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# Process optimization of lipase catalyzed synthesis of diesters in a packed bed reactor

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ABSTRACT

Wax esters are fine chemicals which are produced in low volume but are highly priced with varied industrial applications. They are formulated in numerous personal care products due to their excellent emollient properties. The conventional technology for the preparation of wax esters are based on reaction between long chain carboxylic acids and alcohols at high temperatures in presence of a strong catalyst. This process is unselective and the removal of used catalyst and byproducts requires several stages which increases the production cost. Enzyme technology is an alternative which is eco friendly and requires lesser stages of purification. The present study aims at the enzymatic preparation of wax esters like dibehenyl adipate and dibehenyl sebacate in presence of immobilized lipases from different microbial origin e.g., RM-IM, TL IM, NS40013 and NS 435. Various reaction parameters like temperature, reaction time, mole ratio, enzyme concentration and solvent selection were optimized. The work clearly indicates NS 435 as the most efficient enzyme and can be recycled for 20 times without significant change in product quality. Trial run was given in a packed bed bioreactor using NS435 which produced 89% and 91% of dibehenyl adipate and dibehenyl sebacate, respectively.



## 1. Introduction

Waxes, or wax esters, are esters of long-chain carboxylic acids and long-chain alcohols. In nature they are found as coatings on fruits and in beehives. They are nonhazardous compounds with high degree of biodegradability. The waxes have attracted the industry since they have innumerable applications in the field of high pressure lubricants, pharmaceuticals, cosmetics, personal care products, wood coatings, printing, leather industries, as well as in candles and polishes. The conventional technology for the production of wax esters is based on the reaction between alcohol and carboxylic acid at high temperatures like 150 °C using strong alkaline or acid catalyst. The reaction is unselective and the waste and catalyst residue need to be removed in post-reaction purification.

These naturally occurring wax esters can also be made from renewable resources, like vegetable oils and also by lipase catalyzed esterification reactions [1]. Ester synthesis by means of either soluble or immobilized lipases contributes an interesting application for the food and additives industry [2–4]. When a lipase is immobilised on a water insoluble carrier, its stability is increased compared with an original lipase. The use of immobilized enzymes particularly lipases, in organic media rather than aqueous media has several advantages, i.e., shift in thermodynamic equilibria in favor of the product over the hydrolysis reaction, increases solubility of nonpolar substrate, eliminates side reactions, enzyme and product recovery are easier and also thermostability of enzyme increases [5–6]. Many researchers studied the effect of different solvents on the enzymatic reaction along with optimization of other reaction conditions. Several researchers have investigated lipase isolation, immobilization for preparation of wax esters based on fatty acids like oleic acid and different long chain alcohols [7]. Further sufficient research work is available on study of the kinetics for lipase catalysed wax esters [8,9]. Very little information is available regarding the usage of dibasic acids for preparation of wax esters and scale up of the optimized data in a reactor to be used commercially. Packed bed reactors have several advantages as they are easy to operate, run continuously and facilitates easy separation of products along with improving enzyme stability and reuse which ultimately reduces the overall process cost [10–12]. Development of PBR is suggested for running lipase catalyzed reactions for industrial use [13].

The present work aimed at preparation of two wax esters namely dibehenyl adipate and dibehenyl sebacate, i.e., diesters of adipic acid (1, 6-hexanedioic acid) and sebacic acid

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(1, 10-decanedioic acid) with behenyl alcohol (22 carbon atoms). Four commercial lipases RM-IM, TL IM, NS40013 and NS 435 of different microbial origin and different specificity were screened for their ability to catalyze the esterification reactions. Out of these four enzymes NS 435 was found to be most efficient. Various reaction parameters like temperature, reaction time, acid, alcohol molar ratio, enzyme concentration and solvent selection were optimized for maximum production of the diesters. The study reveals that the enzyme could be successfully recycled for more than 20 times which shows the commercial viability of the process. Further for commercialization of the products synthesized a trial run was given in simple designed packed bed reactor which ultimately produces 89% and 91% of dibehenyl adipate and dibehenyl sebacate, respectively.

## 2. Materials and methods

### 2.1. Materials

The immobilized lipases used; Lipozyme RM-IM (Source: *Rhizomucor miehei,*) 1,3 specific lipase, TL IM (Source: *Thermomyces lanuginosus,*) 1,3 specific lipase, NS40013 random lipase, NS 435 (Source: *Candida Antarctica*), all these were donated by M/s. Novozymes South Asian Pvt., Ltd., Bangalore, India, for research purpose. Adipic acid, sebacic acid, behenyl alcohol, petroleum ether, hydrochloric acid, sodium hydroxide, ethanol, methanol and all solvents used were obtained from E. Merk (India) Ltd., Mumbai, India.

### 2.2. Lipase catalyzed esterification reaction

The esterification reactions were carried out in a round-bottomed flask fitted with an air condenser, containing adipic acid (0.2 g, 1.36 mM) or sebacic acid (0.2 g, 1.0 mM) with behenyl alcohol in presence of 6 ml isooctane under different reaction conditions, e.g., variation of enzymes, enzyme concentration, acid alcohol ratio and temperature. Reactions were stopped by adding 20 mL ethanol and the excess free fatty acid was neutralized with 0.1 M NaOH. The percentage conversion was calculated from the amount of acid consumed in the reaction.

### 2.3. Isolation of mono and di-ester

After esterification reaction, the product mixture contained unreacted dicarboxylic acids, diester, monoester and the unreacted alcohol. After neutralization with 0.1(M) NaOH, the reaction mixture was extracted thrice with petroleum ether to remove all the diesters produced. The mixture was then acidified with 1(N) HCl and extracted thrice with petroleum ether to recover the monoesters formed. The extracts were evaporated to dryness and monoester and diester formed were estimated gravimetrically. Monoesters and diesters were also confirmed by thin layer chromatography (TLC).

### 2.4. Estimation through gas-chromatographic analysis

Purity of isolated monoester and diesters were also confirmed by GLC analysis. Isolated monoester was converted to mono methyl ester of corresponding alcohols and analysed on a Hewlett–Packard-HP 5890A gas chromatograph (carrier gas – $N_2$, flow rate – 30 ml/min., oven temperature was programmed from 100 °C to 190 °C at 5°/min, Inj. temp – 230 °C, detector – FID, temp – 240 °C). Column – 10% DEGS, 6′ × 1/8” i.d. supported on chromosorb WHP.

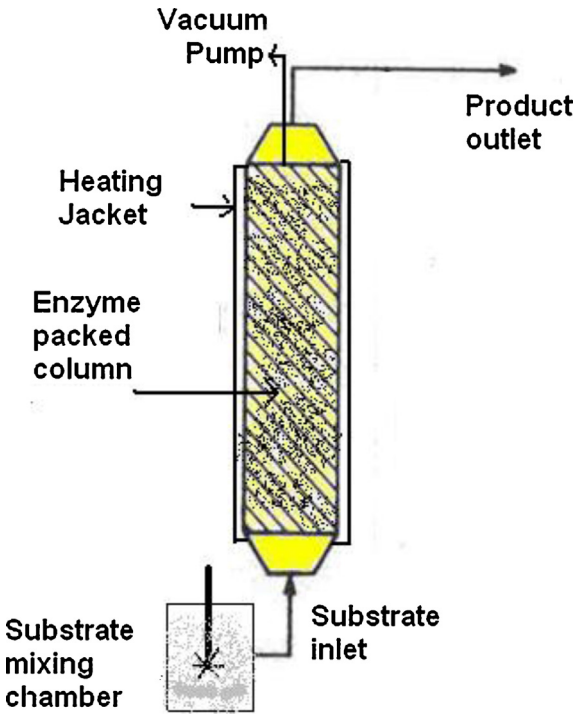


**Fig. 1.** Packed bed reactor.

### 2.5. Packed bed reactor

Trial run was given in a packed bed reactor using the enzyme NS435 with all the optimised parameters. The packed bed reactor apparatus was fabricated [14] in-house and consisted of a glass double walled reaction column of 1 inch diameter packed to approximately 12 inch high with 8.0 g of immobilized lipase NS 435 (immobilised on macroporous acrylic resin). The reaction mixture (mixed previously in a separate chamber for dissolving the substrates) was continuously introduced at a flow rate of 3 mL/min into the column with application of reduced pressure. To achieve a higher rate of conversion the products and water are removed from the reaction mixture in the packed bed column. The water produced in the reaction is continuously removed by the reduced pressure. Fig. 1 shows the packed bed column used under present investigation.

## 3. Results and discussions

Four enzymes namely RM-IM, TL-IM, NS40013 and NS 435 of different microbial origin were screened for their ability to produce dibehenyl esters of adipic and sebacic acid and the obtained results are depicted in Tables 1 and 2. Of the four enzymes under study RM-IM and TL-IM enzymes were observed to be quite inefficient regarding the formation of behenyl adipate esters. The scenarios were different with sebacic acid and behenyl alcohol where RM-IM and TL-IM can produce 48.1% and 53.3% of esters, respectively. NS40013 and NS 435 were found to be quite efficient for both the conversions under study. NS40013 converted 57.3% of adipic and 68.1% of sebacic acid to their respective behenyl esters after 5 h of reaction. But NS 435, the most potential biocatalyst produced 70.7% and 78% of behenyl adipate and behenyl sebacate, respectively. Rest of the study was continued with NS435.

Percent esterification depends on the concentration of enzyme, as more enzymes mean more active binding sites and so the rate of reaction would be higher. Fig. 2 shows that increasing enzyme concentration does not always increase the conversion. 10% enzyme