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Sugaring-out extraction coupled with fermentation of lactic acid

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ABSTRACT

Sugaring-out extraction as a novel phase separation for sugar-based chemicals was postulated to extract lactic acid from the fermentation broth by means of sugars and organic solvents. From the different sugaring-out extraction systems investigated, the isopropanol/glucose system was found to be more favorable. The partition coefficient (1.39) and recovery (84.27%) of lactic acid were obtained by a system consisting of 12% (w/w) glucose and 40% (w/w) isopropanol. The glucose-rich bottom phase can be utilized to produce lactic acid after recovery of isopropanol. The experimental results were comparable with the normal fermentation, e.g. the yield of lactic acid to glucose being 91.1% vs. 94.0%. This provides a novel approach to integrate sugaring-out extraction into fermentation for production of sugar-based chemicals.

1. Introduction

Developing sustainable production strategies for bio-based chemicals has recently gained more and more attention to reduce dependency on fossil sources. Among bio-based chemicals, e.g. ethanol, n-butanol, 1,3-propanediol, 2,3-butanediol, lactic acid, succinic acid, and so on, most of them are produced by microbes from sugars, such as glucose, xylose, fructose and saccharose as substrate in fermentation. These bio-based chemicals can be called as sugar-based chemicals. Lactic acid is a sugar-based chemical that widely used in the food and non-food industries, including the cosmetic and pharmaceutical industries, and for the production of oxygenated chemicals, plant growth regulators, and special chemical intermediates. In addition, it has received growing attention for use as a monomer for the production of biodegradable poly-lactic acid (PLA) [1].

Separation and purification have become the most costly step in the production of lactic acid [2]. Traditionally, lactic acid is recovered as calcium lactate by precipitation [3]. The disadvantages of this process include the production of a large amount of calcium sulfate as a by-product and high sulfuric acid consumption. In order to reduce the cost, many separation techniques have been investigated, for examples, adsorption [4,5], membrane separation [6,7], electrodialysis [8,9], reactive distillation [10], and extraction [11,12]. Among these, extraction is considered to be an effective separation step for large-scale separation of lactic acid from fermentation broths. It can be quite efficient and inexpensive while requiring relatively short processing time. However, lactic acid is poorly extractable in traditional liquid–liquid extraction due to its high hydrophilicity. In recent researches, salting-out extraction systems comprising of hydrophilic organic solvents (such as short-chain alcohols) and inorganic salts, have been successfully used in the extraction of bio-based chemicals from the fermentation broths [13], such as 1,3-propanediol [14–16], 2,3-butanediol [17–19], succinic acid [20], and lactic acid [12,21]. Salting-out extraction could recover 90.6% lactic acid from fermentation broth and remove almost all cells and 86% of the proteins using the system of 14% (w/w) dipotassium phosphate and 30% (w/w) ethanol. Since a large amount of inorganic salt is rich in the bottom phase, the recovery and reuse of inorganic salt become the key step for salting-out extraction. Although methanol precipitation can be used to recover salts (such as ammonium sulfate [14], dipotassium phosphate [16]) from the bottom phase, the methanol present in the crystallized salt must be removed when the recovered salt is reused if the extractant is not methanol. Furthermore, the recovery of target product decreased slowly as reusing times of salt increased [22]. Such problems can be undoubtedly avoided if the phase separation agent is added without the need for recycle.

Sugars are the substrates in fermentations for sugar-based chemicals and can replace inorganic salts to trigger two-phase separation. The firstly reported phase separation phenomenon triggered by the addition of a mono-sugar or a disaccharide into an acetonitrile–water mixture was called as "sugaring-out" in 2008 [23,24]. Addition of water-soluble sugars destroys the hydrogen-bonding of acetonitrile–water and results in the stripping of water molecules from the hydrated acetonitrile molecules. Therefore the acetonitrile molecules were distributed to the top phase and sugar

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to the bottom phase. Until now, sugaring-out has been applied on extraction of acetonitrile, metal ions, biomolecules (proteins and enzymes), antibiotics, and drugs [23–30]. In general, low polarity solutes tend to partition into the upper phase, such as syringic acid, furfural, para-coumaric acid, ferulic acid and 5-hydroxymethyl furfural [23]. However, the application of sugaring-out is unknown in separation of hydrophilic primary metabolites from fermentation broths.

In this study, sugaring-out extraction (SOE) was postulated for the extraction of lactic acid from the fermentation broth by means of sugars and organic solvents. Firstly, the influences of the different sugars and organic solvents on the partition and recovery of lactic acid were investigated. Secondly, a sugaring-out extraction system (SOES) of isopropanol/glucose was chosen to separate lactic acid from the fermentation broth. Finally, sugar as phase-separating agent was considered to be fermented as carbon source for lactic acid production. The feasibility of the reutilization of bottom phase was investigated for lactic acid fermentation. It seems that sugaring-out extraction can couple the fermentation and separation for lactic acid production. This work could act as a typical example of the application of sugaring-out extraction of sugar-based chemicals from fermentation broths.

2. Material and methods

2.1. Chemicals

Considering that the fermentation of lactic acid is usually carried out at pH 5.5, the pH value of simulated fermentation broths and real fermentation broths was constant (pH 5.5). Simulated fermentation broth was prepared at 150 g/L of lactic acid and the pH was adjusted by adding NaOH or H_2SO_4. Glucose is food grade. L-lactic acid standard was purchased from Sigma. Glucose, organic solvents, lactic acid and other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and were analytical grade.

2.2. Fermentation

Lactobacillus rhamnosus CICC 22825 was obtained from China Center of Industrial Culture Collection (Beijing, China). The bacterium was maintained at −70 °C in Lactobacilli de Man Rogosa Sharpe (MRS) medium containing 20% (v/v) glycerol. To prepare the inoculum for fermentation, one centrifuge tube containing glycerol stock was shortly thawed, and then inoculated into a 100 ml conical flask containing 40 ml MRS medium at 37 °C with shaking at 200 rpm for 24 h. Afterward transferring into a 250 ml conical flask containing 100 ml MRS medium at 37 °C with shaking at 200 rpm for 12 h. Batch fermentations were performed in a 5 L fermentor (BIOTECH-5BG, Shanghai) with a working volume of 2 L. The inoculation quantity was 10.0% (v/v).

The medium for batch fermentations was the same as the seed culture medium (MRS) except the concentration of glucose. Batch fermentation was conducted in an autocontrolled fermentor under anaerobic conditions at an initial glucose concentration of 199 g/L. The pH for the fermentation was adjusted to 5.5 by automatic addition of 10 M NaOH. The temperature and agitation speed were maintained at 37 °C and 250 rpm, respectively. The final concentrations of lactic acid, residual glucose and yield were 123.2 g/L, 68 g/L, and 92.9% at fermentation time of 92 h, respectively.

After SOE of lactic acid from fermentation broths as described in the next step, isopropanol was recovered from the bottom phase using reduced pressure distillation. Appropriate amounts of water and nutrients were added into the treated bottom phase, resulting in a glucose concentration of about 180 g/L. The seed was inoculated in a 250 ml conical flask containing 100 ml treated bottom phase and then fermented to produce lactic acid. The pH for the fermentation was adjusted by adding 80 g/L $CaCO_3$.

2.3. Sugaring-out extraction of lactic acid

Simulated fermentation broth as described in Section 2.1 was used to investigate the influence of SOES for the partition behavior of lactic acid. The sugar was dissolved in simulated fermentation broth to obtain sugar mixture. Then, organic solvent was added into the sugar mixture and mixed thoroughly by a vortex mixer. The obtained mixture was stood for 8 h or overnight. The better SOES would be determined according to partition of lactic acid and reuse of the bottom phase for fermentation.

An appropriate SOES would be further verified by using the fermentation broth centrifugated for 15 min at 5000 rpm after batch fermentation. Lactic acid was extracted and concentrated in the solvent-rich top phase. The sugar-rich bottom phase would be reused for fermentation in conical flasks as described in Section 2.2.

2.4. Phase diagram

In our experiments, the phase diagram of isopropanol/glucose system was obtained by turbidity titration method at 298.15 K according to the published paper [14]. Glucose was added to a test tube with deionized water or sodium lactate solution until the solution saturated, and isopropanol was then added dropwise to the tube. The clear mixtures became turbid with the addition of isopropanol drop by drop, and one more drop isopropanol was added, no deposition was observed. The point at which the mixture first became turbid was the turbid point. The compositions of these mixtures were noted and determined by an analytical balance with a precision $\pm 1 \times 10^{-7}$ kg.

2.5. Analytical method

Lactic acid was analyzed by HPLC (Agilent 1100) using a C18 column at 214 nm with an ultraviolet detector, 2‰ phosphoric acid, and acetonitrile (96.5:3.5, v/v) as the mobile phase at a rate of 1 mL/min. Glucose concentration was measured by biosensor SBA-50 (Shandong Academy of Sciences, Shandong, China). The biomass concentration was measured by absorbance at 620 nm using a spectrophotometer (721S, Shanghai Lengguang Technology Co., Ltd., Shanghai, China). The concentration of soluble proteins was determined by Bradford method using BSA as the standard protein [31]. Organic solvents were determined by GC (Shimadzu GC-2010) using a 2 m × Φ5 mm glass column packed with Chromosorb 101 at 180 °C.

The parameters including partition coefficient (K), volume ratio (R) and recovery (Y) were calculated as follows:

$$K = \frac{C_t}{C_b} \tag{1}$$

where C_t and C_b were the concentrations of lactic acid in the top and bottom phase, respectively.

$$R = \frac{V_t}{V_b} \tag{2}$$

where V_t and V_b were the volumes of top phase and bottom phase, respectively.

$$Y = \frac{RK}{1 + RK} \tag{3}$$