REUSE OF CANDIDA ANTARTICA LIPASE B IMMOBILIZED ONTO IRON MAGNETIC NANO Particles FOR THE SYNTHESIS OF FLAVOR ESTERS

MONTEIRO RRC1, FEITOSA MRC2, MOREIRA KS3, SANTOS JCS4, SOUZA MCM5

1,2,3,4,5 University for the International Integration of the Afro-Brazilian Lusophony, Institute for Engineering and Sustainable Development
Contact e-mail address: rodolpho@aluno.unilab.edu.br

ABSTRACT – Enzymes are biocatalysts that allow natural catalysis under mild reaction conditions and are highly selective. In order to increase enzyme stability and facilitate its recovery and reuse, enzymes are immobilized onto magnetic nanoparticles. In this context, this study aims to analyze the maintenance of the catalytic activity of Candida antartica Lipase B immobilized onto magnetic nanoparticles applied to the production of methyl butyrate and ethyl butyrate through esterification. Analyzing the effects of temperature, the best conversion rate was 78.48% for ethyl butyrate at 35 °C, while the conversion rate for methyl butyrate was 80.67% at a substrate concentration of 0.5 mol/L. Analyzing the molar ratio (acid: alcohol), the best conversion rate (44.71%) was obtained for ethyl butyrate using a molar ratio of 1:1.

1. INTRODUCTION

Innovation in traditional chemical processes requires that environmental concerns be addressed in order to mitigate problems. In this sense, the use of toxic chemical catalysts, which are the main mediators of reactions of industrial interest, causes some undesirable reactions that can generate toxic waste to the environment. The catalysis of reactions by biological catalysts is an alternative to the traditional chemical processes. Enzymes are highly selective biological catalysts, which reduce the occurrence of undesirable reactions and require mild reaction conditions (Hasan et al., 2006).

According to Wiseman (1995), enzymes are catalysts of protein nature that have an active region that give them high catalytic activity, due to the high specificity of enzyme-substrate interaction. Lipases are enzymes classified as hydrolases that stand out due to the range of reactions catalyzed in organic systems with low water content, high stability, use in mild reaction conditions and great diversity. Among the lipases, Candida antartica lipase B (CALB) stands out due to its wide range of specificity for substrates, utilization in mild reaction conditions, resistance to organic solvents and thermal stability, as well as acting in a comprehensive range of pH and high stereo specificity (Rodrigues et al., 2008).

Nevertheless, a broad industrial application of CALB is limited due to problems concerning to the stability, recovery and reuse of this biocatalyst. According to Adriano et al. (2005), an
alternative solution to this problem is the immobilization of enzymes. The enzymatic immobilization onto solid nanostructured magnetic supports allows an easy recovery of the enzyme, besides having high surface area, higher temperature tolerance, good chemical reactivity and strong interactions with the enzymes (Lei et al., 2009).

The synthesis of fatty acid esters for use in additives and flavorings through traditional chemical processes implies in a great demand for energy, besides the reactions are slow and non-selective and cause environmental problems. Thus, natural catalysis allows better quality products to be obtained under mild conditions of reaction, as stated by Kiss et al. (2004).

The aim of this study was to analyze the maintenance of the catalytic activity of Candida antarctica lipase B immobilized onto iron magnetic nanoparticles, CALB-NPM, applied to the synthesis of ethyl butyrate and methyl butyrate ester, which are in great demand in the food industry like banana and pineapple aromas, as regards the effects of temperature, substrate concentration and molar ratio (acid: alcohol).

2. MATERIALS AND METHODOLOGY

The enzymes Candida antarctica lipase B used by Souza (2013) and reused here were purchased from Codexis (Redwood, USA) and immobilized onto iron magnetic nanoparticles produced by the co precipitation method. The solution of glutaraldehyde grade II 25% (w / v) and γ-aminopropyltriethoxysilane were obtained from Sigma-Aldrich (St. Louis, USA). The other analytical grade reagents were obtained from Synth (São Paulo, Brazil) and Vetec (São Paulo, Brazil).

Aiming to immobilize CALB onto iron magnetic nanoparticles, the support was initially treated with γ-aminopropyltriethoxysilane solution, according to the methodology described by Netto et al. (2009). After the treatment was finished, the cross-linking of the support was done with glutaraldehyde solution, as described by Andrade et al. (2010). Once this was done, immobilization of the enzyme on the support was started by the contact of 0.01 g of magnetic nanoparticles with 0.5 mL of sodium phosphate buffer (100 mM and pH 7.0), the system was maintained under controlled stirring at 45 rpm. The enzyme-support contact time was 1 hour, being the immobilized enzyme removed from the solution by magnetism at the end of the process.

Then, CALB-NPM was used for the synthesis of ethyl butyrate and methyl butyrate esters through esterification. The reaction medium was composed of heptane (1 mL), which was used as solvent, in addition to butanoic acid and alcohols (methanol or ethanol) at different concentrations of substrates (0.1-1.0 mol/L), temperature (25-55 °C), molar ratio (1:1-1:4), reaction time (8h) and mass of the biocatalyst. The reaction was performed in 2.0 mL eppendorf on orbital shaker (150 rpm).

3. RESULTS AND DISCUSSIONS

The influence of temperature on biocatalysis of reactions is due to the increasing in kinetic energy and enzyme denaturation. Another important parameter for synthesis reactions is the concentration of substrates. Increasing acid and alcohol concentrations have a significant effect on enzyme activity. In addition, mechanisms similar to those of competitive inhibition in lipases are caused by the excess of acid in the reaction medium.
Figure 1 – Effects of temperature, substrate concentration and molar ratio (acid: alcohol) on the synthesis of methyl butyrate and ethyl butyrate using CALB-NPM. Source: Authors (2017)

Figure 1 shows the effect of temperature (25-55 °C) on the synthesis of methyl and ethyl butyrates using CALB-NPM. The best result obtained was for the production of ethyl butyrate at 35 °C (78.48%) and methyl at 45 °C (15.77%). Under the same reaction conditions of this study, Souza (2013) obtained a conversion of approximately 90% at 25 °C.

Still according to Figure 1, a better conversion was observed for the production of methyl butyrate at a substrate concentration of 0.5 mol/L (80.67%). For ethyl butyrate, the highest conversion was using a concentration of 0.2 mol/L (67.95%). Methanol has a lower chain and this can be attributed to this result. In the same reaction conditions of this study, Souza (2013) obtained a higher conversion using a substrate concentration of 0.4 mol/L for ethanol (97%) and 0.5 mol/L for methanol (87.9%).

For the analysis of the molar ratio effect, the acid concentration was considered constant and then the alcohol concentration was considered constant. Figure 1 depicts the profile obtained experimentally for the influence of the different molar ratios on the conversion of ethyl butyrate methyl butyrate. As can be observed, the best result obtained for ethyl butyrate (44.71%) was using a molar ratio of 1:1, whereas for methyl butyrate, the best conversion (41.01%) was obtained using a molar ratio of 1:3.

The enzymes reused in this study went through consecutive cycles of esterification; in addition, the time (four years) and the storage conditions may have influenced the biocatalytic activity and stability of the biocatalyst. In addition, the lower mass of the immobilized enzymes in the eppendorfs may have affected the conversion process.

4. CONCLUSION

In general, the biocatalysis of the esterification reaction for the synthesis of methyl butyrate and ethyl butyrate, using Candida antarctica lipase B immobilized onto iron magnetic nanoparticles, presents high conversion percentages, even when they are used in consecutive cycles. Thus, the use of CALB-NPM for the production of esters is quite advantageous.

However, the storage conditions and the lack of precise control over the various esterification
cycles, which the enzyme was submitted, had a negative influence on the catalytic activity of the biocatalyst, even though the determination of the conversion was carried out under the best conditions of temperature, concentration of the substrates and molar ratio. In addition, the lower amount of available enzyme mass also influences in the conversion rate.

Therefore, the use of immobilized lipases onto iron magnetic nanoparticles for the production of esters of industrial interest through the esterification reaction presents itself as a promising alternative to traditional chemical processes, since biocatalyzed reactions are faster and less aggressive to the environment.

5. REFERENCES

ADRIANO W, SILVA J, GIORDANO R, GONÇALVES L. Stabilization of penicillin g acylase by immobilization on glutaraldehyde-activated chitosan. *Bra. J. of Chem. Eng.*, v. 22, p. 529–538, 2005.

ANDRADE L, REBELO L, NETTO C, TOMA H. Kinetic resolution of a drug precursor by Burkholderia cepacia lipase immobilized by different methodologies on superparamagnetic nanoparticles. *J. of Mol. Cat. B: Enz.*, v. 66 , p. 55–62, 2010.

HASAN F, SHAH A, HAMMED A. Industrial applications of microbial lipase. *Enzyme and Microbial Technology. Elsevier*, v. 39, p. 235–251, 2006.

LEI L, BAI Y, LI Y, YI L, YANG Y, XIA C. Study on immobilization of lipase onto magnetic microspheres with epoxy groups. *Elsevier*, v. 21, p. 252–258, 2009.

KISS M, SEFANOVITS-BÁNYAI É, TOTH A, BOROSS L. Extractive synthesis of ethyl-oleate using alginate gel co-entrapped yeast cells and lipase enzyme. *Eng. in Life Sci*, v. 4, p. 460–464, 2004.

NETTO C, ANDRADE L, TOMA H. Enantioselective transesterification catalysis by Candida antarctica lipase immobilized on superparamagnetic nanoparticles. *Tetra: Asy.*, v. 20, p. 2299–2304, 2009.

RODRIGUES D, MENDES A, ADRIANO W, GONÇALVES L, GIORDANO R. Multipoint covalent immobilization of microbial lipase on chitosan and agarose activated by different methods. *J. of Mol. Cat. B: Enz.*, v. 51, p. 100–109, 2008.

SOUZA MCM. *Imobilização de Lipase de Candida Antarctica do Tipo B em Nanopartículas Magnéticas Visando a Aplicação na Síntese de Ésteres*. 2013. 87 f. Tese (Doutorado em Engenharia Química). Universidade Federal do Ceará, Fortaleza/ Ceará, 2013.

WISEMAN A. *Handbook of Enzyme Biotechnology*. Londres: Ellis Horwood, 1995.