Ultrasound accelerated the enzymatic synthesis of ethyl hexanoate in a solvent free system.

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Abstract

This work focuses on ultrasound assisted lipase catalyzed synthesis of ethyl hexanoate via esterification in solvent free condition. Optimization of various parameters gave a conversion of 94.25 % at ethanol to hexanoic acid ratio 2:1 with 2% enzyme loading, at 50°C and 200 rpm, with 60 W ultrasound power, at 25 kHz frequency, 50 % duty cycle. Further, the time required for the maximum conversion is reduced to 40 min as compared to 120 min of conventional process. The reusability study revealed that enzyme can be recycled more than five consecutive cycles.

Keywords: esterification, lipase, ultrasound, intensification

1. Introduction

Ethyl hexanoate a chain acid ester gives an apple-pineapple flavour. These short chain ethyl esters are generally characterized by their strong fruity flavour and fragrance [1]. In recent years, there is an increasing demand of flavoured compounds in industry sectors, especially food and beverage, cosmetic and pharmaceutical industries. Recently, ultrasound irradiation has been used to accelerate the enzymatic reactions. Since, ultrasound can enhance heterogeneous reactions it is a very useful tool for increasing the rate of enzymatic reactions. The power of ultrasound waves affects the rate of enzymatic reaction. Ultrasound waves of low intensity have a small influence on removing the mass transfer limitation in enzymatic reactions. Ultrasound waves of sufficient high intensity can solve the problem of mass transfer limitation [2]. However, very high intensity of ultrasound can cause inactivation of enzymes. Hence, it is very important to select the right power and frequency of ultrasonic waves for enzymatic reactions. The aim of our research is to reduce the reaction time and increase the conversion of ethyl hexanoate reaction. Optimisation of all the parameters such as molar ratio, enzyme loading, ultrasound power, and duty cycle and reaction temperature affecting the reaction were studied in detail.

2. Experimental

Immobilised lipase, FERMASE CALB 10000 **(***Candida Antarctica* lipase B) was used as catalyst and provided by Fermenta Biotech, India. Ultrasound assisted enzymatic esterification was carried out using an ultrasound water bath manufactured by Dakshin India Pvt. Ltd. A flat bottom glass reactor of 50 mL capacity was used. The reactor was kept at a height of 2 cm from the base of the ultrasound water bath. Stirring was carried out by using six-bladed impeller. An electric motor having speed control facility was used to provide agitation. The ultrasound water bath was provided with a transducer, having operating frequency of 25 kHz and maximum power 200 W. Hexanoic acid and ethanol were first fed into the reactor in a specific molar ratio at desired reaction temperature. After a homogenous phase was formed, immobilized enzymes were added. The water bath temperature, motor stirring speed, ultrasound power, ultrasound frequency and ultrasound duty cycle were set and the reactions were carried out till equilibrium. Samples from the reaction mixture were removed and analysed after every 10 min. The acid value was determined by titrimetric analysis and the results were calculated by the following equation,

$$\text{Acid value} = \frac{56.1 \times N \times V}{W}$$

Where, N is the strength of alkali required to neutralize the acid, V is the volume of KOH in milliliters and W is the weight of the sample in grams.

3. Results and discussion

The molar ratio of hexanoic acid to ethanol was varied from 1:1 to 1:3, enzyme loading 2% (w/w), and temperature 60 ºC at 200 RPM with 80 W ultrasound power and 50 % duty cycle at 25kHz power. The increase in molar ratio from 1:1 to 1:2 increases the percentage conversion to 85.8% of ethyl hexanoate at 40 min of time. By increasing amount of alcohol further i.e. 1:3 ratio, the conversion decreases; which attributed to the inhibitory effect of ethanol on enzyme. The effect of enzyme loading from 1.5 % to 2.5% (w/w) was studied and 2% (w/w) loading was optimized for further study. At a higher dose of enzyme i.e. 2.5% (w/w), a fall in conversion was observed due to agglomeration of enzymes at very high doses which ultimately increases the viscosity of the reaction mass. In order to study the effect of ultrasound power on the enzymatic synthesis of ethyl hexanoate, reactions were carried out at different ultrasound powers i.e., 40, 60 and 80W keeping all other parameters constant. The maximum conversion 90.45 % was observed at 60W at 40 min. However, high ultrasound power (80 W) can bring about vigorous micro turbulence which can affect the enzyme structure causing its deactivation. This reduces the activity of the enzyme which certainly reduces the % conversion. Effect of duty cycle on % conversion was studied at different duty cycles i.e., 30% (3 min on & 7 min off), 50% (5 min on & 5min off) & 70% (7 min on and 3 min off). Increase in the duty cycle from 30% to 50 % duty cycle increases the conversion from 76.35% to 90.45%; while further increasing duty cycle to 70% reduces the conversion to 80.31%. This is due to the denaturation of enzyme by increase in the exposure time under ultrasonic irradiation [3]. The effect of temperature was investigated at reaction temperature 40℃, 50℃, and 60℃. It was observed that, at 50 ℃ the highest conversion 94.25% achieved; while further increase in temperature diminishes the conversion. At higher temperature, cavitation effect reduces as well as there is possibility of deactivation of enzyme. The reusability study was performed at obtained optimized parameters and found that the catalyst retain around 82 % of its catalytic activity after five consecutive cycles. The results observed at ultrasonic and conventional stirring method are represented in Table 1 which showed that ultrasound can be used to reduce the time, enzyme requirement along with solvent. .

Table 1. Comparison of synthesis of ethyl hexanoate using ultrasonic irradiation with that of conventional stirring method

Reaction condition	**Molar ratio**	**Enzyme loading (% w/w)**	**Temperature (℃)**	**Speed of agitation (rpm)**	**Time (min)**	**% Conversion**
Conventional	01:03	2%	60	250	120	84.68
Ultrasound	01:02	2%	50	200	40	94.25

4. Conclusion

The parameters optimised for ethyl hexanoate synthesis were 50 ℃ temperature, 2 % w/w enzyme loading, 2:1 ethanol to hexanoic acid ration, 60W & 25 kHz ultrasound power and frequency respectively, 50 % duty cycle and 200 rpm agitation speed. Under all optimised conditions and after performing purification steps a final conversion of 94.25 % was attained in only 40 min. The reusability of enzymes was studied up to 5 cycles under the above mentioned conditions. In the absence of ultrasonic wave the conversion of 84.68 % was attained at 60 C temperature and 2 h reaction time. Thus, this work shows that application of ultrasound irradiation to enzymatic synthesis of ethyl hexanoate gave better conversion in a shorter period of time.

References

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