PRODUCTION OF HYDROGEN BY DARK FERMENTATION OF MICROALGAE RESIDUES USING A THERMOPHILIC MARINE SEDIMENT AS INOCULUM

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Key words: microalgae, dark fermentation, hydrogen

Introduction. Renewable energy can contribute to reduce the global warming problem and fuel demand. Hydrogen is a clean fuel because during combustion it releases energy and the only product left is water. At the present 90% of the H_2 is generated by steam reforming from hydrocarbons, but this process emits CO_2 and is non renewable. One of the biological alternatives is the production by fermentation of residual sources. Residues of microalgae, remaining from lipid extraction for biodiesel production, are rich in fermentable-nutrients. The main goal of this work was to evaluate the potential of using residual microalgae biomass as substrate for hydrogen production using a thermophilic marine sediment as inoculum.

Methods.
Inoculum. The inoculum was marine sediment from a shallow hydrothermal vent in Nayarit.
Substrate and pretreatments. Biomass of the oleaginous microalga *Scenedesmus obtusiusculus* was used as substrate. Acid treatments varying the HCl concentrations (1.5% and 16%) [1,2], times of digestion (10-70 min) at a temperature of 120 °C were assayed to enhance the biomass biodegradability. Carbohydrates were quantified by reducing sugars (DNS).
Fermentation: Experiments were carried out in 125-ml serum bottles with 12 g of marine sediment and 70 ml of mineral medium with: a) pre-treated microalgae biomass (3.3 g/L); b) microalgae biomass without treatment, or c) glucose (used as control at 4.25 g/L). The bottles were sealed and flushed with N_2. The initial pH was 5. Experiments were incubated at 60°C and performed in triplicate. The production of H_2 was measured by TCD-gas chromatography, and COD [3] was used to determine the substrate consumption.

Results. As it can be observed in Table 1, pre-treatments with HCl 1.5% and 16% exhibit the highest COD and reducing sugars concentrations and Figure 1 shows the accumulated hydrogen production for those treatments.

Table 1. Pre-treatments of microalgae biomass

Treatment	Digestion time (Minutes)	COD_s (g/l)	DNS_s (g/l)
No-treatment	0	0.463	0.019
HCl 1.5%	20	4.87	0.294
HCl 16.2%	30	6.19	0.333
HCl 2% + $MgCl_2$ 2.5%	10	3.74	0.126
NaOH	480	3.95	0.02

The highest accumulated hydrogen was achieved with biomass pretreated with HCl 1.5%. It was twice and four times higher than H_2 obtained with glucose or the non-treated biomass, respectively. There was no H_2 production in experiment with biomass pretreated with HCl 16% due to the high salinity observed after pH adjustment.

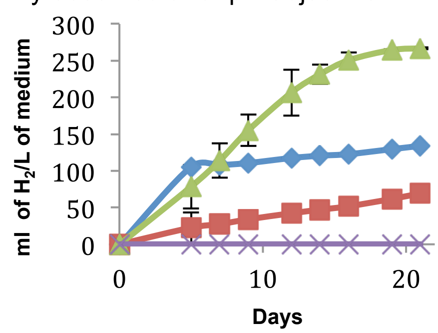

Fig.1 H_2 production for each experiment after 21 days of fermentation ◆glucose,■ non-treated biomass, ▲ HCl 1.5%, **X** HCl 16%.

Conclusions. Integration of different energy production processes such as biodiesel from microalgae lipids and hydrogen from residual biomass can increase the overall energy yields from microalgal biomass. In this work, it was demonstrated that the residual biomass of the oleaginous strain, *Scenedesmus obtusiusculus,* could be used to produce hydrogen after an acid-pretreatment of the biomass.

References.
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