Effects of Ultrasound on Enzymatic Esterification

Vinatoru M., Calinescu I. ,Vartolomei A-M, Gavrila I-A.

Faculty of Applied Chemistry and Material Science, University "Politehnica" of Bucharest, 1-7 Gh. Polizu, Bucharest, 011061, Romania

Introduction

Classical esterification methods have some disadvantages like low purity of products and using an acid as catalyst which is undesired in food and cosmetics preparations, requirement of longer reaction times, unsatisfactory yields, requirement of large amounts of toxic expensive reagents and high temperatures giving an unsafe and sometimes uneconomical approach.The advantages for using enzymatic catalysis for esterification are: high degree of purity for the products, obtaining of green products, regio-, stereo- and substrate specificity [1], moderate reaction temperature and pressure, better stability of lipases, recycling biocatalysts and support regeneration [2-4] A method for intensification of enzymatic esterification is the use of a ultrasound assisted system. The effects of acoustic cavitation on enzyme are thermal effect, that can lead to the enzyme denaturation, the forming of free radicals which can attack the cellular materials and the shear forces produced can deactivate the enzyme and modify the conformational structure of enzymes. But using low ultrasound frequencies the conformational structure of enzymes can be changed from the closed conformation to the opened one, allowing the easy access of the reagens. [5]The main advantages of using ultrasounds to enhance biocatalyzed reactions are: increasing selectivity, using less dangerous solvents, lowering energy consumption for the desired transformation, using renewable and sustainable materials, reducing reaction time, better use of raw material and catalyst. [6]

Materials and Methods

The esterification process was carried out with acetic acid and *i*-amyl alcohol in the presence of an enzyme. The acetic acid was glacial acetic acid from Chemical Company and the *i*-amyl alcohol was provided from Universite Chemie Belgique. The enzyme used as catalyst was Lipozyme 435 from Novozyme, a preparation of a *Mucor miehei* lipase immobilized on a macroporous anion exchange resin. The concentration of *i*-amyl acetate formed from the esterification process was determined by gas cromatography analysis. The equipment used for the ultrasound assisted process in this study consists of a Vibracell 750 processor.

Results

In the present paper a systematic study on the effects of ultrasounds on the enzymatic esterification for aroma esters preparation is described. Thus, by ultrasound assisted enzymatic esterification *i*-amyl acetate was obtained. We studied the effect of temperature, ultrasonic power and duty cycle on the esterification reaction. From figure1 we see that significant improvements in the ester concentration were obtained in comparison to conventional method.

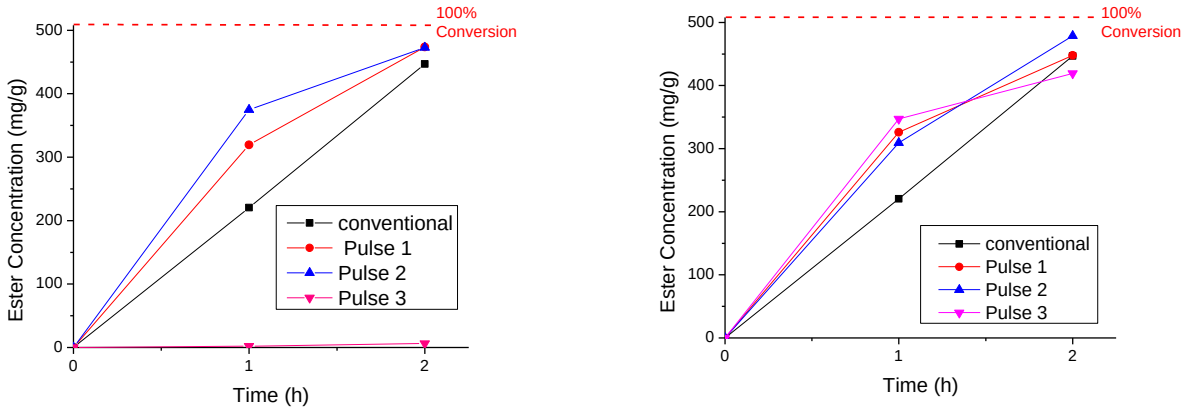

Figure 1. Ester concentration for US intensification with three tipes of pulses (reaction temperacture 50 ^{0}C, molar ratio 1 :2, 0.16mL/min mixture flow , 0.047g enzyme/g mixture) a)US probe at 4,5 cm from the reactor ; b) US probe at 3,5 cm from the reactor ;Pulse 1: 3sec ON/3sec OFF, Pulse 2: 3sec ON/6sec OFF, Pulse 3: 3sec ON/9sec OFF

Conclusion

Ultrasound assisted enzymatic synthesis of isoamyl acetate was performed using Lipozyme 435 in solvent-free system. The results show a favorable perspective of the ultrasound technique to improve the process efficiency and reduce the reaction time. The commercial aroma esters synthesis will be potentially realized due to this ultrasound-promoted esters synthesis method.

References

[1] Krishna H.S., Divakar S., Prapulla S.G., et al., *Journal Of Biotechnology*, volume (87), 193-201, **2001**.
[2] Abbas H., Comeau L., *Enzyme and Microbial Technology*, volume (32), 589-595, **2003**.
[3] Gumel A.M., Annuar M.S.M., Chisti Y., et al., *Ultrasonics Sonochemistry*, volume (19), 659-667, **2012**.
[4] Kwiatkowska B., Bennett J., Akunna J., et al., *Biotechnology Advances*, volume (29), 768-780, **2011**.
[5] Delgado-Povedano M.M., Luque de Castro M.D., *Analytica Chimica Acta*, volume (889), 1-21, **2015**.
[6] Sancheti S.V., Gogate P.R., *Ultrasonics Sonochemistry*, volume (36), 527-543, **2017**.

* mircea.vinatoru@gmail.com