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A novel process for enzymatic synthesis of *N*-lauroyl-β-amino propionitrile using packed bed reactor coupled with on-line separation

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Abstract

N-Lauroyl-β-amino propionitrile is an intermediate for synthesis of sodium *N*-lauroyl-β-alanine, an antimicrobial surfactant. We provide a novel process for enzymatic synthesis of *N*-lauroyl-β-amino propionitrile, using a cascade connection of an enzyme packed bed reactor (EPBR) with a crystallization separator for on-line separation. The substrate solution was fed to the reactor inlet. High-purity crystal product was obtained from the separator outlet with a yield of 91.7% under the optimum conditions. The immobilized lipase can be utilized repeatedly. The solvent and unreacted substrates were recovered and reused on-line.

Keywords: Enzymatic synthesis; *N*-Lauroyl-β-amino propionitrile; Lipase; β-Amino propionitrile; Organic media

1. Introduction

N-Lauroyl-β-amino propionitrile is a precursor of *N*-lauroyl-β-alanine, the latter can be used as an antimicrobial surfactant after saponification in many fields such as food, cosmetics, pharmacy and textile [1–3]. Lipase-catalyzed synthesis in nonaqueous media has been popular since 1990 as a mild synthesis strategy. One of the advantages of enzymatic synthesis of *N*-lauroyl-β-amino propionitrile is, after hydrolysis, there is no sodium laurate in the final surfactant product (Scheme 1). Sodium laurate is an unavoidable by-product in chemical synthesis of sodium *N*-lauroyl-β-alanine, which is synthesized by reacting β-alanine and lauroyl chloride in sodium hydroxide aqueous solution [3].

Izumi et al. [4] used lipase from *Candida antarctica* (CAL) as the catalyst for amidation of β-amino propionitrile to synthesize *N*-lauroyl-β-amino propionitrile. They found that room temperature was the suitable reaction temperature. In a batch reactor, using equimolar reactants in diisopropyl ether and equal weight of deionized water sprayed on the enzyme (7000 U of the lipase per gram of β-amino propionitrile), the highest yield (99.3%, determined by TLC) was achieved after 24 h. In our previous study [5], in a batch reactor at room temperature, the reaction rate was considerably slow despite using such a high amount of lipase (7000 U of the lipase per gram of β-amino propionitrile). Meanwhile, the product precipitated and adhered to the immobilized lipase surface, leading to a great loss of lipase activity during the separation of product and the enzyme. Under optimal reaction conditions, with a lower lipase dosage (200 U/g β-amino propionitrile) and equimolar reactants in diisopropyl ether (35 g β-amino propionitrile/l), the highest conversion (96.8%, determined by HCl titration) of β-amino propionitrile was obtained after 24 h at 70 °C [5].

As mentioned above, it is unavoidable that *N*-lauroyl-β-amino propionitrile can precipitate in batch reactor at room temperature. Both mechanical stirring and the separation process cause the lipase activity loss. Thus if we apply the enzymatic amidation at a high temperature in an EPBR, adjusted the operation parameters to avoid the precipitation, and then

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$$NH_2(CH_2)_2CN + CH_3(CH_2)_{10}COOCH_3 \xrightarrow{CAL} CH_3(CH_2)_{10}CONH(CH_2)_2CN + CH_3OH \quad (1)$$

$$CH_3(CH_2)_{10}CONH(CH_2)_2CN + H_2O \xrightarrow{NaOH} CH_3(CH_2)_{10}CONH(CH_2)_2COONa \quad (2)$$

Scheme 1. Preparation of sodium *N*-lauroyl-β-alanine via enzymatic synthesis of *N*-lauroyl-β-amino propionitrile: (1) lipase-catalyzed synthesis of *N*-lauroyl-β-amino propionitrile; (2) alkaline hydrolysis of *N*-lauroyl-β-amino propionitrile to produce sodium *N*-lauroyl-β-alanine.

separated the crystal product at a low temperature, a high product yield with desired purity as well as low lipase activity loss can be expected. Hence an EPBR coupled with an on-line crystallization separator was used in this study, to utilize the lipase repeatedly, to reuse the solvent and unreacted substrates on-line after simple processing.

2. Experimental

2.1. Chemicals

Immobilized lipase from *Candida antarctica* (CAL) with olive oil hydrolysis activity of 7000 U/g was a gift from Novo Nordisk Company. One unit (U) of olive oil hydrolysis activity is defined as the amount of the lipase-catalyzed production of 1 μmol of free fatty acid per minute at 40 °C, pH 9.

β-Amino propionitrile was synthesized in our lab and distilled before use. Methyl laurate was purchased from Haiyan Fine Chemicals Co., Zhejiang, China (99.1% after rectification, GC). All other reagents were of analytical grade and were purchased from Shanghai Chemicals Co., China. Reactants were dehydrated with 4A zeolite before use, unless otherwise stated.

2.2. Analysis

Water content was determined by Karl Fischer titration (K90290 automatic Karl Fischer volumetric titrator, Koehler Instrument Company, USA). The conversion of β-amino propionitrile was determined by HCl (in isopropyl alcohol) titration and calculated with the following equation:

$$X = 100\left(1 - \frac{A_1}{A_0}\right)$$

Here A_0 was the consumed HCl (mmol) per gram of the reactants solution before reaction and A_1 was the consumed HCl (mmol) per gram of the reaction mixture obtained from the reactor outlet.

2.3. Enzymatic synthesis of N-lauroyl-β-amino propionitrile in batch reactor

For a typical experiment, methyl laurate (20 mmol) and β-amino propionitrile (20 mmol) in diisopropyl ether (40 ml) were homogenized using a magnetic stirrer and heated to 70 °C in a glass reactor. CAL (200 U/g β-amino propionitrile) was then added into the mixture, which was stirred at 70 °C. The reaction was stopped with addition of isopropanol when the conversion of β-amino propionitrile reached a constant.

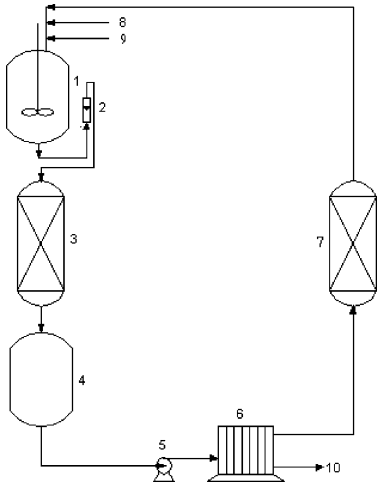

Fig. 1. The scheme of the reaction system: 1, elevated tank; 2, flow meter and valve; 3, EPBR; 4, crystallization tank; 5, slurry pump; 6, crystallization separator; 7, absorption column; 8, fresh methyl laurate; 9, fresh β-amino propionitrile and solvent; 10, crystal product.

2.4. Enzymatic synthesis of N-lauroyl-β-amino propionitrile in EPBR

As shown in Fig. 1, the reaction system consists of an elevated tank (1) (for mixing and storing raw reactants, equipped with a refluxing tube and a mechanical stirrer), an EPBR (3) (0.7 cm in diameter and 5–8 cm in height, filled with CAL), a crystallization tank (4), a crystallization separator (6) and a methanol absorption column (7) filled with $CaCl_2$. Equimolar of methyl laurate (8) and β-amino propionitrile in diisopropyl ether (9) (400 ml/g β-amino propionitrile) were well mixed in the elevated tank and pre-heated to 65 °C. The reactant solution was then delivered through the EPBR (65 °C) with a designed flow rate (2) to the crystallization tank, where the crystal *N*-lauroyl-β-amino propionitrile precipitated at 25 ± 1 °C. The slurry was pumped to the crystallization separator by a slurry pump (5) and separated by the filter film in the separator. The precipitate (10) was washed with cold 95% ethanol and dried for subsequent hydrolysis to produce sodium *N*-lauroyl-β-alanine; while the filtrate was delivered to an adsorption column to remove methanol, the by-product. After the determination of β-amino propionitrile content, the adsorption column effluent was mixed with fresh reactants and solvent to make-up for loss and then returned to the elevated tank. The temperature at all other parts in the system,

excluding the section from the elevated tank to the outlet of EPBR, was maintained at 25 ± 1 °C.

3. Results and discussion

3.1. Flow style and substrate concentration

A downstream flow was applied to avoid back-mixing. The reactant stream flowed from the elevated tank to the EPBR. The flow rate was monitored by a flow meter. The product concentration increased along the reactor vertically from the inlet to the outlet, where the product content in the reaction mixture was the highest. Therefore an appropriate initial substrates concentration is required to ensure a reasonable product concentration at the reactor outlet to prevent precipitation.

In a batch reactor, 35 g of β-amino propionitrile per liter diisopropyl ether was the optimal substrate concentration [5]. However, in the EPBR, the product slightly precipitated at the reactor outlet if the initial substrate concentration was above 2.7 g of β-amino propionitrile per liter diisopropyl ether. Thus a diluted initial substrate concentration (2.5 g of β-amino propionitrile per liter diisopropyl ether) was applied based on previous experiments (data not shown), the product solubility under the suitable reaction temperature (as shown in Fig. 2), and the operation flexibility.

3.2. Reaction temperature

As shown in Fig. 2, the optimal reaction temperature in a batch reactor was 70 °C. However, in a column reactor, diisopropyl ether would be partly vaporized at 70 °C during the reaction, which generated bubbles in the column reactor. Considering the solubility of *N*-lauroyl-β-amino propionitrile, the reaction rate, as well as the enzyme thermostability (data not shown), the reaction and delivery (from elevated tank to the reactor) temperature was set to 65 °C. Under this condition, neither solvent vaporization nor product precipitation was observed during the reaction.

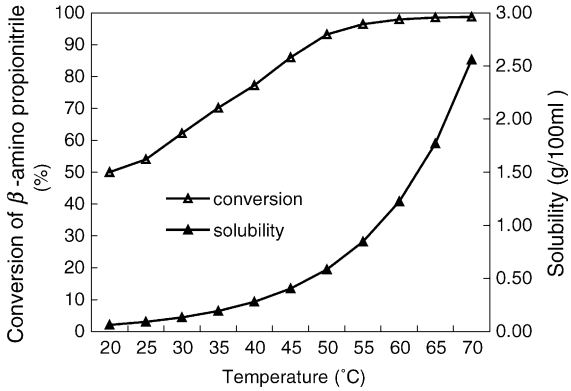

Fig. 2. The solubility of *N*-lauroyl-β-amino propionitrile in diisopropyl ether and the effect of temperature on conversion of β-amino propionitrile in batch reactor. Reaction conditions: β-amino propionitrile, 0.35 g; methyl laurate, 1.07 g; diisopropyl ether, 10 ml; 0.01 g of CAL (200 U/g β-amino propionitrile); water, 0.021 g, 24 h.

3.3. Water dosage

Water is essential for lipase-catalyzed reactions [4,6,7]. CAL is usually used together with equal weight of deionized water according to Novo Nodisk product brochure. Some investigators also used this protocol for CAL-catalyzed amidation of β-amino propionitrile [4]. For example, 0.4 g of CAL associated with 0.4 g of water was added in a system containing 0.4 g of β-amino propionitrile and 20 ml of diisopropyl ether [4]. For an enzymatic reaction performed in nonpolar solvent system, higher conversion is usually obtained at low initial water content in the reaction mixture [8]. As indicated in Fig. 3a, the optimal initial water content in batch reactor is 6% water in β-amino propionitrile. While as shown in Fig. 3b, without addition of water, the conversion of β-amino propionitrile (95.6%) was not significant lower than that (97.2%) at the optimal water dosage. This can be explained that the commercial CAL was not a "dry" lipase but contained 2% of water (w/w) and so the reaction without addition of water in β-amino propionitrile also resulted in high conversion. Therefore, no water was added to initialize the reaction in

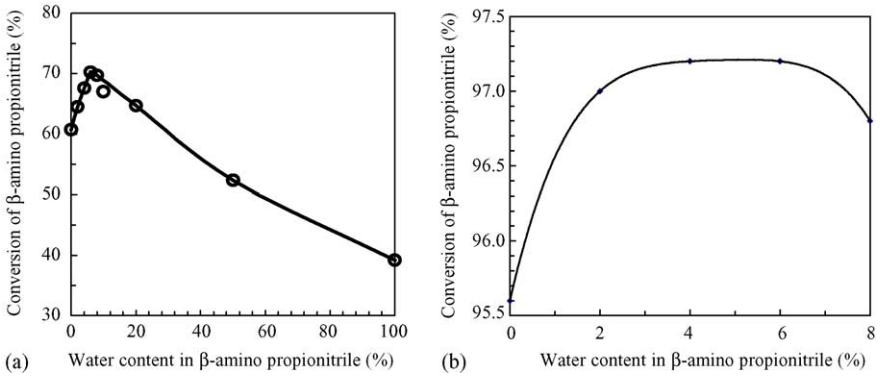

Fig. 3. The effect of water content on the conversion of β-amino propionitrile in tank reactor. Reaction conditions: β-amino propionitrile, 0.35 g; methyl laurate, 1.07 g; 10 ml of diisopropyl ether; (a) 0.15 g of CAL (3000 U/g β-amino propionitrile), 35 °C, 6 h; (b) 0.01 g of CAL (200 U/g β-amino propionitrile), 70 °C, 24 h.

Table 1
The effects of reactor bed height and space velocity on total conversion of β-amino propionitrile

Run	Reactor bed height (cm)	Flow rate of reactant mixture (ml/min)	Space velocity (min^{-1})	Conversion (%)
1	3.5	2.7	1.98	68.6
2		2.3	1.70	78.7
3		1.9	1.38	89.9
4	4.5	2.4	1.40	81.6
5		1.9	1.10	90.7
6		1.7	0.98	91.7
7		1.0	0.58	94.3
8	6.0	2.3	0.99	91.4
9		1.9	0.82	92.7
10		1.5	0.67	94.0

Packed bed reactor, equimolar of reactants, 65 °C, β-amino propionitrile:diisopropyl ether = 1:400 (w:v).

the column reactor. Additionally, the water activity of the reaction system was the same as that in the atmosphere since the system had been contacted with the atmospheric environment through the venting tube (refluxing tube) of the elevated tank.

3.4. Effect of packed bed height on total conversion of β-amino propionitrile

As mentioned in Section 3.1, flow rate is very important for a sufficient product concentration at the column reactor outlet, and the reactor bed height likewise. These two parameters are synergic; therefore, they were investigated simultaneously.

Table 1 illustrates the effects of reactor bed height and space velocity on the conversion of β-amino propionitrile at the reactor outlet. To determine the suitable operation parameters, a high conversion, less lipase consuming (lower bed height) and shorter operation time (higher space velocity) are required.

As shown in Table 1, the conversions in run 5 to run 10 are similar. The conversions in run 7 and run 10 are higher (around 94%), but the space velocities are only around 60% of that of run 6 and run 8; space velocities and the conversions in run 6 and run 8 are almost same, but run 6 consumed 25% less lipase. Therefore, reaction conditions in run 6 were taken as the suitable operating parameters because of the considerable high conversion, low enzyme amount and short operation cycle. In this run, the conversion of β-amino propionitrile at the reactor outlet was 91.7% at a flow rate of 1.7 ml/min with the reactor bed height of 4.5 cm. The raw crystal product was then washed with cold (5–10 °C) 95% ethanol. No trace of β-amino propionitrile or methyl laurate was found after drying the crystal under vacuum. No obvious loss of the product was observed after the washing and drying.

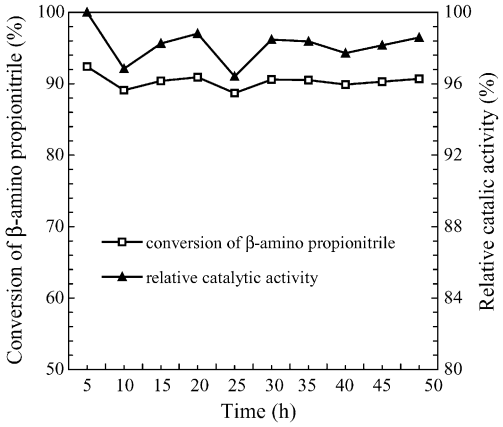

Fig. 4. The conversion of β-amino propionitrile vs. operation time. Packed bed reactor, equimolar of reactants, 65 °C, β-amino propionitrile:diisopropyl ether = 1:400 (w:v).

3.5. Operation stability of the system

It was observed that the conversion of β-amino propionitrile was decreased if the filtrate from the crystallization separator was recycled without removing methanol. This is because methanol inhibited the lipase activity. Thus, in order to remove methanol, an adsorption column filled with $CaCl_2$ was inserted between the crystallization separator and the elevated tank. After removing methanol, the filtrate was mixed with the fresh reactants to the required substrate concentration before it was fed to the elevated tank. No obvious ill effect on the conversion was observed. Fig. 4 illustrated that the conversion of β-amino propionitrile did not drop significantly even after 50 h reaction.

4. Conclusion

CAL-catalyzed amidation of β-amino propionitrile to *N*-lauroyl-β-amino propionitrile in a packed bed reactor coupled with on-line separation system was investigated. The conversion of β-amino propionitrile at the reactor outlet reached 91.7% under following operation parameters: flow rate of 1.7 ml/min, reactor bed height of 4.5 cm (reactor: 0.7 cm in diameter and 6.5 cm in height), reacted at 65 °C, and crystallized at 25 °C.

The conversion of β-amino propionitrile remained steady (around 91%) up to 50 h. The final crystal product can be purified simply with an ethanol wash and vacuum drying.

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