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Development of a continuous L-lysine bioconversion system for cadaverine production
Continuous bioconversion of L-lysine into cadaverine using barium alginate packed reactor

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ABSTRACT

Cadaverine, a five carbon diamine (1,5-diaminopentane), plays a role as a building block of polyamides and it can be made by fermentation or direct bioconversion. To improve its production by increasing reusability of immobilized enzyme and avoid separation of enzyme in bioconversion, a continuous L-lysine bioconversion process for cadaverine production has been developed. Various divalent cations, alginate concentrations, cell density with alginate and flow rate of feed were examined to maximize the lysine decarboxylase activity of the whole-cell immobilized beads. Under the selected conditions, 123 h of continuous cadaverine production has been performed and 5.5 L of 819 mM cadaverine were produced with 14 mL reactor resulting in 466.5 g of cadaverine. Cadaverine production was possible with small volume reactor maintaining relatively high concentration of substrate.

Introduction

Q4 Increasing energy demands along with environmental problems have stimulated development of sustainable industry. Therefore, as an alternative to petrochemical production, biochemical production has received attentions [1–5]. However, economic feasibility has prevented its successful industrialization. Compared with petrochemical production, biochemical production has several drawbacks, such as relatively low production titer, purity, and productivity [6]. Therefore, to overcome the economic barrier, developing a cost-efficient bioprocess is an important step in the industrialization of biochemical production.

Cadaverine, a five carbon diamine (1,5-diaminopentane), plays a role as a building block of polyamides such as PA 5.4, 5.6, 5.10, and 5.12, which can be alternatives to petroleum based polyamides [7]. In particular, bio-based PA 5.10 has mechanical properties comparable to those of the well-established petrochemical polyamides PA 6 and PA 6.6, suggesting it to be a possible replacement for conventional petrochemical nylons. As the global market for petroleum-based polyamides has increased, and is currently ~6.6 million tons per year, interest in biological cadaverine production has also increased [7]. Cadaverine is produced by decarboxylation of L-lysine, which is an essential amino acid synthesized by bacteria and plants, by lysine decarboxylase (E.C. 4. 1. 1. 18). Metabolic engineering has enabled *Escherichia coli* and *Corynebacterium glutamicum* to overproduce cadaverine with various substrates [8–12]. Particularly, using a L-

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lysine over-producing *C. glutamicum* as a host strain, high titers (103 g/L) and molar yields (50%) were achieved [13].

Direct bioconversion of concentrated L-lysine into cadaverine using whole-cell biocatalysts is another possible cadaverine production process [14,15]. Because lysine production has been successfully commercialized, biotransformation using commercial lysine as a substrate could be considered. *E. coli* whole-cell biocatalysts overexpressing lysine decarboxylase achieved a 92–99% conversion ratio with L-lysine over 1 M (mol/L) [14–16]. In addition, immobilized whole-cell biocatalyst enabled repetitive reactions by increasing the stability of the *E. coli* whole-cell catalyst [17]. Accordingly, it is important to develop and evaluate processes for industrialization of cadaverine production [14].

In this study, we designed a continuous cadaverine production system based on an immobilized *E. coli* whole-cell biocatalyst. A bead-packed column reactor was designed for continuous L-lysine bioconversion into cadaverine by optimizing the reaction efficiency through adjusting immobilization and operating conditions. The simple and small reactor (14 mL working volume) was operated for 123 h with a constant flow rate and achieved continuous cadaverine production. This report suggests the feasibility of cadaverine production using immobilized whole-cell biocatalysts and provides insight into bulk cadaverine production using a continuous bioconversion reactor.

Immobilized cells have long been applied to continuous production systems [18–24]. These systems have potential for industrial production using various types of packed-bed reactors. Whole-cell bioconversion of L-lysine into cadaverine is a rapid reaction that converts over 90% of 1 M L-lysine into cadaverine within 2 h [15]. We reported previously that immobilization of the whole-cell biocatalyst using barium alginate was stable and effective for repetitive reactions [17]. Therefore, as a proof of concept, we designed a continuous process using a packed-bed column reactor and investigated its potential for industrial production of cadaverine (Fig. 1). The designed glass reactor had a working volume of 14 mL and height of 14 cm, and was able to be packed with about 1500 2-mm-diameter beads. The beads encapsulate *E. coli* whole-cells overexpressing lysine decarboxylase (CadA). Feed would pass through the bead packed reactor with constant flow rate. As it undergoes reaction, carbon dioxide (CO_2) gas is emitted to top of the reactor and product would be collected to the effluent storage.

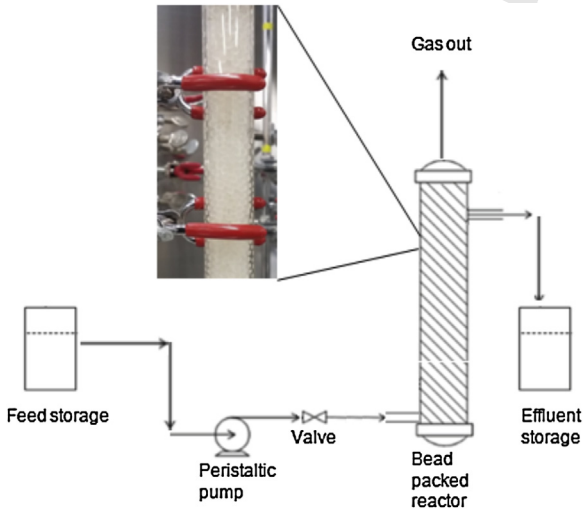

Fig. 1. Schematic diagram of continuous cadaverine production by the L-lysine bioconversion system. The bead packed reactor contains alginate beads encapsulating *E. coli* whole-cell lysine decarboxylase (CadA).

Because barium alginate immobilization reduced the conversion rate by 50% [17], further optimization was required. The interaction between divalent cations and alginate determines the structure and complexation of alginate gels because of the different affinity of alginate towards cations [25,26]. Therefore, we screened out divalent cations to improve activity. The lysine decarboxylase activity of immobilized whole cells using six divalent cations, Mg^{2+}, Cu^{2+}, Zn^{2+}, Sn^{2+}, Co^{2+}, Mn^{2+} and the equal amount of free cell for control was compared to that of a barium alginate whole-cell catalyst (Fig. 2A). Except Mg^{2+}, they formulated sphere beads while their intensity was different from each other. Unfortunately, however, only alginate gels using Sn^{2+} or Ba^{2+} retained their shape after 1 M L-lysine bioconversion. Also, barium alginate exhibited the highest activity among the alginate beads. Because calcium alginate beads have been reported to be unstable [17], use of Ba^{2+} for whole-cell immobilization of the *E. coli* overexpressing lysine decarboxylase would be the most effective cation. For further improvement, the sodium alginate concentration was varied (Fig. 2B). At alginate concentration with less than 1.5%, no significant reduction in whole-cell activity was observed. However, above 1.5% alginate, the whole-cell activity decreased. In general, a low alginate concentration in beads results in higher permeability but lower strength [27,28]. Likewise, an alginate

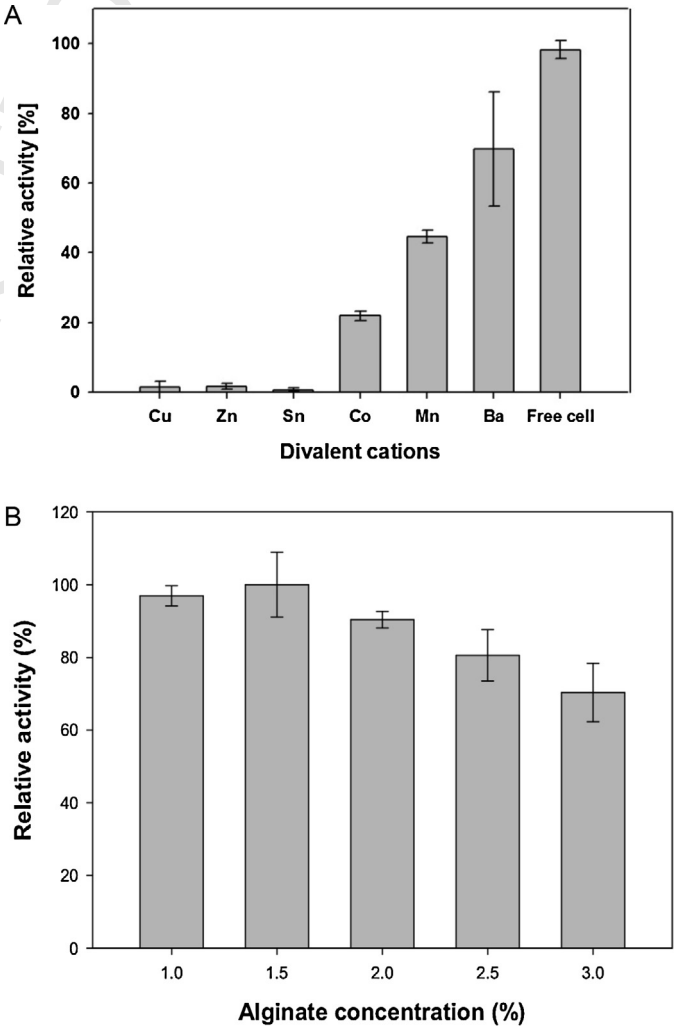

Fig. 2. Optimization of whole-cell immobilization. A: Effect of divalent cations, B: Effect of alginate concentration on lysine decarboxylase activity. The *error bars* represent standard deviations of three independent experiments.

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