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Extraction of 1,3-propanediol from glycerol-based fermentation broths with methanol/phosphate aqueous two-phase system

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ABSTRACT

An aqueous two-phase system consisting of methanol and phosphate was used to extract 1,3-propanediol from glycerol-based fermentation broth. Methanol not only extracted 1,3-propanediol effectively from the broth through forming aqueous two-phase system with phosphate, but also allowed the recovery of phosphate by dilution crystallization through adjusting the pH. At 35% (v/v) methanol, saturated concentration of phosphate and a pH of 10.7, the partition coefficient and recovery of 1,3-propanediol reached 38.3 and 98.1%, respectively. The recovery of phosphate in the bottom phase reached 94.7% when 1.5 volume of methanol was added to the salt-rich phase after adjusting the pH to 4.5. At the same time, the main byproduct, 2,3-butandiol was also extracted with high efficiency. In addition, cells and proteins could be simultaneously removed from the fermentation broth, and the removal ratios for cells and proteins reached 99.85% and 92.4%, respectively.

1. Introduction

1,3-Propanediol (1,3-PD) is a chemical intermediate in organic synthesis and an important monomer for the production of high performance polyester such as polytrimethylene terephthalate (PTT), which has been given much attention by the fiber and textile industry [1,2]. Microbial production of 1,3-PD has attracted much investigation and made considerable progresses in fermentation [2–5]. However, compared to the high reduction in the cost of fermentation during the past 20 years, no efficient separation method is available for cutting down the large cost of conventional steam stripping and vacuum distillation [6]. The cost of downstream processing can incur a very high portion (up to about 50–70%) of the production cost, and the recovery of 1,3-PD therefore, becomes a bottle-neck for developing a commercially viable process for its separation from the fermentation broth. The hydrophilicity and high boiling point of 1,3-PD (214 °C at atmospheric pressure), as well as its low concentration in the fermentation broth have made it difficult to develop an efficient method that is applicable to industrial scale for separating 1,3-PD from fermentation broth, which has complex composition.

Previously reported separation techniques mainly include steam stripping and vacuum distillation [7], exchange resins [8,9], solvent extraction [10–14], reactive extraction [15–17], zeolite membranes [18–20] and ion activated carbon or molecular sieve adsorption [21,22]. In the conventional steam evaporation and distillation, the energy consumption is too high to implement its application. Liquid–liquid extraction has been attracting much attention, but no extractants reported are good enough for extensive production [10–14], because the hydrophilic 1,3-PD in dilute broth is not apt to enter into the hydrophobic solvents, unless a large amount of solvent is added to a concentrated broth. Reactive extraction has been developed in order to solve the hydrophilicity problem associated with 1,3-PD. However, the inactivation of catalyst (a strong acidic resin) has to be avoided by desalination of the fermentation broth through electrodialysis [15–17]. This complicated process and the anticorrosion devices needed due to acidity of the catalyst are the main problems of this method for large scale. In addition, desalination and deproteinization [23–25] are required before evaporation because at a high salt concentration the denaturation of biomacromolecules can make the broth very viscous during distillation, leading to high consumption of energy and difficulty for a smooth operation.

All the separation methods and techniques mentioned above have some limitations or drawbacks. Most of them are complicated processes and usually require high energy input. A novel aqueous two-phase extraction (ATPE) composed of short chain alcohol/salt system is expected to meet all these criteria and decrease the cost of extraction, as it possesses certain advantages over traditional ones, such as low cost of extractants, easy recovery of hydrophilic organic solvents by evaporation and obviating the back-extraction. Our previous work has shown that using an ATPE composed of ethanol/ammonium sulfate, this novel technology can be used to separate 1,3-PD from fermentative broth [26]. However, a large

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amount of methanol is mixed with ethanol during the recycling of ammonium sulfate. This would disturb the formation of two phases. Furthermore, it is very difficult to separate the two alcohols from each other due to their similar polarities and boiling points. Additional energy and alcohol lost are inevitable during the desiccation of ammonium sulfate after recycling, and this will restrict the application of ethanol/ammonium-based ATPS at an industrial scale.

In this paper, methanol/phosphate ATPS was used instead of ethanol/ammonium sulfate ATPS to separate 1,3-PD from the fermentation broth for resolving the problem that the alcohol loss and energy expenditure was too high due to the difficulty to separate the extractant and the solvent for crystallizing salt. In this system, the extractant and the solvent for crystallizing salt were unified because methanol not only was a more effective extractant than ethanol, but also can be used to recycle the salt effectively by dilution crystallization after adjusting the pH. Furthermore, the influences of the phase forming components on the partition of 1,3-PD and removal of proteins and cells, as well as the recycling of salt were also studied. This study provided a simple and effective method for the direct extraction 1,3-PD from fermentation broths by an aqueous two-phase system.

2. Materials and methods

2.1. Materials

1,3-PD standard was 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). Bovine serum albumin (BSA) was purchased from the Shanghai Institute of Bioproducts, Ministry of Health of China. Coomassic Brilliant Blue G250 was obtained from the Shanghai Boao Biotechnology Corp. The cellulose triacetate hollow fiber dialyzer with effective surface area of 1.5 m^2 and cut-off molecular weight of 5000 was purchased from NISSHO Corp, Osaka, Japan. All other chemicals were of analytical grade.

2.2. Fermentation

Fed-batch fermentation was carried out under anaerobic condition using a chemically defined medium [5]. Glycerol was fed into the bioreactor to maintain a residual glycerol concentration of 10–20 g/L. The temperature, pH and agitation speed were maintained at 37 °C, 7.0 and 300 rpm, respectively. The concentrations of 1,3-PD, 2,3-butandiol, and glycerol in the fermentation broths were 65.06 g/L, 10.62 g/L, and 12.39 g/L, respectively

2.3. Phase diagram of ATPS comprised of methanol/K_2HPO_4

The phase diagram was obtained by using a turbidity titration method [27]. A series of tube containing different amounts of K_2HPO_4 and distilled water were prepared. After the K_2HPO_4 had dissolved, methanol was then added drop-wise to each tube placed on an analytical balance with a precision $\pm 10^{-7}$ kg for measuring the amount of added methanol. Methanol was added drop-wise and the mixture was shaken for 3 min after each drop. When the mixture first became turbid after addition of methanol, and further addition of methanol caused no precipitation, the point at which the mixture first became turbid is considered the turbid point. The total amount of added methanol was precisely measured by weighing, and the concentrations of methanol and K_2HPO_4 at different turbid points were calculated from the following equations:

$$w_1 = \frac{m_1}{m_1 + m_2 + m_3}$$

$$w_2 = \frac{m_3}{m_1 + m_2 + m_3}$$

where w_1 and w_2 represent the mass fraction of methanol and K_2HPO_4, respectively. m_1, m_2, and m_3 represent the amount of added methanol, K_2HPO_4, and water, respectively. A phase diagram curve was plotted and the effect of 1,3-PD on the phase separation (as examined by phase diagram) was investigated by adding a known amount of K_2HPO_4 to different tubes, each containing 100 g/L 1,3-PD. The turbid points for the solution were then determined by adding methanol drop-wise as described above.

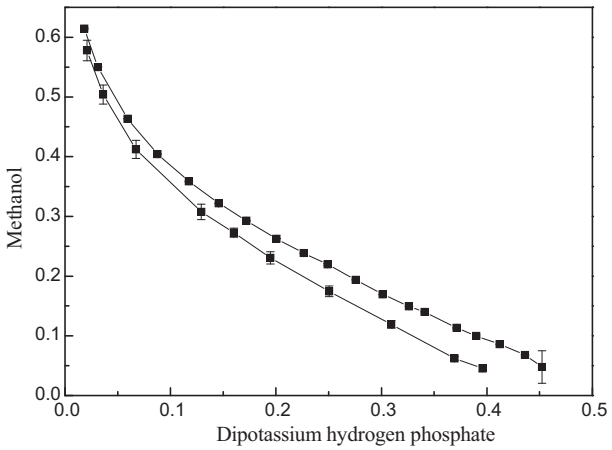

Fig. 1. Effect of 1,3-PD on phase diagram of methanol/K_2HPO_4-based ATPS. Symbols: (●) distilled water; (■) 100 g/L 1,3-PD solution.

2.4. Partition behavior of 1,3-PD in ATPS of methanol/phosphate

The fermentation broths were first filtered through a cellulose triacetate hollow fiber, which could remove all the cells and most of the proteins. Solid K_2HPO_4 and methanol were then added into the separate clarified filtrates to form aqueous two-phase systems consisting of 6.5–24% (w/w) methanol and 18–35% (w/w) K_2HPO_4. The mixture was held for 8 h at room temperature. The partition of 1,3-PD in ATPS for unfiltered fermentation broth was studied by adding solid K_2HPO_4 (to saturation) to the broth containing 10–35% (v/v) methanol. The effect of pH was investigated for unfiltered fermentation broths by adding saturated amounts of K_2HPO_4 and KH_2PO_4 or K_2HPO_4 and KOH in different molar ratios to the broth containing 35% (v/v) methanol. The same experiments were carried out for clarified filtrate. The concentrations of compounds in the top and bottom phases were analyzed by gas chromatography. The partition coefficient (K) was defined as the ratio of the concentration of compound in the top phase to that in the bottom phase. The recovery (Y) is the mass ratio of compound partitioned in the top phase to the total amount of compound. The effects of cells and proteins on the extraction of 1,3-PD and their removals from the ATPS were examined.

2.5. Recovery of phosphate in the salt-rich phase

After extraction with ATPS consisting of 35% methanol and saturated phosphate, phosphoric acid was added to the bottom phase for adjusting the pH to 4.5. Methanol was then added to the bottom phase in the amount that was 0.25–2.5 times the volume of the bottom phase, to crystallize the salt. The solid salt was recovered through filtration. The recovery (Y_2) of phosphate was defined as follows:

$$Y_2 = \left(\frac{1 - V_2 \times C_2}{V_1 \times C_1}\right) \times 100\%$$

where V and C are the volume and phosphate radical concentration, respectively, while the subscripts 1 and 2 represent bottom phase and the filtrate after salt crystallization, respectively.

2.6. Analytical methods

The concentrations of 1,3-PD and 2,3-butandiol (2,3-BD) in the samples were determined by gas chromatography (SHIMAZU GC-2010, FID-detector, 2 m × φ5 mm glass column packed with Chromosorb 101 and operated with N_2 as carrier gas at flow rate of 30 mL/min, detector temperature of 200 °C and column temperature of 170 °C) [25]. Protein concentration was determined by Bradford method [28] using bovine serum albumin (BSA) as the standard. The biomass concentration was measured by absorbance at 650 nm [29]. The concentration of glycerol was determined by an enzymatic kit [26]. The concentration of phosphate was determined spectrophotometrically with phosphomolybdate blue [30].

3. Results and discussion

3.1. Phase diagram of ATPS of methanol/K_2HPO_4

The phase diagram for methanol/K_2HPO_4-based ATPS was determined both in distilled water and 1,3-PD solution. As shown in Fig. 1, the concentration of methanol for forming-phase in 1,3-PD solution was less than that in distilled water under the same