Journal of Bioscience and Bioengineering
VOL. 117 No. 4, 464–470, 2014

ELSEVIER

www.elsevier.com/locate/jbiosc

2,3-Butanediol recovery from fermentation broth by alcohol precipitation and vacuum distillation

Sangjun Jeon,[1,‡] Duk-Ki Kim,[1,3,‡] Hyohak Song,[1,*] Hee Jong Lee,[1] Sunghoon Park,[2] Doyoung Seung,[1] and Yong Keun Chang[3]

Research and Development Center, GS Caltex Corporation, 104-4 Munji-dong, Yuseong-gu, Daejeon 305-380, Republic of Korea,[1] Department of Chemical and Biomolecular Engineering, Pusan National University, Busan 609-735, Republic of Korea,[2] and BioSystem Engineering Laboratory, Department of Chemical and Biomolecular Engineering, KAIST, Yuseong-gu, Daejeon 305-704, Republic of Korea[3]

Received 13 May 2013; accepted 12 September 2013
Available online 18 October 2013

This study presents a new and effective downstream process to recover 2,3-butanediol (2,3-BD) from fermentation broth which is produced by a recombinant *Klebsiella pneumoniae* strain. The *ldhA*-deficient *K. pneumoniae* strain yielded about 90 g/L of 2,3-BD, along with a number of by-products, such as organic acids and alcohols, in a 65 h fed-batch fermentation. The pH-adjusted cell-free fermentation broth was firstly concentrated until 2,3-BD reached around 500 g/L by vacuum evaporation at 50°C and 50 mbar vacuum pressure. The concentrated solution was further treated using light alcohols, including methanol, ethanol, and isopropanol, for the precipitation of organic acids and inorganic salts. Isopropanol showed the highest removal efficiency, in which 92.5% and 99.8% of organic acids and inorganic salts were precipitated, respectively. At a final step, a vacuum distillation process enabled the recovery of 76.2% of the treated 2,3-BD, with 96.1% purity, indicating that fermentatively produced 2,3-BD is effectively recovered by a simple alcohol precipitation and vacuum distillation.

[**Key words:** 2,3-Butanediol; Fermentation; *Klebsiella pneumoniae*; Recovery; Alcohol precipitation]

2,3-Butanediol (2,3-BD) is a four-carbon diol which is synthesized as one of the fermentation end-products via condensation of two pyruvic acids. With the depletion of fossil resources and concerns over environmental pollution, there has been substantial growing interest in the production of 2,3-BD from renewable resources, since it has wide industrial applications. For example, 2,3-BD can be easily converted to a fuel additive, methyl ethyl ketone, having a heating value of 27,198 J/g, or to a platform chemical, 1,3-butadiene, which is used in the manufacture of synthetic rubber (1–3). Fermentative 2,3-BD production has been widely studied for over 100 years, and there have been significant advances in the development of 2,3-BD producing microorganisms and fermentation processes (4,5). However, the recovery of 2,3-BD from fermentation broth still remains a challenging task making fermentative production techniques economically unfeasible in many instances.

Under microaerobic conditions, *Klebsiella pneumonia* and *Klebsiella oxytoca* naturally produce high titers of 2,3-BD and lactic acid as major fermentation end-products, along with relatively small amounts of by-products, including ethanol, acetoin, formic acid, acetic acid, and succinic acid (6,7). The inactivation of the lactate dehydrogenase in the wild-type strains of *Klebsiella* species by UV-induced mutagenesis increases the 2,3-BD productivity to economically feasible levels (8,9). Also, various fermentation media were developed to maximize the formation of 2,3-BD, as well as to minimize the cost of fermentation (10,11). However, the industrial media generally contain a wide variety of ingredients as sources of carbon, nitrogen, and minerals (12), which may inhibit cost-effective 2,3-BD recovery from fermentation broth. In addition, pH buffering agents should be added to the medium since organic acids are produced as by-products. Therefore, there are a number of impurities which must be removed, including nutrients, pH buffering agents, and by-products from fermentation broth, for the development of a commercially applicable fermentative 2,3-BD production process.

Suspended solids, such as cells, can be easily removed from fermentation broth through simple filtration or centrifugation processes, while the removal of dissolved impurities, especially organic acids and inorganic salts, requires much more complicated processes. Many methods, such as ion exchange, electrodialysis, and membrane filtration have been proposed to remove or extract the dissolved organic acids and inorganic salts from fermentation broth (13–16). However, these methods have inherent problems which must be circumvented. For example, ion exchange processes generally generate a large amount of wastewater for resin regeneration (US 7919658). In the case of electrodialysis, membranes are still too expensive for large-scale applications (17,18). Although it has been reported that reverse osmosis combined with distillation can reduce the recovery cost (19), a single usage of reverse osmosis for the separation of 2,3-BD is neither economic nor efficient. Furthermore, many filtration steps, such as micro-, ultra-, or nano-

* Corresponding author. Tel.: +82 42 866 1711; fax: +82 42 866 1749.
E-mail address: hyohaks@gscaltex.com (H. Song).
‡ The first two authors contributed equally to this work.

1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2013.09.007

filtrations, should be implemented prior to reverse osmosis, to prevent membrane fouling and to maintain an appropriate flux across the membrane. These multiple filtration steps may dramatically decrease the recovery yield of 2,3-BD.

In this paper, we report a truly practical methodology for the recovery of 2,3-BD from fermentation broth, based on alcohol precipitation. The recovery process mainly consists of four steps: pretreatment, concentration, alcohol precipitation, and purification. The fermentation broth of the *ldhA*-deficient *Klebsiella pneumoniae* strain contained about 90 g/L of 2,3-BD as the major end-product. Isopropanol precipitation resulted in the recovery of 2,3-BD with a purity of 96.1%, and a recovery yield of 76.2%.

MATERIALS AND METHODS

Microorganisms and fermentation *K. pneumoniae ldhA*-deficient strain was constructed by disrupting the *ldhA* gene encoding lactate dehydrogenase in *K. pneumoniae* KCTC 12132BP (Korean Collection for Type Cultures, Daejeon, Korea), and used for 2,3-BD fermentation. The construction process of the *ldhA*-deficient strain was described in detail in the previous work (20). Cells were routinely cultivated in a complex medium which contained (per liter): 5 g yeast extract (Becton Dickinson, Le Pont de Claix, France), 0.05 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$, 0.001 g $MnSO_4 \cdot H_2O$, 0.001 g $CaCl_2 \cdot 2H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 6.6 g $(NH_4)_2SO_4$, 8.7 g K_2HPO_4, and 6.8 g KH_2PO_4. Fed-batch fermentation was carried out in a 6.6 L BioFlo/CelliGen 310 bioreactor (New Brunswick. Scientific Co., Edison, NJ, USA) containing 2.7 L complex medium with 300 mL inoculum. The initial glucose concentration was 500 mM and a concentrated solution, containing 700 g/L glucose, was fed into the bioreactor using a peristaltic pump (Cole–Parmer, Vernon Hills, IL, USA) before the residual glucose becomes depleted. The aeration rate and agitation speed were maintained at 3.0 L/min and 150 rpm, respectively, based on the previous researches (7,8,11). The temperature was set at 37°C, and the pH was stabilized to 6.5 ± 0.1 by the automatic addition of 5 N NaOH.

Fermentation broth pretreatment Cells were removed from the fermentation broth by microfiltration (LPJ-PEH-005-N3, $L = 250$ mm, ROKI TECHNO, Tokyo, Japan). The pH of the cell-free fermentation broth was adjusted to 5.0, 6.0, 7.0, and 8.0, respectively, by adding 1 N NaOH or 1 N HCl. After pH adjustment, vacuum evaporation was performed under a pre-determined optimum condition (50°C and 50 mbar) to concentrate 2,3-BD. Once the concentration of 2,3-BD reached approximately. 500 g/L, the concentrated solution was cooled down and stored at room temperature before further experimentation.

Precipitation and recovery Precipitation of organic acids and inorganic salts was conducted by the addition of light alcohols, including methanol (99.9%, Merck KGaA, Darmstadt, Germany), ethanol (99.9%, Merck KGaA), and isopropanol (99.9%, Mallinckrodt Baker, Phillipsburg, NJ, USA), as solvents to the concentrated solution, respectively. The mixture was gently shaken for 10 min, and was then allowed to stand at room temperature overnight. The content of the precipitate in the mixture was determined by weighting the filtered precipitate through a 0.45 μm membrane which was dried in an oven at 80 ± 0.5°C overnight, and cooled down in a desiccator to room temperature. The recovery of solvents and the removal of water from the filtrate were conducted at 70°C and 13 mbar. The purified 2,3-BD was finally obtained by vacuum distillation at 130°C and 13 mbar.

Analytical methods Glucose, 2,3-BD, and by-products, including formic, acetic, lactic, and succinic acids, as well as ethanol, and acetoin, were quantified by high-performance liquid chromatography (Agilent 1200, Agilent Technologies, Waldbronn, Germany) equipped with a refractive index detector and an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, Hercules, CA, USA). The column was eluted by 0.01 N H_2SO_4 at a flow rate of 0.6 mL/min, and the oven temperature was set at 80°C. Inorganic salts, including sodium, ammonium, potassium, magnesium, calcium, chloride, phosphate, and sulfate were measured by ion chromatography (Dionex ICS1100, Thermo Scientific, CA, USA) equipped with a conductivity detection cell. IonPac CS12A analytical (4 × 250 mm) and IonPac AS22 analytical (4 × 250 mm) columns were used for cation and anion analysis, respectively. The CS12A and AS22 columns were continuously eluted by 20 mM methanesulfonic acid and a mixture of 4.5 mM Na_2CO_3 and 1.4 mM $NaHCO_3$ at flow rates of 1.0 and 1.3 mL/min, respectively. Cell growth was monitored by measuring the optical density at 600 nm using a UV–visible spectroscopy system (Ultrospec 3000, Pharmacia Biotech, Uppsala, Sweden). The cell concentration, defined as dry cell weight (DCW), was determined from the predetermined standard curve, in which an OD_{600} of 1.0 is equivalent to 0.4528 ± 0.0309 g DCW/L.

RESULTS AND DISCUSSION

Fig. 1 shows the schematic diagram of 2,3-BD fermentation and recovery system employed in this study. The system mainly consists of 2,3-BD fermentation, cell removal, pH adjustment, concentration, alcohol precipitation and recovery, and 2,3-BD purification.

2,3-Butanediol fermentation The fed-batch fermentation of the *K. pneumoniae ldhA*-deficient strain produced 89.8 g/L of 2,3-BD in 65 h (Fig. 2A). The DCW reached a maximum level of 16.5 g/L at 8 h, and continuously decreased to a final concentration of 8.7 g/L due to dilution of the fermentation broth by the supplementation of concentrated glucose solution and pH control agent (5.0 N NaOH). In order to maintain optimum levels of glucose and pH, about 750 mL of 700 g/L glucose and 250 mL of 5.0 N NaOH were added to the bioreactor, respectively, throughout the fermentation. As

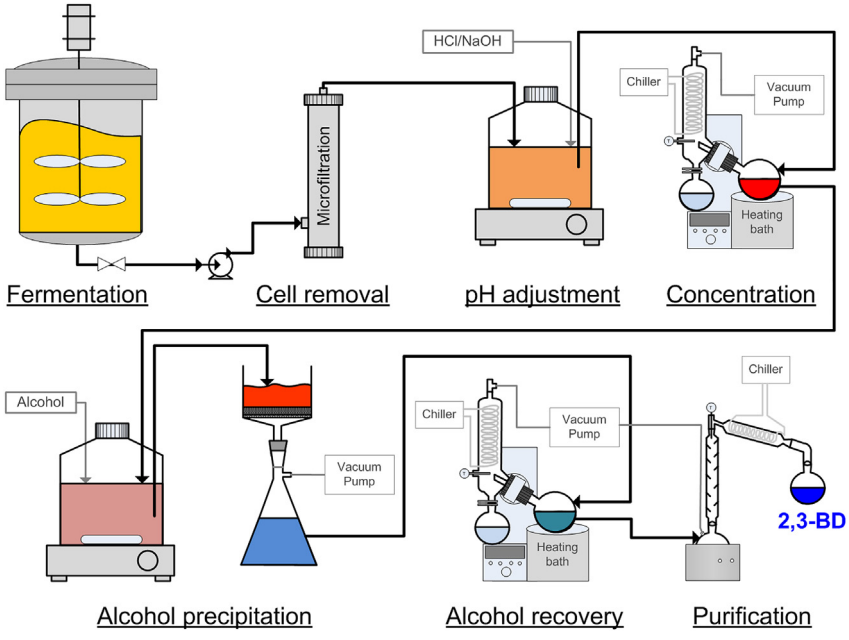

FIG. 1. Schematic diagram of 2,3-BD fermentation and recovery system.