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Research paper

Integrated ABE fermentation-gas stripping process for enhanced butanol production from sugarcane-sweet sorghum juices

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ABSTRACT

The biobutanol production by ABE (acetone-butanol-ethanol) fermentation with an integrated product recovery system by gas stripping was studied. A mixture of industrial sugarcane-sweet sorghum juices was fermented using *Clostridium acetobutylicum* DSM 792. Empirical models were developed to determine an operational condition for the integrated process that contributes both to mitigate the butanol inhibitory effect and to obtain a highly concentrated butanol condensate. A Monod model supplemented with a term describing product inhibition was employed to describe cell growth, butanol formation, and substrate consumption, respectively. Gas stripping was described by a first order model. The models showed satisfactory agreement with the experimental data in terms of cell growth, sugar consumption, and butanol production and extraction. A gas recycle flowrate in the range 0.3–0.6 vvm allowed to maintain butanol concentration below the inhibitory concentration (8 g/L) and to obtain a concentrated butanol condensate after phase separation, which could reduce energy consumption in the final product recovery. In a fed-batch fermentation coupled with *in situ* gas stripping, total sugar conversion and 18.6 g/L butanol distributed 42% in the fermentation broth and 58% in the condensate, were obtained.

1. Introduction

During the last decades, there has been an increasing interest for the production of chemicals and fuels from renewable resources. Reasons for this include climate change, global warming, and energy security [1]. Butanol is considered an attractive biofuel as it has clearly superior properties to ethanol due to its higher energy density, less volatile and explosive and less hygroscopic [1–4], in addition to being an important chemical precursor for paints, polymers and plastics, and widely used solvent [5]. Biobutanol can be produced by the acetone-butanol-ethanol (ABE) fermentation by various *Clostridium* spp., in which a solvent mixture is produced, generally in the ratio 3:6:1 acetone-butanol-ethanol respectively. The economic viability of fuel biobutanol production largely depends on the costs of raw materials and energy consumed. Energy consumption can be high due to the low butanol titer and purity reached in the fermentation broth [1,3].

The raw material utilized in biobutanol production is one of the major cost factors in its production [3–5]. Both sugarcane and sweet sorghum can offer more advantages than other crops since they produce a residue (bagasse) which can be burnt for steam production to satisfy the energy demand of the industrial processes [6,7]. Therefore, raw materials with high carbohydrate content, efficient transformation processes energetically optimized, and an accessible, low cost energy source are needed.

Fuel bioethanol is currently produced in the north of Uruguay mainly from sugarcane. Sweet sorghum juice alone or mixed with sugarcane juice is also used to extend the plant facility working time. Since both biobutanol and bioethanol could be produced from the same feedstock, its production could take place in the same facility using similar equipment (Alur SA, Bella Unión, http://www.alur.com.uy/agroindustrias/bella-union/, access on 11/21/2016).

One of the major challenges in biobutanol production is the intensive energy consumption in product recovery caused by the low product concentrations reached in the fermentation broth [8]. This is attributed mainly to cell toxicity or fermentation inhibition by butanol [9–12]. Furthermore, the butanol yield and productivity are also low, due to the co-production of acetone and ethanol. In order to solve this problem, extensive research and development efforts have been made. Some approaches that have been proposed include improve butanol tolerance of strains by mutagenesis and metabolic engineering [1,5,13,14], to couple butanol production

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with an *in situ* extraction process. Different techniques for *in situ* butanol recovery process (adsorption, liquid-liquid extraction, pervaporation, vacuum flash fermentation and gas stripping) have been evaluated as promising techniques to improve productivity by mitigating butanol inhibition [15–19]. Even these techniques are more economically competitive comparing to conventional distillation for butanol recovery from the dilute fermentation broth, the deficiency in recovering a high titer of butanol is still the challenge.

Gas stripping is a physical separation process where target compounds are removed from an aqueous solution by bubbling gas through it [20]. It has many advantages over the other methods: simple operation, no harm to culture, and does not requires any chemicals nor membranes. It also collaborates with the agitation and homogenization of the system [10,13,21–27]. However, its potential in butanol recovery and energy saving for biobutanol production has not yet been fully explored nor optimized [13,28]. Most of previous studies on integrated ABE fermentation with *in situ* gas stripping have been conducted at relatively low butanol concentrations (5 g/L or less) to minimize butanol toxicity [9]. Several works reported that the gas flowrate [24,28] and the butanol titer in the feed solution affect gas stripping process [20,28]. Xue et al. [28] reported that it is necessary to conduct gas stripping at a butanol titer higher than 8 g/L in the feed solution in order to obtain a condensate with a butanol titer higher than its solubility in water (~80 g/L at 20 °C), which will result in phase separation and in a more energy-efficient butanol recovery process. On the other hand, the recycling of large amount of gas in industrial reactors would be uneconomical. The effect of gas recycle rate should be evaluated in order to optimize gas stripping conditions as an interest in commercialization this technology.

The aim of this work was to evaluate butanol production from a mixture of sugarcane-sweet sorghum juices using *C. acetobutylicum* DSM 792 in an integrated process (fermentation with *in situ* gas stripping) that contributes both to mitigate the butanol inhibitory effect and to obtain a highly concentrated butanol condensate. Kinetics models describing butanol formation by fermentation and butanol extraction by gas stripping were developed to study the effect of gas recycle rate on the performance of the fermentation coupled with *in situ* gas stripping and to determine the most favorable operational condition.

2. Materials and methods

2.1. Experimental assays

2.1.1. Characterization of raw material

A mixture of concentrated industrial juices of sugar cane (75%) and sweet sorghum (25%) was provided by Alur SA (Bella Unión, Uruguay) and it was stored at 5 °C. Table 1 shows its composition.

Table 1
Composition of concentrated sugarcane-sweet sorghum juices.

Component	Unit	Value
Sucrose	%	75
Glucose	%	5
Fructose	%	3
Ethanol	%	0.69
Acetic acid	%	0.11
Butyric acid	%	0.34
Succinic acid	%	0.15
cis-Aconitic acid	%	0.13
trans-Aconitic acid	%	1.05
Furfural	%	nd
5-Hidroximetilfurfural	%	nd
Phosphorous	%	<0.5
Nitrogen (Kjeldahl)	%	0.31
Aluminum	%	<0.002
Calcium	%	0.18
Cooper	%	<0.0005
Iron	%	0.0047
Magnesium	%	0.11
Manganese	%	0.0012
Potassium	%	0.76
Sodium	%	0.04
Zinc	%	<0.0005
Ash	%	2.5
Moisture	%	32
Density (20 °C)	kg/m^3	1330

% percentage in dry weight except moisture.
nd: no detected (detection limit: 0.003%).

2.1.2. Microorganism, inoculum preparation and fermentation media

Clostridium acetobutylicum DSM 792 was used in all fermentations. A stock culture was maintained in Reinforced Clostridial Medium (RCM) at 4 °C. A pre-culture was prepared by inoculating 10 mL of the stock culture into a serum bottle containing 100 mL RCM medium and incubating at 37 °C for 24–48 h until active growth was observed.

Glucose-based and the industrial media were used, containing 50–60 g/L of total sugar concentration expressed as glucose equivalent and 1% (v/v) P2 solutions. The P2 solutions contains K_2HPO_4 50 g/L, KH_2PO_4 50 g/L, ammonium acetate 220 g/L, *p*-amino benzoic acid 0.1 g/L, thiamine 0.1 g/L, biotin 0.001 g/L, $MgSO_4.7H_2O$ 20 g/L, $MnSO_4.H_2O$ 1 g/L, $FeSO_4.7H_2O$ 1 g/L, NaCl 1 g/L.

The seed culture for the fermentation was prepared in 250-mL bottles containing 100 mL of the same medium used in the fermentation but adjusted to reach half of the total sugar concentration. The pH was adjusted to 6.0 ± 0.1. The medium supplemented with 1 g/L of yeast extract was swept with O_2-free N_2 over the headspace of the bottles. It was sterilized at 121 °C during 15 min. On cooling to 37 °C, 1% (v/v) of filter-sterilized P2 stock solutions were added followed by inoculation with 10% (v/v) highly active cells that were grown in RCM. The culture was incubated at 37 °C, 150 rpm for 20–24 h until the cell density reached an optical density (OD) value of ~3.

2.1.3. Batch fermentation

Fermentation tests with glucose-based medium were performed in bottles of 250 mL with 100 mL of medium containing 50–60 g/L of glucose. The pH was adjusted initially to 6.0 ± 0.1. The medium was swept with O_2-free N_2 over the headspace of the bottles. It was sterilized at 121 °C during 15 min. On cooling to 37 °C, 1% (v/v) of filter-sterilized P2 stock solutions were added, followed by inoculation with 9% (v/v) highly motile cells. The bottles were incubated in an orbital shaker at 150 rpm and 37 °C. Samples were withdrawn at intervals for analysis. Tests were conducted in duplicate.

A batch fermentation was also performed in a 5 L-bioreactor (Infors HT, Switzerland) containing 2 L of the industrial juices diluted to reach 75 g/L of total sugar concentration. The pH was adjusted to 6.0 ± 0.1. The medium was swept with O_2-free N_2 over the headspace of the bioreactor. It was sterilized at 121 °C during 15 min. On cooling to 37 °C, 1% (v/v) of filter-sterilized P2 stock solutions were added followed by inoculation with 6% (v/v) highly active cells. Fermentation was performed at 37 °C and 150 rpm. No antifoam was necessary during the fermentation.

2.1.4. Fed batch fermentation coupled with in-situ gas stripping

A schematic diagram of the integrated reactor set up is shown in Fig. 1. Prior to the gas stripping experiment, the condenser and gas-circulation line were flushed with O_2-free N_2 gas. A 5-L bioreactor (Infors HT, Switzerland) containing 2.5 L of the industrial medium