; glycerol; anaerobic digestion; biorefinery; hydrogen; methane

INTRODUCTION

Bioresources are increasingly the target of research around the world mainly due to their potential for the mitigation of greenhouse gas emissions. Biodiesel is a fuel produced from biomass sources which is used as an alternative to fossil fuels to achieve lower emissions of carbon dioxide. Currently, biodiesel is produced from vegetable oils, animal fats, and waste cooking oils, but these sources do not provide the large production rates necessary to supply current demand. Microalgae can achieve a high production yield of oil per unit cultivation area (e. g., 9-fold higher than palm [1]) and is thus a promising alternative oil source for biodiesel production.

Following growth and harvesting, extraction of oil from microalgae results in two fractions. One is the oil itself that is subsequently converted to biodiesel through a transesterification process with methanol, generating glycerol as a by-product [2]. The other fraction is the defatted cellular components of the microalgae, mainly protein and carbohydrates. Both these and waste glycerol can be regarded as potential substrates for anaerobic digestion.

One aim of this work was to evaluate the energy contained in the defatted residues of the microalga *Chlorella protothecoides*, grown in heterotrophic conditions, recoverable through an anaerobic digestion process, in the frame of the biorefinery concept.

The glycerol obtained from the biodiesel production process, in admixture with the algae fed to the digestion process was also a subject of research. The effect of glycerol addition to the microalga residues on the anaerobic digestion process was evaluated as a means to selectively inhibit methane production, in order to establish an expeditious method to obtain bio-hydrogen.

METHODOLOGY

The microalga *Chlorella protothecoides* was chosen to produce biodiesel because this microalga can accumulate up to 60% of its dry weight in oil [3]. The microalga was grown in a fermenter in heterotrophic aerobic conditions in the dark, at 28 °C, during 5 days as described before [4]. *C. protothecoides* biomass was harvested by centrifugation, and the biomass pellet was freeze-dried, milled, and subsequently subjected to the process of oil extraction with hot n-hexane. The dried residue was then used for the anaerobic digestion experiments. The anaerobic sludge inoculum was obtained from the anaerobic digestion reactor of a municipal wastewater plant in Portugal.

EXPERIMENTAL SETUP

The anaerobic digestion assays were conducted in 71.5-mL batch vials, with 40 mL of suspension and a remaining initial headspace of 31.5 mL. The suspension comprised the anaerobic sludge inoculum (84% v/v) and different combinations of substrates: a control without substrate (D0), glycerol (D1), microalga residues (D2) and a mixture of glycerol and microalga residues (D3). All assays were performed in duplicate. The reaction mixtures were prepared under N₂, closed with butyl rubber stoppers and aluminum caps and placed in a thermostatic bath under mesophilic conditions at 37 ± 1° C, for a period of up to 50 days. No pH control chemicals were added and no other corrections or pretreatments were performed on the substrates.

ANALYTICAL METHODS

Total and volatile solids, gas volume and gas composition were determined according to standard methods [5]. All measured gas volumes are presented at standard temperature and pressure (STP) conditions, specifically 0 °C and 1 atm.

RESULTS

Gas production was observed in all anaerobic digestion vessels (Fig.1), except in D1. In D1 a maximum value of only 44.6 mL of biogas was obtained after 6 days, and from then on the biogas volume was unaltered up to the 50th incubation day. In contrast, in the other three digesters D0, D2 and D3 biogas production continued to increase until close to the 50th day.

The comparison of the accumulated gas volumes obtained in the experiments clearly suggests that microalga residues (D2) are a suitable substrate for anaerobic digestion, with a maximum of 240 mL of biogas accumulated after 50 days. It is also noticeable that the addition of glycerol to the microalga residues (D3) had a negative effect on the gas production, reducing it to 87 mL, a level comparable to that obtained in the control run, 70 mL.

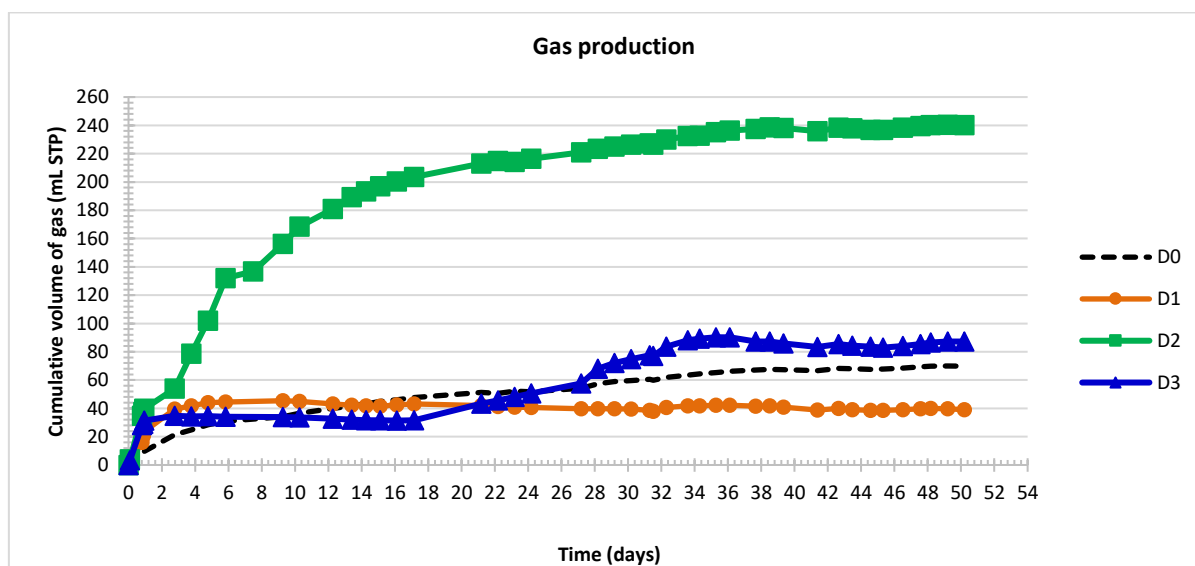


Fig. 1. Cumulative gas production in the anaerobic digestion experiments: D0-control without substrate, D1- glycerol, D2-microalgae residues, D3- glycerol and microalgae residues.

The increase in biogas volume in D2 was accompanied by an increase in its methane content up to a maximum of 65 % (v/v) obtained on the 10th day and maintained up to the 50th day. The control (D0) digester had a lower maximum methane content, 53 % (v/v), and the D1 digester registered a very low methane content in the produced biogas, 20 % (v/v). The lowest maximum methane content was observed in unit D3 with only 7 % on the 10th day, the methane content subsequently decreasing until it became undetectable on the 30th day. Simultaneously, starting on the 10th day, the hydrogen content increased until it reached a maximum of 16 % (v/v), on the 30th day. No hydrogen gas was formed in the other digesters.

CONCLUSIONS

The effluents generated in different steps of the process of biodiesel production from the oil content of the heterotrophic microalga *Chlorella protothecoides*, i.e., defatted alga residues and glycerol, are here shown to be promising feedstocks for renewable energy production through anaerobic digestion. The tested methodology additionally makes it possible to diversify the type of biofuel produced. While the digestion of microalga residues as a single substrate revealed a methanogenic potential of 498.4 mL CH₄/g VS of microalga residues, the combination of the two substrates showed a hydrogen production potential of 39.9 mL H₂/g VS of microalga residues. Innovative results were obtained in the latter case, namely in the utilization of glycerol as a selective inhibitor of methanogenesis.

References

- [1] Chisti, Y., Biodiesel from microalgae. *Biotechnol. Adv.* 25 (2007), 294–306.
- [2] Halim, R., Danquah, M.K., Webley, P.A, 2012. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnol. Adv.* 30 (2012), 709–32.
- [3] Xiong, W., Li, X., Xiang, J., Wu, Q., High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production, *Appl. Microbiol. Biotechnol.* 78(2008), 29–36.
- [4] Santos, C. A., Nobre, B., Lopes, T., Pinheiro, H. M., and Reis, A., Dual-mode cultivation of *Chlorella protothecoides* applying inter-reactors gas transfer improves microalgae biodiesel production, *Journal of Biotechnology* 184(2014), 74–83.
- [5] APHA, Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF, Washington, 2000.