Biochemical Engineering Journal 117 (2017) 112–120

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Extractive fermentation for enhanced isopropanol and *n*-butanol production with mixtures of water insoluble aliphatic acids and oleyl alcohol

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form
29 September 2016
Accepted 3 October 2016
Available online 4 October 2016

Keywords:
Isopropanol
n-Butanol
IB fermentation
Dodecanoic acid
Liquid–liquid extractive fermentation

ABSTRACT

Liquid–liquid extractions using the mixtures of aliphatic acids and oleyl alcohol as extractants are investigated to improve isopropanol and butanol (IB) production in fed-batch fermentation. Extractive fermentation with a mixture of tetradecanoic acid (30%) and oleyl alcohol improved IB production by 63.41% compared with fermentation without extraction. A mixture of dodecanoic acid (35%) and oleyl alcohol was 24.75% more effective that the tetradecanoic acid system. The IB yield and productivity were 0.53 g/g and 0.35 g/L/h, respectively for the dodecanoic acid system. The accumulated IB in the dodecanoic acid containing extractant reach a relatively high level of 65.92 g/L from four sequential continuous fed-batch fermentation batches. Online extractive fermentation using the mixture of dodecanoic acid and oleyl alcohol as extractant can recover and accumulate IB at high concentration, which will facilitate subsequent IB purification and significantly improve IB production as a future technique reserve.

1. Introduction

Isopropanol and butanol (IB) continue to receive attention as potentially biofuels and chemical feedstocks because these chemicals can be produced from renewable biomass by *Clostridium* species [1,2]. The production of these alcohols is severely restricted by their cytotoxicity, which result in low alcohol concentrations and yields in conventional alcohol fermentations [3]. Substantial research and development has focused on finding solutions to overcome the toxicity of these solvents. One strategy is to enhance the alcohol tolerance of *clostridia* producer strains *via* metabolic engineering [4] or adaptive evolution domestication [5]. Another strategy is to integrate product recovery with fermentation to remove solvents *in situ* prevent their inhibitory effect, which is called extractive fermentation.

At present, extractive fermentation techniques used in alcohol production include adsorption, pervaporation, perstraction, gas stripping, and liquid–liquid extraction. Adsorption is an effective butanol recovery technique by exploiting sorptive materials as the adsorbents [6]. Liu et al. [7] investigated butanol adsorption using macroporous resin for butanol removal and obtained a high solvents titer of 130.7 g/L. Pervaporation and perstraction are membrane–based techniques, which first selectively remove solvents by permeation and then recover them by condensation and distillation [8,9]. Yen et al. [10] controlled the butanol concentration in the range of 8–12 g/L in fermentation broth during the pervaporation process using the permeable membrane which made of hydrophobic polyether amide polymer. Qureshi and Maddox [9] used silicone tubing as a membrane and oleyl alcohol as an extractant to recover solvents at high concentration level of 98.97 g/L by perstraction process. Gas stripping is a simple technique of butanol separation, which can strip away the solvents by spraying oxygen free nitrogen or fermentation gases through the fermentation broth [11]. Xue et al. [11] achieved a highly solvents concentration of 227.0 g/L by two–stage *in situ* gas stripping. The advantages and disadvantages of all above mentioned extractive fermentation techniques have been summarized distinctly in a recent report [12].

The liquid–liquid extractive fermentation method, which captures and removes products from the aqueous fermentation broth by using other solvents that have higher product distribution

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http://dx.doi.org/10.1016/j.bej.2016.10.006
1369-703X/

coefficient, is preferred because of its low–energy consumption and cost-effectiveness compared to any other extraction process [13–15]. The process of liquid–liquid extraction consumes less energy because of the low operation temperature and agitation speed. The advanced distillation technology will be employed for the subsequent products purification, which can saves operation energy consumption [16]. In order to reduce the cost of extractants, many studies have focused on finding the effective and low-cost agents. Some organic solvents have been used for extractive fermentation include alkanes, long chain fatty alcohols, and vegetable oils. Liquid–liquid extraction can be used especially for some liquid products with high boiling points and low saturated vapor pressures. These properties make separating the extracts from the aqueous medium by distillation or gas stripping difficult because large amounts of water will be extracted with the products. Liquid–liquid extractive fermentation is considered as a potential method for separating poorly volatile liquid substances from aqueous fermentation broth, and many of solvents have been produced by this method, including acrylic acid [16], glycols [17], 1,3-propanediol [18], 2,3-butanediol [19], and butanol [13].

Three criteria are required for extractive IB liquid–liquid fermentation; namely, the extractant should be insoluble in water, have high IB selective capture ability, and be biocompatible with the fermentative organism. Several solvents have been identified on the basis of their distribution coefficients for butanol and tested to improve the extractive fermentation process. Some of the organic extractants used at the laboratory scale for *in situ* liquid–liquid extraction include oleyl alcohol [20], decanol [21], dodecanol [22], dibutyl phthalate [23], and polypropylene glycol [24]. Furthermore, vegetable oils have been investigated for butanol extraction in fermentations, including castor, soy, corn, olive, cocos, rapeseed, linseed and sesame oil [25]. Derivatives of vegetable oils including biodiesel [26], methylated crude palm oil [27] and methylated sunflower oil [28] have also been successfully applied to extract butanol from fermentation broths. Vegetable oils are rich in aliphatic acids which have high butanol selective capture abilities and can produce esters that play an important role in the butanol extraction process [25,29]. The purpose of methylating vegetable oil was to convert the aliphatic acids into the corresponding esters to obtain water insoluble and low viscosity butanol extractants. However, the butanol capture ability of aliphatic acids is better than esters because of higher solvent polarity caused by the carboxyl group in molecular structure [30]. An applicable aliphatic acid is needed as an extractant to further improve butanol extraction from fementaiton broths.

Aliphatic acids have relatively higher butanol distribution coefficients than vegetable oils and esters, and this property should translate to the isopropanol distribution coefficient because of the similar molecular structure and chemical properties of isopropanol and butanol. The high distribution coefficients of aliphatic acids allow extraction of both isopropanol and butanol from aqueous fermentation broth, even at low alcohols concentrations. But short chain aliphatic acids are unsuitable extractants because they are soluble or slightly soluble in water. Aliphatic acids that have ten or more carbon atoms, including decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid and eicosanoic acid, are insoluble in water and suitable for butanol extraction. However, these long chain aliphatic acids are solid at room temperature condition, and cannot be used directly as extractants. Dissolving solid aliphatic acids in a solvent to form a mixed extractant is a feasible way to effectively use long chain aliphatic acids for butanol extraction. Oleyl alcohol has been successfully used for butanol extractive fermentation in single or mixed form [13,31], and solid aliphatic acids can be easily dissolved into oleyl alcohol. Thus, an aliphatic acid/oleyl alcohol mixed extractant is potentially useful because of the high biocompatibility and butanol extraction ability of the constituents.

The present study investigates the feasibility of extractant mixtures of aliphatic acids and oleyl alcohol in a liquid–liquid extractive fermentation to enhance IB production from *Clostridium beijerinckii* (*C. beijerinckii* ATCC 6014), then recover and accumulate IB at higher concentration to facilitate the subsequent IB purification. The alcohol capture ability of decanoic acid, dodecanoic acid, tetradecanoic acid and hexadecanoic acid are evaluated. Aliphatic acids with higher IB distribution coefficients are used in fed-batch extractive fermentations. The fed-batch fermentation integrated with liquid–liquid extraction produces a highly concentrated alcohol product and enhances yield of IB.

2. Materials and methods

2.1. Microorganisms, chemicals and media

C. beijerinckii (ATCC 6014) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Aliphatic acids, oleyl alcohol and other chemicals used for gas chromatography (GC) standards were purchased from Macklin Biochemical Technology Inc. (Shanghai, China). All vitamins used in media were purchased from Sigma-Aldrich Co. (USA), and other components were purchased from Sangon Biotechnology Inc (Shanghai, China).

Subculture medium was reinforced clostridial medium (RCM) consisting of 3.0 g/L yeast extract, 10.0 g/L beef extract powder, 10.0 g/L peptone, 1.0 g/L soluble starch, 5.0 g/L glucose, 0.5 g/L cysteine hydrochloride, 5.0 g/L sodium chloride and 3.0 g/L sodium acetate. Fermentation medium was P2 medium containing 50.0 g/L glucose, 1.0 g/L yeast extract, phosphate buffer (0.5 g/L KH_2PO_4, 0.50 g/L K_2HPO_4, 2.2 g/L ammonium acetate), mineral salts (0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $MnSO_4{\cdot}H_2O$, 0.01 g/L $FeSO_4{\cdot}7H_2O$, and 0.01 g/L NaCl), vitamins (1.0 mg/L *para*-aminobenzoic acid, 1.0 mg/L thiamin, and 0.01 mg/L biotin), and maintained at pH 6.0. The carbon sources and yeast extract were sterilized at 115 °C for 20 min. Phosphate buffer, mineral salts and vitamins were sterilized by filtration.

2.2. Extractant screening

The distribution coefficients of isopropanol and butanol in the chemicals screened were measured by small scale extraction in shake flasks at room temperature (approximately 28 °C) for 6 h from fermentation broth with an aqueous-to-organic phase volume ratio of 1:1. The agitation speed was controlled at approximately 150 rpm/min to prevent emulsification. The distribution coefficients of isopropanol and butanol are defined as: distribution coefficient = [mass concentration in organic phase]/[mass concentration in aqueous phase]. The cytotoxicity of extractants was tested by adding an equal volume of extractants to the culture medium and fermenting the bacteria at 36 °C in 100-mL serum bottles.

2.3. Fermentation system with liquid–liquid extraction

Our extractive fermentation system consisted of a 5 L bioreactor (BIOSTA A plus, Sartorius Stedim Biotech, Germany), 0.5 L fibrous-bed bioreactor (FBB), peristaltic pump, extraction apparatus, latex tube and on-line sensing control system for the bioreactor. The FBB and extraction apparatus connected to the bioreactor in series by a recirculated latex tube loop. The extraction apparatus was installed downstream of the FBB and upstream of the bioreactor with the peristaltic pump installed between the bioreactor and FBB (Fig. 1). The FBB was made of a jacketed glass column packed with a spirally wound cotton towel (35 × 40 cm; about 5 mm thickness; with > 95%