BIOCHAR FROM MICROALGAE AS A SUPPORT FOR LIPASE IMMOBILIZATION AIMING TO OBTAIN A SUSTAINABLE BIOCATALYSTS

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ABSTRACT

The present work aimed to evaluate the use of biochar obtained from microalgae biomass as a support for lipase immobilization, aiming its use in transesterification reactions for the biodiesel production. Initially, the microalgae was cultivated for a period of 7 days, then the biomass was recovered. Its transformation into biochar occurred through pyrolysis, in which the biomass was subjected to temperatures of 400 °C and 500 °C in an inert atmosphere for a period of 1 h. The biochar obtained was pretreated and activated with epichlorohydrin solution. Then, five different sources of lipases were immobilized on biochar through covalent bonding method, and the lipase from *Burkholderia cepacia* (LBC) showed the highest catalytic activity. After selecting the best lipase, LBC was immobilized in biochar produced at 500 °C, reaching an activity of 4052.11 U g^{-1}, which indicated that the calcination temperature increased the surface area of the support. Finally, the best biocatalyst was used in transesterification reactions, showing a biodiesel conversion yield of 100% in the first 72 h, and a half-life of 301.3 h. Therefore, it was concluded that biochar is a promising support for the immobilization of lipases, especially LBC, and for the production of biofuels.

Keywords: Microalgae. Biochar. Lipase. Immobilization. Biocatalyst.

1 INTRODUCTION

Biochar is a material with a high carbon content, produced by thermochemical processes, such as pyrolysis. It has been seen as a promising product in mitigating several significant global problems, such as global warming, pollution and soil changes. Obtaining biochar from biomass is a process that provides the storage of captured atmospheric carbon, reducing by up to 12% the amount of gases that contribute to the greenhouse effect, generated from anthropogenic activities, such as CO_2, which can suffer an annual reduction of up to 3.7 gigatons from biochar production.[1]

Biochar can be obtained from various raw materials, including microalgal biomass, which has several applications, including its role as a material that corrects the soil, making it more fertile. Furthermore, microalgal biochar has characteristics that also make it an effective adsorbent material used to remove impurities and pollutants from wastewater, such as its high surface area, high porosity, various functional groups present on its surface, environmental sustainability and stability.[2] Currently, biochar has gained attention as an alternative for the production of heterogeneous biocatalysts, in order to replace conventional chemical catalysts, with the aim of producing biodiesel and other bioproducts.

Furthermore, it is important to highlight one of the main advantages of heterogeneous catalysts, such as enzymes immobilized on biochar, compared to homogeneous catalysts. This advantage consists in the fact that heterogeneous catalysts can be easily recovered because they are in a different physical state of the reaction medium in which they are inserted, allowing them to be reused and facilitating the purification of the products obtained.[3]

In this context, the current study aimed to promote alternative routes to produce a heterogeneous biocatalyst, in which different sources of lipase were immobilized in microalgal biochar, aiming to determine the immobilized derivative with the best catalytic activity and use it in transesterification reaction, in order to verify its efficiency in the biodiesel production. This is a study that uses microalgal biomass as a precursor material for obtaining a sustainable and environmentally friendly biocatalyst.

2 MATERIAL & METHODS

The present work used biomass from the marine microalgae *Dunaliella salina*, obtained through *Banco de Algas Marinhas do Instituto Oceanográfico da Universidade de São Paulo*, to obtain microalgal biochar. The experiments were carried out with commercial preparations of cell lipases microbial species, such as: *Candida antarctica* B (CALB L), kindly donated by Novozymes, *Pseudomonas fluorescens* (Lipase AK), *Burkholderia cepacia* (LBC), *Candida rugosa* and lipase from animal cells (pig pancreas, Type II) purchased commercially from Sigma Co. (St. Louis, MO, USA). Among the lipases tested, the one that showed the best catalytic activity was applied in the transesterification reaction, aiming to obtain biodiesel. The following topics show the methodologies used to obtain the biocatalyst and the conditions of the biodiesel reaction in which it was used.

Cultivation of microalgae: Modified Guillard f/2 culture medium was used to cultivate microalgae in bubble column photobioreactors, which has a useful volume of 10 L, maintained at a temperature of 25°C, using 10% inoculum, LED lamps to provide adequate lighting and an air compressor (Boyu ACQ 007) with a power of 75 W and a capacity of 100 L min^{-1} connected by latex tubes to promote system aeration. Biomass harvesting was carried out by adding the flocculating agent $Al_2(SO_4)_3$, in a proportion of 2 mL L^{-1}. Subsequently, the biomass was filtered with distilled water, aiming to remove excess salts.

Biochar production: The microalgal biomass pyrolysis step was carried out using a methodology adapted. Initially, the residual microalgal biomass was subjected to a drying step at 100 °C until it reached humidity below 10%. Then, pyrolysis was carried out in a tubular furnace under N_2 flow (0.5 L min^{-1}), in which the sample was heated at a heating rate of 10 °C min^{-1} until reaching the final temperature of 400 °C, in which it was maintained for 1 h. Finally, the sample already converted into biochar was cooled until it reached room temperature. A temperature of 500 °C was tested too, in order to verify its influence in the surface area and enzyme immobilization.[4]

Surface Area Analysis (B.E.T): The surface area of the support used was measured using the methodology Brunauer, Emmett and Teller (B.E.T), in which the adsorption/desorption technique was adopted nitrogen physics at a temperature of 77.3 K. The samples underwent preheating 100 °C for 3 hours under vacuum.

Preparation and activation of the support: The biochar was pre-treated by immersion in a nitric acid solution (1% v/v HNO_3) and heated to 75 ºC under stirring for 1 h. Subsequently, the material was filtered and washed with distilled water until a neutral pH was obtained. The support was dried at 105 ºC for 24 h. After that, the support was silanized with γ-aminopropyltriethoxysilane solution (γ-APTS at 0.5% v/v). The pre-treated biochar was activated by 2.5% v/v epichlorohydrin solution, prepared with 0.1 mol L^{-1} buffer solution with pH 7, being the support/solution ratio of 1:10 m/v. Then, the mixture was stirred for 1 h at room temperature. After this period, the biochar was recovered by vacuum filtration, being washed with distilled water and buffer solution (pH 7). Finally, the filtered support was taken to the oven at 60 °C for 24 h for drying.

Immobilization of lipases: The enzymes were immobilized using the covalent bond method, in which the treated and activated biochar was immersed in hexane, in a support/liquid ratio of 1:10 m/v, a mixture that was kept under gently stir for 2 h. For each gram of support, 100 µL of aqueous solution was added with 5 mg mL^{-1} of polyethylene glycol (PEG-1500) and an amount of enzyme corresponding to the ratio of 0.3 g of enzyme per gram of support. Thus, the mixture containing enzyme and support was stirred for 2 h, followed by static contact for a period of 18 h at 4 °C. Finally, the biocatalyst was recovered by vacuum filtration. The hydrolytic activities of the biocatalysts were determined by a method of hydrolysis of olive oil.[5]

Biodiesel production reaction and purification: Biodiesel was produced by transesterification, using lipase with better catalytic activity immobilized in microalgal biochar. The reaction was carried out using babassu oil and ethanol as acyl group acceptor, in a glass reactor of 50 mL, hermetically sealed, at 45 °C and magnetic stirring. A mass of catalyst corresponding to 15% m/m in relation to the reaction medium was used and the oil/alcohol molar ratio was 1:12. Each batch of the reaction was carried out for 72 h, with the samples taken every 24 h, which were subsequently purified. Biodiesel purification was carried out using Amberlite BD10DRY adsorbent. [6]

3 RESULTS & DISCUSSION

Firstly, lipases from different species were immobilized, by covalent bond method, on preteatred and activated biochar prepared at 400 °C, as shown in Table 1.

Table 1 Hydrolytic activity of different lipase sources immobilized on biochar.

Lipase sources	Hydrolytic activity (U g^{-1})
Burkholderia cepacia (LBC)	2746.28
Pseudomonas fluorescens (AK)	1929.87
Candida rugosa (CR)	2070.77
Porcine pancreas (Pancreática)	746.64
Candida antarctica (CALB)	557.39

As seen in Table 1, it is possible to verify that the activity values ranged from 557.39 to 2746.28 U g^{-1}, depending on the source of lipase. The lipase that presented the highest hydrolytic activity was LBC. The difference observed in the catalytic activities of each immobilized enzyme occurs due to the difference between the structures of each lipase analyzed in relation to the structure and nature of the support, as this directly affects the interaction between it and the enzyme. One example is a work where authors immobilized lipase from *Burkholderia cepacia*, by covalent bonding, on niobium oxide (Nb_2O_5) support, and the higher activity obtained was 1827.6 U g^{-1}. [7]

Soon after, the lipase with greater hydrolytic activity (LBC) was immobilized in biochar, pretreated and activated, produced at 500 °C. In this case, the hydrolytic activity of the immobilized derivative was 4052.11 U g^{-1}. In this way, it was possible to verify that the temperature in which pyrolysis occurred influences considerably the characteristics of the support and, consequently, the biocatalyst, influencing its catalytic activity. It is worth highlighting that the best conditions for producing catalysts or supports through pyrolysis also vary according to the raw material used.[8,9] In this case, from the characterization of the biochar produced at 400 and 500 °C, through the B.E.T method, it was possible to verify that the surface area of the support produced at each temperature was, respectively, 24 and 60 m^2 g^{-1}. This justifies the increase in hydrolytic activity in the biocatalyst produced with biochar obtained at 500 °C, since the greater surface area allows a greater enzymatic adherence.

After selecting the best enzyme and the best biochar production condition, the biocatalyst with the best catalytic activity (LBC immobilized in pre-treated and activated biochar produced at 500 °C) was used in transesterification reactions, aiming to obtain biodiesel. The reactions were carried out in four cycles of 72 hours and the Figure 1 shows the results obtained.

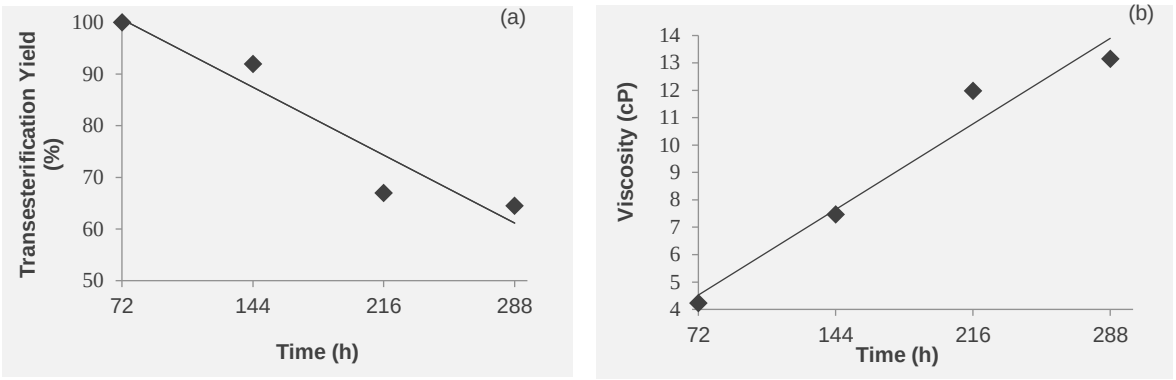

Figure 1 Operational stability of LBC immobilized in microalgal biochar in consecutive babassu oil transesterification responses: (a) transesterification yield and (b) consistency of the transesterified product at the end of each cycle.

At the end of the first cycle, the transesterification yield obtained was 100%, with a viscosity value of 4.24 cP. In the following batches, the yield of transesterification gradually reduced, reaching 92% at the end of the second cycle, 67% at the end of the third cycle and reaching, at the end of the fourth cycle (288 h), a yield of 64.5%. Regarding the viscosity of the final product, obtained at the end of each cycle, a gradual increase was observed according to the number of batches carried out. Thus, it is observed that there is an inversely proportional relationship between transesterification yield and viscosity, since the higher the yield, the lower the viscosity of the product. In this way, in the lowest yield of the reaction (64.5%), the product formed reached a viscosity of 13.15 cP. From the results obtained in Figure 1, it was possible to calculate the half-life for the biocatalyst, of the order of 301.3 h.

4 CONCLUSION

From the experiments carried out and the results obtained, it was possible to conclude that the biochar production conditions, as well as the type of enzyme, significantly influence the activity of the biocatalyst obtained. As it was possible to observe, among the lipases tested, the one from *Burkholderia cepacia* showed the greatest catalytic activity when immobilized in biochar (2746.28 U g^{-1}), and its activity increased when immobilized in biochar produced at 500 °C (4052.11 U g^{-1}). The application of this biocatalyst in transesterification reactions to obtain biodiesel revealed a yield of 100% in the first 72 h, as well as a half-life of 301.3 h. According to the above, biochar from microalgae shows to be a promising support for the immobilization of enzymes, in addition to standing out for being a biodegradable material.

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