

## ORGANIC-INORGANIC SOLID HYBRID AS SUPPORT FOR PIG PANCREATIC LIPASE IN BIODIESEL PRODUCTION

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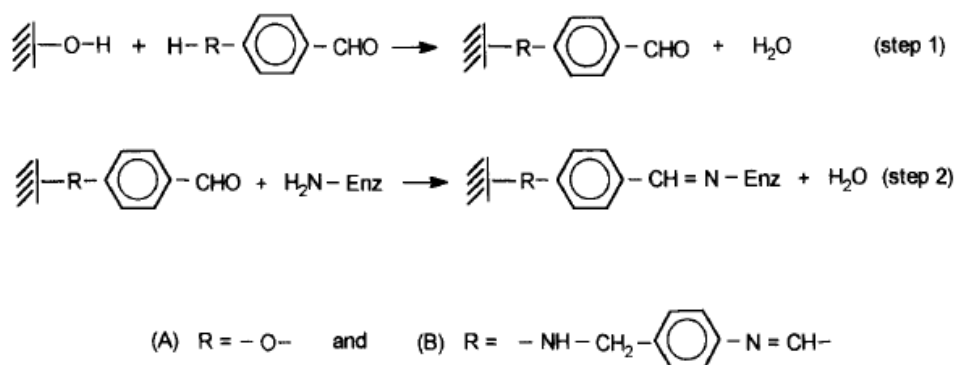
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### Introduction.

The weaknesses associated to the current procedure of production of Biodiesel by basic homogeneous catalysis could be overcome by using immobilized *Lipases*, this would allow, not only the reutilization of the enzymes, but rather it would also facilitate the experimental procedure for the isolation and purification of the products of the reaction.

In this communication it is carried out a study about the possibilities of application of an enzymatic procedure using an immobilized *Pig Pancreatic Lipase (PPL)*, as a really affordable biocatalyst from an economic point of view. For the production of Biodiesel, the reaction of transesterification of sunflower oil has been chosen as a reaction test, evaluating the behavior of the PPL in low different pH environments, temperature and relative concentration of ethyl alcohol regarding the oil. Likewise, it has been studied the behavior of the ethanol and other alcohols of short chain, and used oils. For the covalent immobilization of the *PPL*, we use like support  $\text{AlPO}_4$ -Sepiolite (20-80% in weight), previously activated in agreement with the synthesis in solid phase for steps picked up in the Figure 1, [1-4].



**Figure 1.** General outline for the immobilization of the enzyme PPL, through the  $\epsilon$ -amine group of the lysine residue. The activation of the amorphous  $\text{AlPO}_4$  supported by microwaves heated with tereftaldicarboxaldehyde (step 1), and before the covalent immobilization of the enzyme through the lysine residuals (step 2).

### Experimental.

The support  $\text{AlPO}_4$ -Sepiolite (20-80% in weight), it is obtained adding natural Sepiolite given by Tolsa S.A (Vallecas, Madrid) to the means of reaction where the precipitation of the aluminum phosphate has begun [1-4]. The immobilization of the PPL is carried out to ambient temperature following the methodology described when putting in contact 0.5 g of previously activated inorganic solid and functionalized with 0,04 g of PPL in the reaction flask (50 mL) with methanol 6 mL, keeping in refrigerator during 24 hours, with occasional agitation.

Enzymatic transesterification reactions have been carried out by magnetic shaking in a 50 mL round bottom flask, at controlled temperature (20-80°C). The reaction mixture consists on 9.4 g of sunflower oil, 0,5 g of support with the immobilized PPL. The samples take to different reaction times (6-48 hours). Esters are

quantified with a gas chromatograph HP 5890 Series II Gas connected to a capillary column HT5, 0.1 UM (25 m x 0.32 mm, SGE).

### **Conclusions.**

In accordance with the results obtained we can conclude that the enzyme PPL is fixed in a stable way in the investigated inorganic support (95%) although at the same time the efficiency of this enzyme is quite reduced (approximately 50%). However, the immobilized enzyme experiences a smaller influence of the experimental reaction conditions with regard to the native enzyme and shows a remarkable resistance to the deactivation.

The free PPL shows a very important influence of pH and temperature in the transesterification reaction with ethanol. However, no influence of relative proportion oil/ethanol was obtained when immobilized PPL was tested. When different alcohols: 1 and 2-propanol, 1 and 2 butanol, t-butanol and 1-pentanol were used in the transesterification reactions, clearly it was shown a high sensibility of the PPL regarding the structure of the substrates. According to previous references [5] methanol is not a good substrate in this process. Regarding the use of waste cooking oils, there was not any difficulty in using them only a slight decrease in the activity was detected in the transesterification with ethanol.

The application of this immobilization system offers diverse advantages, like the reutilization, the separation easiness and, mainly the extraordinary stability that the PPL acquires after its immobilization, regarding the most habitual parameters in reaction: temperature, pH, etc, obtaining also a glycerin of high purity.

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