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A novel and environment-friendly bioprocess of 1,3-propanediol fermentation integrated with aqueous two-phase extraction by ethanol/sodium carbonate system

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ABSTRACT

An integrated fermentation–separation process for the production of 1,3-propanediol (1,3-PD) was investigated. Aqueous two-phase system (ATPS) not only recovered 97.9% of 1,3-PD, but simultaneously also removed 99.1% cells, 81.9% proteins, 75.5% organic acids, and 78.7% water. Furthermore, after extraction the bottom phase of ATPS was used to adjust the pH of the culture during fermentation, leading to 16% and 126% increases in the concentrations of 1,3-PD and lactic acid, and dramatic decreases in the concentration of acetic acid and formic acid. The total mass conversion yield of three main products (1,3-PD, 2,3-butanediol, and lactic acid) from glycerol reached 81.6%. The salt-enriched phase could also be used to absorb carbon dioxide (CO_2), resulting in 94% recovery for carbonate. Finally, process simulation using the program PRO/II showed the use of ATPS reduced 75.1% of the energy expenditure and 89.0% of CO_2 emissions.

1. Introduction

1,3-Propanediol (1,3-PD) is an important monomer for the production of high performance polyester such as polytrimethylene terephthalate (PTT). It has attracted a great deal of attention in the fiber and textile industries due to its excellent physical properties (good tensile elastic recovery, good dye ability) [1–3]. In the last decade, microbial conversion of glycerol and/or glucose into 1,3-PD has been the focus of research and development due to its renewable resource and high level of safety under mild reaction conditions [4–9]. However, apart from the considerable progress in fermentation, research in the separation of 1,3-PD from the fermentation broth has not progressed smoothly due to its high boiling point (214 °C at atmospheric pressure) and hydrophilicity, as well as the low concentration in the complex fermentation broth. Although many separation techniques have been investigated during the past 20 years, such as steam stripping and vacuum distillation, solvent extraction, reactive extraction, ion exchange resins, activated carbon or molecular sieve adsorption and zeolite membranes [10–13], all the separation techniques and methods have some limitations or drawbacks [14]. Most of them require complicated processes and high energy input, resulting in a large portion of the total cost.

Liquid-liquid extraction has attracted much attention, but no available extractants are good enough for commercial application due to the low partition coefficient of 1,3-PD [10,15–18]. Some previous works have shown that a novel aqueous two phase extraction (ATPE) or salting-out extraction composed of short chain alcohol/salt systems such as ethanol/ammonium sulfate, ethanol/phosphate and methanol/phosphate systems can effectively extract 1,3-PD from fermentation broth. However, recycling the huge amount of salt in the bottom phase is an issue that needs to be resolved at the commercial level. Dilution crystallization was an effective method, but it needs 1.5-2 times the amount of methanol, resulting in large energy consumption for methanol recovery [12,19,20]. An effective and economical method of recycling the salt is the key factor to the commercial application of ATPS in the extraction of 1,3-PD from fermentation broth.

Another potential problem that can restrict the commercial production of 1,3-PD is the environmental concerns, especially since the process is accompanied by a large amount of carbon dioxide (CO_2) emission. Even until now, the emission of CO_2 from the commercial production of 1,3-PD has not been recognized because the level of CO_2 produced from 1,3-PD fermentation is lower than that produced from chemical synthesis. However, CO_2 emission associated with 1,3-PD separation is very high due to the high consumption of energy. As a result, almost no study has been undertaken to address this question.

Recently, the main study on capture and sequestration of CO_2 is focus on the conventional petrochemistry and usually includes two main steps. The first step is the adsorption or absorption of

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CO_2 by means of physical, chemical or biotechnological methods [21–25]. The second step is CO_2 fixation, such as ocean fertilization, biological carbon sequestration, and mineral carbonation [22,26–28]. However, most of them are part of the production process and consume additional energy and require additional equipment investment. Given the fact that CO_2 emission will come under stricter regulation world-wide, an integrated method to capture and sequester CO_2 upon its production can undoubtedly make it a commercially attractive venture.

In this paper, an aqueous two-phase system (ATPS) composed of ethanol/sodium carbonate was used to separate 1,3-PD from the fermentation broth. Influences of the phase forming components on the partition of 1,3-PD and the removal of proteins, organic acids and cells were studied. The possibility of recycling the bottom phase, which contained most of the sodium carbonate and glycerol, was also investigated. Furthermore, the use of the residual salt-enriched phase to absorb CO_2, resulting in the coupling of salt recovery with the capture and sequestration of CO_2, was also instigated. This study provided a simple and environmentally friendly method for the production of 1,3-PD via fermentation, and its subsequent separation from the fermentation broth by ATPS.

2. Materials and methods

2.1. Materials

1,3-PD and organic acid standards were purchased from Sigma Chemical Co. *Klebsiella pneumoniae* (CGMCC 2028) was isolated from soil and preserved in China General Microbiological Culture Collection Center (CGMCC, Beijing, China). Coomassie Brilliant Blue G250 was purchased from Shanghai Boao Biotechnology Corp. Cellulose triacetate hollow fiber dialyzer with effective surface area of 1.5 m^2 and cut-off molecular weight of 5000 was manufactured by NISSHO Corp, Osaka, Japan. All other chemicals were of analytical grade. The concentration of 1,3-PD, 2,3-BD, ethanol and residual glycerol in the fermentation broth were 51.8, 2.44, 11.2 and 18 g/L, respectively.

2.2. Phase diagram of ATPS composed of ethanol/sodium carbonate

The phase diagram of ATPS was obtained by using a turbidity titration method [19]. Sodium carbonate dissolved in de-ionized water was added to a test tube, and ethanol was then added dropwise to the tube placed on an analytical balance with a precision $\pm 10^{-7}$ kg for measuring the amount of added ethanol. After each drop, the mixture was vortexed for 3 min. When one drop of ethanol was added, the mixture became turbid (which indicated the presence of a second phase), and an additional drop of ethanol was added which caused no solid precipitation, the point at which the mixture first became turbid was considered as the turbid point. The total amount of added ethanol was precisely measured by weighing, and the concentrations of ethanol and sodium carbonate at different turbid points were calculated from the following equations and the phase diagram curve was plotted.

$$w_1 = \frac{m_1}{m_1 + m_2 + m_3} \quad (1)$$

$$w_2 = \frac{m_2}{m_1 + m_2 + m_3} \quad (2)$$

where w_1 and w_2 represent the mass fraction of ethanol and sodium carbonate, respectively. m_1, m_2, and m_3 represent the amount of added ethanol, sodium carbonate, and water, respectively. The effect of 1,3-PD on phase separation (as examined by phase diagram) was investigated by adding sodium carbonate to various solutions of 1,3-PD. The turbid points for the solution were then determined by adding ethanol dropwise as described above.

2.3. Partition behavior of 1,3-PD in ATPS

The fermentation broth was first filtered by a cellulose triacetate hollow fiber, which could remove all the cells and most of the proteins. Solid Na_2CO_3 and ethanol were added to the clarified filtrate to form aqueous two-phase systems consisting of 5–26% (w/w) ethanol and 10–22.5% (w/w) Na_2CO_3. The mixture was held for 3 h at 37 °C. The partition of 1,3-PD in ATPS for the clarified filtrate was studied by adding solid Na_2CO_3 to a solution containing 5–25% (w/w) ethanol to saturation level. The same experiment was carried out for unfiltered fermentation broth. A four-stage extraction was also conducted by infusing the top phase of the ATPS (consist of 10% ethanol and sodium carbonate concentration set at saturated level) counter-currently into 5 times volume of the salt-saturated bottom phase in the four extractors [29]. The concentrations of 1,3-PD and 2,3-butandiol (2,3-BD) in the top and bottom phases were analyzed by gas chromatography. The concentrations of organic acids in the top and bottom phases were analyzed by high performance liquid chromatography (HPLC). The partition coefficient (K), the recovery (Y), and the enriched factor (E) defined as follows:

$$K = \frac{C_t}{C_b} \quad (3)$$

$$Y = \left(\frac{V_t \times C_t}{V_t \times C_t + V_b \times C_b}\right) \times 100\% = \left(\frac{R \times K}{R \times K + 1}\right) \times 100\% \quad (4)$$

$$E = \frac{C_t}{C_f} \quad (5)$$

where C_t, C_b and C_f represent the concentration of compound in the top phase, the bottom phase and the fermentation broths, respectively. V_t, and V_b represent the volume of the top phase and the bottom phase, respectively. R represents the volume ratio of the top phase and the bottom phase.

2.4. Fermentation

Cells were grown in 250-mL shake-flasks containing 100 mL medium at 37 °C for 12 h. Fed-batch fermentation was conducted in a 5-L stirred bioreactor containing 3 L chemically defined medium [7], with inoculation volume of 10% (v/v). The temperature and the agitation rate were controlled at 37 °C and 200 rpm, respectively. Initial glycerol concentration was 40 g/L, but additional glycerol was added to maintain the concentration between 15 and 25 g/L during fermentation. A micro-aerobic environment in the bioreactor was maintained by sparging air at 0.02 vvm. The pH was controlled at 7 by automatic addition of 5 M NaOH, 2.5 M Na_2CO_3, and the bottom phase of the ATPS, respectively. The product formation rate (q_p) and the substrate uptake rate (q_s) were calculated as follows:

$$q_p = D\left(C_p - \frac{n_{p0}}{V}\right) \quad (6)$$

$$q_s = D(C_{s0} - C_s) \quad (7)$$

where D is the dilution rate, C_p and C_s is the product and the residual glycerol concentration, respectively. C_{S0} is the glycerol concentration in the feed medium, n_{p0} is the molar amount of the production in the added bottom phase, V is the volume of the fermentation broth. The metabolic flux distributions of the product were obtained by dividing the product formation rates (q_p) by the glycerol uptake rate (q_s) and multiplying by 100.