Chemical Engineering and Processing 110 (2016) 106–113

Contents lists available at ScienceDirect

Chemical Engineering and Processing: Process Intensification

journal homepage: www.elsevier.com/locate/cep

Analysis of process intensification in enzyme catalyzed reactions using ultrasound

M. Ajmal[a,*], G. Fieg[a], F. Keil[b]

[a] Institute of Process and Plant Engineering, Hamburg University of Technology, Am Schwarzenberg-Campus 4, 21073, Hamburg, Germany
[b] Institute of Chemical Reaction Engineering, Hamburg University of Technology, Eißendorfer Str. 38 21073, Hamburg, Germany

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 20 September 2016
Accepted 7 October 2016
Available online 11 October 2016

Keywords:
Lipozyme CALB
Ultrasound (US)
Intensification
Esterification
Ultrasonic reactor

ABSTRACT

Intensification of enzyme catalyzed reactions using US is analyzed by use of enzyme catalyzed esterification as model reaction. Lipozyme CALB L is taken as model enzyme in free and immobilized form. This allowed the clarification of the underlying mechanisms of the phenomena. Process intensification was tested both with low frequency (high amplitude) and high frequency (low amplitude) US. Reaction behavior with US was compared with stirring. For the immobilized enzyme the reaction rate increased by a factor of 2.2 by applying low frequency US, however, the cavitation from US damaged the enzyme carrier particles. For the free enzyme low frequency US produced a 22 times faster reaction rate compared with stirring, and the enzyme molecules were not damaged and could be reused. With high frequency US no improvement of reaction rate was observed. The ultrasonic conditions necessary for achieving intensification were identified.

1. Introduction

Lipozyme CALB is a commercially available lipase enzyme from Novozymes and is widely used in industrial applications. CALB is available in two forms i.e. free enzyme (Lipozyme CALB L) and immobilized form (Lipozym-435). Processes based on these enzymes are well established in industry. Marchetti et al. [1] have shown that CALB is a very effective catalyst for esterification reactions. However, the time required for achieving reaction equilibrium is around 72 hours. It will be of great benefit if the time to reach the equilibrium is reduced as it will have a positive impact on production rate of industrial processes. One of the possibilities to achieve this is by improving the activity of CALB. Application of low frequency US (<100 kHz) to improve the activity of immobilized CALB, i.e. Lipozym-435, has been discussed in the literature, and an activity improvement by a factor of 2–3 (compared with stirring) has been reported [2–8]. Subhedar and Botelho et el. [9] have shown that use of US even suppresses the need for excess alcohol. This improvement in activity has been attributed to different phenomena such as faster mass transfer and change in enzyme structure (to make it more active). As stated by Kwiatkowska et al. [10], experimental evidence for the exact mechanism of US action on enzymes remains to be elucidated. In order to determine the mechanism of this phenomenon it is necessary to study the effect of US on free enzyme Lipozyme CALB L also. Indeed, studies regarding US effect on Lipozyme CALB L free enzyme are scarce [11]. To the best of our knowledge this is first study that is comparing the activity of Lipozyme CALB L and Lipozyme-435 under the influence of US. Regarding the effects produced by US in a reaction medium Moholkar et al. [12] and Cravotto et al. [13] have given a detail discussion.

For sonication of enzymes ultrasonic baths [4] and ultrasonic probe arrangements [2,3, and 14] have been used which operate at lower frequencies. In an ultrasonic bath reaction mixtures are held in a beaker/flask which is placed inside the bath at a position where the ultrasonic intensity is maximum [15,16]. The details of these systems can be read in the works of Sutkar and Gogate et al. [17] and Gogate and Kabadi [18]. The problem with this system is that ultrasonic wave needs first to cross the liquid inside the ultrasonic device and then to cross the wall of the sample container. As a result, actual ultrasonic effects transferred to the reaction medium are far lower. Therefore, positioning the glass beaker in a bath at a place of maximum ultrasonic intensity does not ensure that correspondingly higher intensity will be achieved inside reaction medium also. According to Martínez [16] many ultrasonic applications related to baths can be linked to the heat that is transmitted to the sample–rather than to actual ultrasonic effects, i.e. cavitation. By using ultrasonic probe systems US is directly introduced into reaction medium. Due to high intensity and low

* Corresponding author.
E-mail addresses: majmal@encos.de, mhdajmal@hotmail.de (M. Ajmal).

http://dx.doi.org/10.1016/j.cep.2016.10.002
0255-2701/

frequency, probe systems can generate strong cavitation in the medium. The cavitation bubbles imploding near the carrier particles will have a detrimental effect on particle integrity [19]. However, this aspect has not been discussed in the previously mentioned studies. A recent review from Povedano et al. [20] and Kwiatkowska et al. [10] states that the role of US frequencies on the effect caused by this energy on enzyme activity has been poorly considered.

To understand the intensification phenomena of lipase catalyzed reactions, the effect of low and high frequency US on immobilized and free enzyme is studied by taking esterification as a model reaction. The research concept is depicted in Fig. 1. Lipozyme CALB L is used as free enzyme while Lipozyme-435 as immobilized enzyme. Although enzyme molecule in both cases is same (CALB), but in immobilized form the enzyme behavior is different (as it is immobilized in active conformation). Therefore, the behavior of immobilized enzymes towards US shall be different than free enzymes. We hope that from this comparison it will be possible to elucidate the mechanism of CALB activity improvement caused by US. This comparison will also demonstrate for which form of enzyme it is more effective to employ the US. The intensification effect is studied using two different ultrasonic reactors. These reactors are capable of operating at various frequencies and intensities. US is directly introduced into the reaction medium to avoid any loss of ultrasonic energy due to dampening or reflection. The reaction volumes studied are large enough (150–600 ml) to facilitate the scale up of the observed phenomena.

2. Experimental methods

2.1. Reaction system and materials used

Esterification is a class of reactions that are of high industrial importance and have been a topic of research for different research groups [21,22], among others. Therefore, esterification of oleic acid with *n*-hexanol was chosen as a model reaction. No solvent was added to the reaction. The products of esterification are hexyl-oleate and water.

$$\underset{\text{oleic acid}}{C_{17}H_{33}COOH} + \underset{1-\text{hexanol}}{C_6H_{14}O} \overset{\text{Lipase}}{\longleftrightarrow} \underset{\text{Hexyl}-\text{Oleate}}{C_{24}H_{46}O_2} + \underset{\text{Water}}{H_2O}$$

Oleic acid (Edenor PK 1805) was a gift from BASF SE Germany (formerly Cognis Germany). N-hexanol of 99.7% purity was a gift from Sasol Germany GmbH. NaOH was purchased from Carl Roth Germany. Lipozyme CALB L and Lipozym-435 were a gift from Novozymes A/S Denmark. All chemicals were used as received without any further processing or purification.

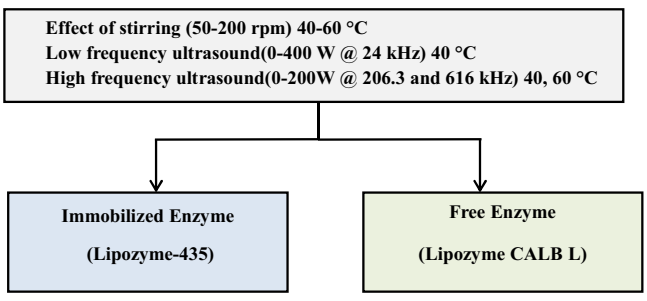

Fig. 1. Research concept for studying the intensification of CALB enzyme.

2.2. Ultrasonic reactors used

Three different reactor configurations were used. In configuration 1 only stirring was applied while in configuration 2 and 3 US was employed. In configuration 2 (Fig. 2a) high frequency US was employed. Stirring was also used in this case as US alone was not capable to keep enzyme particles suspended. In configuration 3 (Fig. 2b) low frequency US was employed however, stirring were not required in this case. In the following, detailed specifications corresponding to each reactor are given.

In configuration 1 reaction behavior was studied under stirring (magnetic stirrer) in absence of US. Experimental arrangement of Fig. 2b was used for this purpose (by removing the sonotrode). In stirring experiments reaction temperature was maintained by circulating water at the correct temperature through jacket of the reactor (for US experiments cooling was required). The temperature inside reactor was monitored using a thermocouple.

The ultrasonic reactor corresponding to configuration 2 is shown in Fig. 2a. It consists of an ultrasonic generator (LVG 60), transducer (USW51) and a glass reactor (with heating/cooling jacket) from L-3 Communications ELAC Nautik GmbH, Germany. Transducer specifications are given in Table 1. The transducer was fitted at the bottom of glass reactor. Through the valves provided in the jacket of the glass reactor, it was possible to connect it with cooling/heating bath (Julabo F12) for maintaining the required temperature. Detailed characterization of high frequency ultrasonic fields is reported by Sutkar and Gogate [23].

The ultrasonic reactor corresponding to configuration 3 is shown in Fig. 2b. It was sonicated with ultrasonic transducer UP400S from Hielscher Ultrasonics GmbH, Germany. The transducer specifications are given in Table. 2. Due to lower frequency and higher intensity compared to configuration 2 UP400S was capable of producing cavitation in the reaction medium used. Ultrasonic generator and transducer are integrated into one assembly. For transfer of US into the reaction medium an ultrasonic horn of 14 mm was used whose specifications are given in Table 2. Kanthale and Gogate et al. [24] have described in detail the mapping of cavitation activity generated by ultrasonic horns. The reaction mixture was filled into a glass reactor from NORMAG Labor- und Prozesstechnik GmbH, Germany (Fig. 2b). This glass reactor also had a heating/cooling jacket for maintaining the required temperature. Ultrasonic horn was inserted into reaction medium from the top of the reactor. The contents of the reactor could also be agitated with a magnetic stirrer (Heidolph RZR 2000).

The reaction volume for all experiments was kept constant at 300 ml (except where mentioned) in order to eliminate any influences arising from variation of this parameter. An equimolar mixture of oleic acid and 1-hexanol was used (192 g oleic acid and 69.5 g alcohol for a 300 ml reaction volume). After filling reactants into the reactor heating/cooling was turned on to achieve the required reaction temperature. In the case of US, cooling was required in order to remove the heat produced from US. In every experiment, after reaching the desired reaction temperature, two samples were taken before addition of enzyme. For the next steps of the experiment samples were taken from the reactor after predefined intervals of time and were immediately analyzed by titrating against 0.1 molar NaOH. Thymolphthalein was used as indicator. The samples were collected using an Eppendorf pipette and were weighed in a balance to determine errors/variations in sampling amount. The amount of NaOH consumed was used to calculate conversion of oleic acid against time.

2.3. Determination of rate constant

Previous studies from the Institute of Process and Plant Engineering at TUHH have shown that kinetic data from enzyme