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# Efficient enzyme-selective synthesis of monolauryl mannose in a circulating fluidized bed reactor

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ABSTRACT

The selective synthesis of 6-*O*-lauryl mannose by lipase-catalyzed esterification of D-mannose with lauric acid in acetone was achieved in a circulating fluidized bed reactor. The effects of operational parameters were investigated and optimized. Lipase of 6 g, expansion ratio of 1.4, mannose of 3.2 mmol/L, lauric acid of 80 mmol/L and substrates flow rate of 1.0 mL/min in acetone at 50 °C were predicted to be the optimum conditions. The average yield in continuous operation for the first 5 days was above 30%, corresponding to a productivity of 59.26 g/L d. Then it decreased to about 25% until 7.5 days later. No diester was detected during the process. It is shown that the new reactor would play an important role in the selective synthesis of monolauryl mannose.

## 1. Introduction

The use of immobilized lipase for synthesis of fatty acid sugar esters has increased dramatically over recent years [1–4]. Enzyme-catalyzed synthesis of various esters is recognized as an alternative way to the chemical one which has the disadvantage of poor selectivity [5–9] such as formation of diesters [10–12]. It has proved that monolauroyl sugar esters like monolauroyl sucrose exhibit better foaming ability and interfacial properties than dilauroyl sucrose [13,14]. Additionally, 6-*O*-lauroyl sucrose and 6-*O*-lauroyl maltose have antibacterial activities while dilauroyl sucrose failed to show the same ability [15], therefore it is valuable to develop the selective synthesis of monolauroyl sugar esters by enzymatic method.

The continuous reaction is preferred to the batch one due to its high efficiency and its convenience of repetitive usage of enzymes. The continuous stirred tank reactors (CSTRs) [16] are widely used for laboratory and small scale assays, but its productivity is relatively low. The weak contact pattern between substrates and enzymes, which is put in a small bag fixed on the reactor to avoid the separation of enzymes and breaking of enzymes granules, is perhaps the main reason for its low productivity. For example, the average yield of monolauroyl maltose was 9.2 g/d L-reactor when continuous stirred tank reactor was used [17]. The packed bed reactors (PBRs) [18] are easier to operate but the productivity is also low. The mass transfer in PBRs is not improved compared with CSTRs owing to the low voidage of packed bed. Furthermore, PBRs might suffer from issues associated with high pressure drop and nonuniform flow distribution. It has been reported that the productivity was 1.25 kg/d L-reactor for the synthesis of lauroyl erythritol using PBR [19]. Thus, the fluidized bed reactors (FBRs) are perhaps more suitable and applicable for continuous operation with immobilized enzymes than CSTRs and PBRs [20].

A liquid-solid circulating fluidized bed reactor (LSCFBR) was used for continuous enzymatic polymerization of phenol [21]. It was operated at very high liquid velocity and the enzyme particles were recycled with the liquid. For the biosynthesis of sugar esters LSCFBR should be modified because the violent collision between particles and tube walls or between particles may also damage the enzymes. Instead of recycling between columns and tubes, the enzyme particles stayed in fluidized bed and only the liquid substrates are recycled. Such reactor may avoid the mechanical damage of enzyme particles due to collision and there is no need of enzyme separation from solution. Furthermore, the residence time of substrates and the fluidizing velocity can be regulated and control separately. Thus, in a circulating fluidized bed reactor (CFBR), a purer and more reproducible product and a far greater productivity from a fixed amount of enzyme than the one achieved in the batch process may be expected.

As a continuation of our interest in enzymatic reactions for selective synthesis of sugar esters [3,12,16], herein we report on the use of a circulating fluidized bed reactor for the selective synthesis of monolauryl mannose ester. The effect of operation parameters such as the enzyme amount, expansion ratio, substrates flow rate and concentration of lauric acid on the yield of monolauroyl mannose were investigated.

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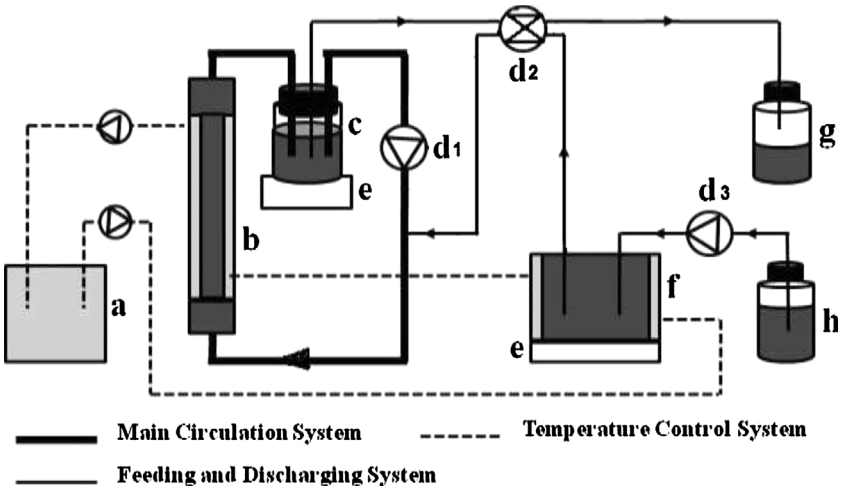


**Fig 1.** Schematic diagram of the experimental apparatus: a, thermostatic water baths; b, fluidized bed; c, fluid reservoir; $d_1$, $d_2$, $d_3$, peristaltic pump; e, magnetic stirrer; f, dissolving tank; g, gathering tank; h, charging stock tank.

## 2. Materials and methods

### 2.1. Materials

Novozyme 435 (lipase B from Candida antarctica, immobilized on a macroporous acrylic resin, 10,000 PLU/g) was purchased from Novo Nordisk Co., Ltd. (Shanghai, China). D-mannose, lauric acid, methanol, acetone, acetonitrile, and *n*-hexane of analytical grade were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and their purity was > 98%. The 4 Å 1/16 molecular sieve was purchased from Shanghai UOP Molecular Sieve Co., Ltd (Shanghai, China). All solvents were dehydrated with 4 Å 1/16 molecular sieves for at least 24 h.

### 2.2. Circulating fluidized bed reactor

The continuous monolauryl mannose production was performed in an upflow circulating fluidized bed reactor. As shown in Fig. 1, a circulating fluidized bed bioreactor is composed of three systems, namely, a Main Circulation System, a Feeding and Discharging System and a Temperature Control System. The main part of the reactor was a glass column (10 × 300 mm) with a jacket. The column was filled with immobilized lipase (2–6 g). A substrate solution was prepared in a stainless steel tank (80 × 100 mm) with a jacket by dissolution of mannose and lauric acid (20 ~ 100 mmol/L) in acetone. The substrate solution was pumped into the column to realize the fluidization by the first HL-1 peristaltic pump ($d_1$) in a Main Circulation System. The HL-2 peristaltic pump ($d_2$) and the second HL-1 peristaltic pump ($d_3$) was used as feed and discharge pump which supplied the substrate solution into the column and removed the reaction mixture. The temperature of the system was maintained at 50 °C by recycling the water prepared in a water baths in the two jackets.

A continuous enzymatic reaction was carried out in the column with immobilized lipase as the reaction vessel. The enzyme particles were fluidized by recirculation of the substrates solution and all the particles kept in the column by the control of fluidization velocity.

### 2.3. Experimentation

The flow rate of the first peristaltic pump was determined by the fluidization velocity which was between the minimum fluidization velocity and the sedimentation velocity. As the fluidization velocity is increased, the voidage of the system was also elevated, as a high value of voidage is advantageous for overcoming the problem of mass transfer. Since the voidage is not easy to measure, it was expressed by the expansion ratio using the following equation (Eq. (1)).

$$R = H_f/H_{mf} = (1 - \varepsilon_{mf})/(1 - \varepsilon_f) \quad (1)$$

Where $H_f$ and $H_{mf}$ represent the height of the fluidized bed at operating velocity and that at the minimum fluidization velocity, respectively; $\varepsilon_f$ and $\varepsilon_{mf}$ represent the void fraction in the two cases, respectively.

Another parameter of importance is the superficial residence time of the substrate solution, which should be at its optimum to guarantee the synthesis of monoester but prevent the formation of diesters. The residence time, $\tau_0$, was calculated using the equation:

$$\tau_0 = V_{tot} \times \varepsilon/v$$

where $V_{tot}$ is the fluidized bed volume, $\varepsilon$ the void fraction, and $v$ the substrates flow rate.

As the solubility of mannose in acetone is poor [22], the experimentation utilized a sufficient quantity of the sugar to saturate the acetone solution with mannose (3.2 mmol/L) in the solution tank.

### 2.4. Analysis

#### 2.4.1. High-performance liquid chromatography

The reaction mixture was analyzed by HPLC using a Sunfire-C18 column (4.6 × 250 mm, Waters, USA) eluted with methanol/water (90:10, v/v) at 1 mL/min, and the eluate was monitored by a Waters 2420 evaporative light scattering detector (ELSD). ELSD conditions were as follows: drift-tube temperature 45 °C, sprayer temperature 36 °C, carrier gas pressure 20 psi, and gain 1. The calibration curves were prepared using the purified products according to the methods described in our previous report [11].

All samples were performed in triplicate. Sigma Stat® software version 2.0 (Jandel Scientific/SPSS Inc., Chicago, IL, USA) was used to determine the mean differences ($p < 0.05$) with analysis of variance (ANOVA).

#### 2.4.2. FT-IR, NMR and mass spectrometry analysis

The infrared spectra were taken on Nicolet Nexus FT-IR. $^1$HNMR and $^{13}$C NMR spectra were recorded on a Varian INOVA spectrometer at 30 °C in $CD_3OD$. Chemical shifts were referred to the methanol multiplet, centered at 3.30 ppm for $^1$H NMR and 49.0 ppm for $^{13}$C NMR. The $^1$H NMR spectra showed chemical shifts and coupling constants within ± 0.005 and ± 0.5 Hz, respectively. Mass spectra were obtained by Mass Spectrometry (Waters Platform ZMD 4000, Milford, MA, USA) with positive EI mode, and the ionization conditions were as follows: capillary voltage 3.87 kV; cone voltage 30 V; and extractor voltage 7 V. The source block temperature was 120 °C, and the desolvation temperature was 300 °C. The electrospray probe flow was adjusted to 70 mL/min. Scanning of sample was recorded over the range of *m/z* 200–1000 with a scan time 1 s and an interscan delay of 0.1 s.