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Aqueous two-phase system: An alternative process for recovery of succinic acid from fermentation broth

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ABSTRACT

Aqueous two-phase system was studied for the extraction of succinic acid (SA) produced by *Actinobacillus succinogenes* from the fermentation broth. Various hydrophilic solvents and inorganic salts were used to form the aqueous two-phase system for SA extraction, among which an acetone/ammonium sulfate aqueous two-phase system was investigated in detail, including examination of the phase diagram, effects of phase composition and pH on partitions, removal of cells and proteins from the fermentation broth, and recovery of ammonium sulfate. Under optimized conditions, with the fermentation broth pH value of 2 and aqueous two-phase system composed of 35% (w/w) acetone and 15% (w/w) ammonium sulfate, the recovery of SA from the fermentation broth was 94.4% with the removal of 93.6%, 98.1%, and 78.5% glucose, cells, and proteins, respectively. The total SA yield was 77.3% and the purity of SA was 98.7%. With the addition of methanol, 95.9% of ammonium sulfate in the salt-rich phase could be recovered, which indicated less waste discharge in SA extraction using the aqueous two-phase system.

1. Introduction

In the last 20 years, succinic acid (SA) produced from biorefinery has become increasingly attractive [1–3]. As one of the C4 bulk chemicals, SA has been listed as a standalone compound in the US Department Energy's top ten platform chemicals that could be produced from carbohydrates [4]. It is promising that the bio-based SA industry could be competitive to the established oil-based SA industry, because fermentative production of SA has the advantage of consuming carbon dioxide which makes it possible to reduce environmental pollution. In addition, it is also recognized that the fermentation route to SA consumes 30–40% less energy than the current petroleum-based route [5]. Nowadays, efficient fermentative SA producers such as *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, *Mannheimia succiniciproducens*, recombinant *Escherichia coli*, and *Corynebacterium glutamicum* have been exploited, among which *C. glutamicum* Δ*ldhA*-pCRA717 has been reported to achieve the highest SA concentration of 146 g/L with a yield of 0.95 g/g glucose at a laboratory scale [6]. However, an economical fermentation process must be combined with low-cost and efficient product recovery and purification methods. It is generally recognized that about 50–60% of the total costs for SA production are generated by downstream processing [7,8]. Therefore, besides strain improvement and fermentation process optimization, downstream processing is a technological challenge and an economic bottleneck for an efficient commercial microbial production of SA.

The downstream processing of bio-based SA usually includes three main steps [7]. The first step is the removal of microbial cells, mostly involving microfiltration or centrifugation. The second step is the removal of impurities and primary separation of SA from the fermentation broth. The last step is vacuum evaporation and crystallization. Various unit operations have been applied for the recovery of SA from the fermentation media, such as precipitation with ammonia or calcium hydroxide [8,9], membrane separation with ultrafiltration, nanofiltration, and electrodialysis [10–14], use of hollow fiber membrane contactor [15], reactive extraction [16–20], ionic liquids extraction [21], use of emulsion liquid membranes [22], ion exchange and sorption [23–25], direct crystallization [26,27], and esterification method [28,29]. Nevertheless, none of these methods have been proved to be simple and efficient enough with regard to yield, purity, and energy [30]. Generation of large amounts of byproducts such as $CaSO_4$ during precipitation with calcium or calcium oxide, serious membrane pollution, excessive energy consumption, or low selectivity, low yield, and low purity of SA are the main disadvantages in downstream processing. Liquid–liquid extraction is extensively used in the chemical industry due to its simplicity, low energy consumption, and ease of scale-up. However, it is difficult to determine an

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effective solvent for the recovery of SA from the fermentation media due to its high hydrophilic and low yield. In a previous study using *M. succiniciproducens*, a SA purity of 99.8% and an overall yield of 71.3% were obtained by employing reactive extraction [18]. An optimized reactive extraction process, in which expensive amines used in the separation process should be efficiently recycled, has been considered for application in the near future for the industrial production of SA [7].

Aqueous two-phase system (ATPS) is a separation method for the extraction and purification of biomolecules from different sources [31–35]. Some studies have reported that this method has the advantages of versatility, high efficiency, high yield, improved purification factor, selectivity, low cost, and fast mass transfer rates [31]. Recently, when compared with the conventional ATPS made of dual polymer systems or polymer/salt systems, a novel ATPS, comprising hydrophilic organic solvent such as short-chain alcohols and inorganic salt, has been successfully used in the extraction of bulk chemicals [35], such as 2,3-butanediol and 1,3-propanediol [36–41], from the fermentation broth. The novel system uses cheaper extractants instead of expensive polymers, and has the advantages of low interfacial tension, high resolution, and easy recovery of hydrophilic organic solvent through evaporation. In the present study, the application of ATPS in the extraction of SA from the fermentation broth was investigated. Among the various organic solvent/inorganic salt systems examined, an acetone/ammonium sulfate system was chosen due to its high SA distribution coefficient. The effects of fermentation broth pH, phase components on the partitions of SA, and removal rates of glucose, acetic acid, cells, and proteins were studied. In addition, recycling of ammonium sulfate was also investigated to develop a cost-effective downstream processing. Subsequently, a complete simple process of separation of SA from the fermentation broth was proposed.

2. Materials and methods

2.1. Materials

SA, acetic acid, glucose, ammonium sulfate, acetone, methanol, and all other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of analytical grade. Coomassie Brilliant Blue G250 was purchased from the Shanghai-Sangon Biotech Co., Ltd. (Shanghai, China). The fermentation broth was obtained from glucose-based fed-batch fermentation by *A. succinogenes* CGMCC1593 in a 5-L stirred fermentor (BIOFLO 110, the New Brunswick Scientific) under anaerobic conditions as previously described [39,40]. The fermentation medium contained the following (per L): glucose, 50 g; corn steep liquor, 25 g; K_2HPO_4 $3H_2O$, 2.5 g; NaH_2PO_4 $2H_2O$, 2.5 g; $MgCl_2$, 0.2 g; $CaCl_2$, 0.2 g; Vitamin B12, 20 μg; Vitamin B6, 50 μg; riboflavin, 50 μg; lipoic acid, 50 μg; niacin, 50 μg; thiamine, 50 μg; folic acid, 50 μg; pantothenate, 50 μg; p-aminobenzoate, 50 μg; and biotin, 100 μg. A concentrated solution containing 60% glucose was fed into the fermentor to maintain the glucose concentration within 4%. The pH was adjusted to 6.5 with magnesium carbonate. The cultures were sparged with CO_2 gas at 0.10 vvm to maintain an anaerobic environment, and the agitation speed was maintained at 200 rpm. Fed-batch fermentation was conducted at 37 °C for 48–56 h. The concentrations of SA, acetic acid, and glucose in the fermentation broth were 55.2–56.2, 4.4–8.4, and 20.2–34.4 g/L, respectively.

2.2. Partition of SA in ATPS

The partition experiments in ATPS were performed at a 20-g scale using 50 g/L pure SA solution. A total of 4 g of various salts were dissolved in 25-mL graduated tubes containing 12 g of SA solution, and subsequently, 4 g of hydrophilic organic solvents were added to the solutions. The mixture was shaken for 3 min and allowed to stand for 10 h at room temperature to reach liquid–liquid equilibrium. The concentrations of SA in both the top and bottom phases were analyzed by high performance liquid chromatography (HPLC). The partition coefficient (K) of SA was defined as the ratio of the top-phase SA concentration (C_t) to the bottom-phase SA concentration (C_b). Furthermore, V_t represented the volume of top phase and V_b represented the volume of bottom phase. The phase ratio (R) indicated the ratio of the top-phase volume to the bottom-volume. The recovery yield (E) was calculated by dividing the SA mass partitioned in the top phase by the SA mass in the initial fermentation broth, i.e. $E = \frac{RK}{(1+RK)} \times 100\%$ [37].

2.3. Phase diagram of acetone/ammonium sulfate ATPS

The phase diagram of acetone/ammonium sulfate ATPS was obtained using a turbidity titration method. This method was based on the transition between a clear and turbid solution when crossing the binodal curve. After adding ammonium sulfate dissolved in de-ionized water to a tube, the tube was placed on an analytical balance and acetone was subsequently added to the tube with a precision of $\pm 1 \times 10^{-4}$ g for measuring the amount of added acetone. After each addition, the mixture was shaken for 3 min. When the solution turned turbid, it indicated the existence of two liquid phases. The turbid point was defined as the time point when the mixture first became turbid. The compositions of the mixture at each turbid point on the binodal curve were calculated by the following equations:

$$w_1 = \frac{m_1}{m_1 + m_2 + m_3}, \quad w_2 = \frac{m_2}{m_1 + m_2 + m_3}$$

where w_1 and w_2 are the mass fractions of acetone and ammonium sulfate, respectively; and m_1, m_2, and m_3 are the amounts of added acetone, ammonium sulfate, and water, respectively. The effect of SA on the phase diagram was investigated by using 50 g/L SA solution, instead of de-ionized water to dissolve ammonium sulfate. The turbid points were determined using the method described earlier.

2.4. Effects of both ammonium sulfate and acetone concentrations on the partition of SA in ATPS

The effects of ammonium sulfate and acetone were investigated by adding solid ammonium sulfate and acetone to the centrifuged fermentation broth to form ATPS consisting of 5–30% (w/w) acetone and 5–30% (w/w) ammonium sulfate. The same experiment was also performed with uncentrifuged fermentation broth. The removal ratios of the cells and proteins were determined at the optimized condition. The selectivity coefficient of SA to glucose (denoted as Glu) and that of SA to acetic acid (indicated as AA) were defined as follows:

$$\text{The selectivity coefficient of SA to Glu} = \frac{\text{The partition coefficient of SA}}{\text{The partition coefficient of Glu}}$$

$$\text{The selectivity coefficient of SA to AA} = \frac{\text{The partition coefficient of SA}}{\text{The partition coefficient of AA}}$$

The removal of glucose and acetic acid was defined as follows:

$$\text{The removal of Glu} = \frac{\text{Glu in bottle phase}}{\text{The whole Glu}}$$

$$\text{The removal of AA} = \frac{\text{AA in bottle phase}}{\text{The whole AA}}$$