ETHYLIC BIODIESEL PRODUCTION USING INTRA AND EXTRACELLULAR LIPASES FROM THERMOPHILIC FUNGI

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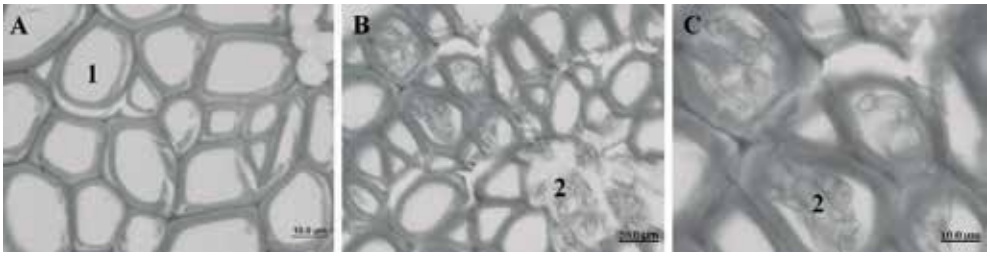

Figure 1. Growth of Thermomucor indicae seudaticae N31 on loofah sponges analyzed by light optical microscope. (A) Sponges without fungi; (B) and (C) whole cells immobilizedin LS; 1. Loofah cell walls appear in dark gray (larger cells) and 2. hyphae of the fungus T. indicae-seudaticae N31 appear in light gray (smaller cells) (Ferrarezi et al. 2014)

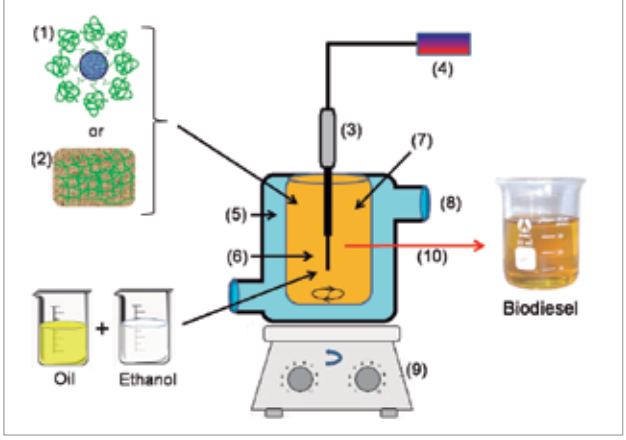

Figure 2. Experimental set-up for enzymatic biodiesel production with ultrasound: (1) immobilized enzyme, (2) immobilizes mycelium, (3) ultrasound probe, (4) power supply, (5) thermostated vessel, (6) reaction vessel, (7) solvent, (8) water inlet/outlet, (9) stirring equipment, (10) Product of reaction after reaction time and removal of glycerol phase (Borges, 2012)

The proposed project aimed to study the use of lipases produced by thermophilic fungi on ethylic biodiesel production. The enzyme lipolytic and transesterification ability were valued in its free form and immobilized in polymeric supports and in immobilized fungal mycelia. Lipases were characterized according to specific reactions and its kinetic characteristics. Besides hydrated ethanol, others such as soybean oil, castor oil and animal fat were used as raw materials to produce biodiesel. The solid state fermentation (SSF) for enzyme production were investigated in constructed bioreactors (bench/pilot). Investigations were also conducted aiming the effect of ultrasound irradiation in the transesterification reaction in the production of biodiesel. The final product were characterized by GC-MS, thermogravimetry, calorimetry, and standards methods for biodiesel.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This study aimed at selecting fungal strains capable of producing lipolytic enzymes with transesterification property to be used for the production of biodiesel via ethylic transesterification. The ability of transesterification of enzymes was evaluated in both free, immobilized on supports and also, immobilized in its own hyphae. The results obtained so far are presented below. Several strains of fungi with esterifying activity and positive for the synthesis of biodiesel were isolated and some fungi have been identified which include: *Thermomucor indicae-seudaticae*, *Rhizomucor pusillus*, *Myceliophtora sp.*, *Thermomyces lanuginosus*, *Fusarium verticillioide*, *Acremonium sp.*, *Aspergillus sp.* and *Thermomyces sp*. Some lipases have already been characterized for specificity and kinetics. Oils from different botanical sources have being used as feedstock for biodiesel production. Different lipases were immobilized in different supports (Quilles et al. 2015), and some had their hyphae immobilized on their own culture medium as *R. pusillus* and *Thermomucor* indicae-seudaticae (Ferrarezi et al. 2014). The enzyme from *Rhizomucor pusillus* was also immobilized (Ferrarezi et al. 2013), and showed 80 and 70% respectively in yield in the conversion of substrates into esters. The analyzes of the esters were confirmed by gas chromatography. A solid state fermentation (SSF) bench bioreactor was constructed and successfully operated for lipase production by the fungus *Myceliophtora spp* (Casciatori et al. 2013). Investigation was also conducted on the effect of ultrasound irradiation in the transesterification reaction in the production of biodiesel from soybean oil and ethanol using the immobilized enzyme from *Thermomyces lanuginosus* and *Acremonium sp* (Borges 2012; Ohe 2011). The results showed that the temperature of 30 to 35 °C and 5% of enzyme were the optimum conditions for the catalysis. The highest conversion values were observed with soy oil. The irradiation of ultrasound (10%) led to an increased conversion of ethyl esters from 92 to 99%, showing a positive effect on the production of biodiesel by the enzymes.

MAIN PUBLICATIONS

Quilles JCJ, Brito RR, Borges JP, Aragon CC, Fernandez-Lorente G, Bocchini-Martins DA, Gomes E, Da Silva R, Boscolo M, Guisan JM. 2015. Modulation of the activity and selectivity of the immobilized lipases by surfactants and solvents. *Biochemical Engineering Journal*. **93**: 274-280.

Ferrarezi AL, Ohe THK, Borges JP, Brito RR, Siqueira MR, Vendramini PH, Quilles JC, Da Costa CCN, Bonilla-Rodriguez GO, Boscolo M, Da Silva R, Gomes E. 2014. Production and characterization of lipases and immobilization of whole cell of the thermophilic *Thermomucor indicae seudaticae* N31 for transesterification reaction. *Journal of Molecular Catalysis*. B, Enzymatic (Print). **107**: 106-113.

Ferrarezi AL, Pivetta DH, Bonilla-Rodriguez GO, Da Silva R, Guisan JM, Gomes E, Pessela BC. 2013. Partial purification, immobilization and preliminary biochemical characterization of lipases from *Rhizomucor pusillus*. *Advances in Enzyme Research*. **01**: 79-90.

Casciatori FP, Laurentino CL, Lopes KCM, Souza AG, Thoméo JC. 2013. Stagnant effective thermal conductivity of agro-industrial residues for solid state fermentation. *International Journal of Food Properties*. 16: 1578-1593.

Janaina Pires Borges. Produção de lipases por fungos termofílicos e mesofílicos e uso na produção de biodiesel etílico. 2012. Dissertação (Química) Dissertação (Química) – IBILCE/Universidade Estadual Paulista.

Da Silva R, Ohe THK, Gomes E, Thomeo JC, Borges JP, Boscolo M. Optimization of ethylic biodiesel production by ultrasonic irradiation using soybean oil and lipase of Thermomyces lanuginousus. *Annais of 34th Symposium on Biotechnology for Fuels and Chemicals*. v.34. New Orleans, USA. 2012.

Da Silva R, Ohe THK, Ferrarezi AL, Borges J P, Gomes E, Thoméo JC, Boscolo M. Effect of ultrasonic irradiation in enzymatic transesterification reaction for biodiesel production. ISAF: International Symposium on Alcohol Fuels. Verona, Italy, 2011.

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