A preliminary study of biodiesel production by *in situ* enzymatic transesterification of microalgae *Desmodesmus subspicatus*

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Abstract

To produce biodiesel from microalgae biomass by enzymatic transesterification eliminating the oil extraction step.

Introduction

Microalgae are simple photosynthetic microorganisms that are excellent feedstock source to produce biofuels because their adaptation capacity in many different mediums, high productivity of biochemical compounds and do not compete with agriculture areas, as other oil plants. The use of microalgae as an alternative to produce biodiesel is increasing because its high lipids levels and production capacity in alternative culture media.¹ The biodiesel production using microalgae is a technological capability technique, however, still has high costs.² Based on this, the objective of this work was to produce biodiesel by in situ enzymatic transesterification from the microalgae Desmodesmus subspicatus, aiming the elimination of the oil extraction step and consequently reducing the production costs.

Results and Discussion

Microalgae *D. subspicatus* was cultivated in tubular photobiorreactors, under continuous illumination and temperature controlled, with N:P:K medium (18:6:18 m/m). Its biomass was separated by centrifugation and, after frozen, lyophilized. The lipid yield was 7,6%. *In situ* biotransformation tests were carried in incubator shaker with orbital agitation 55 rpm, at 55°C, starting with 1g of lyophilized biomass, 10% of enzyme (Novozym 435[®]), methanol and water, with a factorial design 2², as shown in Table 1. The use of methanol had double action: break the microalgae cell wall and as a reactant of the transesterification. Thin Layer Chromatography was used to monitor the conversion, using distilled soybean biodiesel (B100) as standard.

After 48h of reaction, biodiesel conversion in the sample 1 was observed. This sample obtained better performance because it used less amount of alcohol and water, showing similar yields of biodiesel than the samples using higher reagents concentration. The

samples were analyzed by Gas Chromatography fallowed by Mass Spectrometry (GC/MS), to identify the methyl ester composition of each sample. They also were analyzed by Gas Chromatography with Flame Ionization Detection (GC/FID) to evaluate the percentage of each present methyl ester.

Table 1. Results of biodiesel conversion by *in situ* transesterification

Sample	Ratio molar	Water (mol)	Biodiesel
	methanol:oil		(mg/g)
1	74:1	0.0055	33.6
2	148:1	0.0055	19.6
3	74:1	0.05	30.6
4	148:1	0.05	34.1

Commercial standards of the main saturated and unsaturated methyl esters were used to identification. It was observed, in the sample 4, the formation of many methyl esters, mostly C6:0 (0.6%), C8:0 (1.3%), C10:0 (1.7%), C12:0 (0.4%), C14:0 (1.0%), C16:0 (16.6%), C16:2 (18.0%), C16:3 (3.5%), C18:0 (0.6%). C18:1 (2.0%), C18:2 (44.3%) and C18:3 (9.9%). Microalgae biodiesel contains minority fatty acid methyl esters (FAME) with saturated. unsaturated and/or branched chains. Besides the FAME, other components were extracted, such as hydrocarbons and alcohols, but in lower concentrations.

Conclusion

The tests had satisfactory results using microalgae biomass from *D. subspicatus* to produce biodiesel. Increments in the culture media can increase the biomass lipid levels, what could improve the achievement of fatty acid methyl esters by *in situ* transesterification.

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