Bioprocess intensification for isopropanol, butanol and ethanol (IBE) production by fermentation from sugarcane and sweet sorghum juices through a gas stripping-pervaporation recovery process

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ABSTRACT

Butanol and isopropanol are important commodity chemicals with a variety of applications. One of the main obstacles for biobutanol production by IBE (isopropanol-butanol-ethanol) fermentation is the intensive energy consumption for product recovery by conventional distillation due to low butanol titer in fermentation broth caused by butanol toxicity to cells. In the present study, butanol production by batch IBE fermentation coupled to an *in situ* gas stripping-pervaporation process to recover the butanol is proposed using *Clostridium beijerinckii* DSM 6423 and a mixture of sugarcane-sweet sorghum juices as substrate. Gas stripping was used to continuously remove butanol from the fermentation broth, followed with pervaporation to further concentrate

butanol. The strategy used allows alleviating butanol inhibition and to recuperate a condensate containing high butanol concentration (559 g/L). A kinetic model describing butanol production by IBE fermentation was developed. Kinetic parameters and experimental data were used to estimate the energy consumption of the sugarcane-sweet sorghum IBE production process. It was found that although the IBE production process showed less energy consumption (15%) than the butanol production process by ABE (acetone-butanol-ethanol) fermentation, a substantial improvement is still necessary for the process to be energetically/economically attractive.

Keywords: biobutanol, gas stripping, IBE fermentation, pervaporation, sugarcane, sweet sorghum

1. Introduction

There is growing interest in the production of chemicals and fuels from renewable resources due to climate change, global warming and energy security [1]. *n*-Butanol is a four-carbon alcohol known both as an advanced biofuel and as a commodity chemical. It can be produced through acetone-butanol-ethanol (ABE) or isopropanol-butanol-ethanol (IBE) fermentation in which a solvent mixture is produced. The co-production of acetone in the ABE process is not desirable because is corrosive to rubber engine parts and has poor fuel properties [2]. Butanol production through ABE fermentation has also been considered economically risky due to a potential oversupply of acetone [3]. Alternatively, isopropanol can be produced instead of acetone by some *Clostridium* species. Isopropanol is an important commodity chemical with a variety of applications and the solvent mixture

produced by fermentation (IBE) can be used as a fuel [4–6]. The microorganism best known as natural IBE producer is *Clostridium beijerinckii* DSM 6423 [3,4,7,8].

Major challenges in biobutanol production are the cost of the raw material and the intensive energy consumption in product recovery stages of the entirely IBE production process [9–11]. Sugarcane and sweet sorghum are crops whose juices contain high amounts of soluble fermentable sugars, and many essential nutrients for microbial growth [8,12]. Both, mainly sugarcane, are currently used for fuel bioethanol production in Uruguay. Furthermore, a residue (bagasse) is produced when juices are extracted, which can be burnt for steam production to meet the energy demand of industrial processes [13,14]. The low butanol concentrations that are reached in the fermentation broth due to cellular toxicity or product inhibition, requires a high energy consumption in the product recovery [15–17]. Alternative separation technologies have been studied to coupled butanol production with an *in situ* extraction method to mitigate butanol inhibition [18–20], such as liquid-liquid extraction [21], gas stripping [22,23], pervaporation [24,25], and flash vacuum [26].

Among butanol recovery methods, gas stripping and pervaporation are the most promising alternatives, and both have advantages and disadvantages. Gas stripping allows the removal of volatiles from the fermentation broth, does not requires chemicals or membranes, its operation is simple and does not harm the culture [16,27–29]. Its main disadvantage is its low selectivity [30]. Pervaporation is a separation process in which a feed solution is in contact with one side of the membrane, and the permeate is removed as a low-pressure vapor on the other side. The driving force is given by a vacuum system on the permeate side [19,31,32]. It presents high selectivity and less energy requirement [18,30]. The main disadvantage of pervaporation is the operating cost due to membrane fouling when used as an *in-situ* extraction method because of the presence of cells,

residual sugars and other components of the fermentation broth. While sugar conversion could be improved by extracting butanol with an *in-situ* extraction method, obtaining higher butanol concentrations with low energy consumption remains the challenge. By using both methods, their advantages could be combined and enhanced. In the present study, an integrated *in situ* gas stripping-pervaporation process is proposed, where gas stripping is used to continuously remove butanol from fermentation broth, followed by pervaporation to further condense butanol.

The energy consumption of several industrial processes has been successfully modeled and predicted using computer simulations. Various researchers have reported models for butanol production by ABE fermentation using Aspen Plus software from different raw materials such as sugarcane, sugar cane molasses, and corn [33–38]. Some researchers have specifically studied the use of energy of the butanol purification stages. Mariano et al. [17] have evaluated flash fermentation technology whereas Cai et al. [39,40] evaluated the use of energy of a gas stripping-distillation, gas stripping-pervaporation-distillation and two stage pervaporation-distillation processes. However, there are no energy evaluations for the butanol production by IBE fermentation from sugarcane and sweet sorghum juices reported in the literature to the authors' knowledge.

In this work, butanol production by batch IBE fermentation coupled to an *in situ* gas stripping-pervaporation process to recover the butanol was evaluated using *C. beijerinckii* DSM 6423 and a mixture of industrial sugarcane-sweet sorghum juices as substrate. Repeated-batch fermentations were also carried out. A kinetic model describing butanol production by IBE fermentation was developed. The kinetic parameters obtained and the experimental data of raw material composition, batch and repeated-batch fermentations and purification stages, were combined into a model to estimate the energy consumption of the integrated process using Aspen Plus software.

2. Materials and methods

2.1. Experimental assays

2.1.1. Medium, microorganism and inoculum preparation

A mixture of industrial sugarcane and sweet sorghum juices, 75 and 25%, respectively, provided by Alur-Bella Union, Uruguay, was utilized as culture medium. The microorganism used was *C. beijerinckii* DSM 6423. The inoculum preparation using the industrial sugarcane-sweet sorghum juices is described elsewhere, as well as the composition of the juices used [41].

2.1.2. Batch fermentation without and with in situ *gas stripping*

Fermentation experiments were performed in a 5 L-bioreactor (Infors HT, Switzerland) containing 2.5 L of industrial juices diluted to reach a total sugar concentration of 60 g/L (expressed in glucose equivalents) and supplemented with yeast extract (1 g/L). The pH was initially adjusted to 6.0 ± 0.1 with NaOH 1 M, and the medium was swept with O_2-free N_2 (Linde, Uruguay), over the headspace of the bioreactor, followed by sterilization at 121 °C for 15 min. When it reached room temperature, 1% (v/v) of filter-sterilized buffer and mineral P2 stock solutions and a commercial vitamin complex solution (Dispert ®, 1% (v/v)) were added. The P2 buffer and mineral solutions contained: K_2HPO_4 50 g/L, KH_2PO_4 50 g/L, ammonium acetate 220 g/L and $MgSO_4.7H_2O$ 20 g/L, $MnSO_4.H_2O$ 1 g/L, $FeSO_4.7H_2O$ 1 g/L, NaCl 1 g/L,

125 respectively. The vitamin complex solution composition was: thiamine mononitrate 0.12
126 g/L, riboflavin 0.020 g/L, pyridoxine hydrochloride 0.020 g/L, calcium pantothenate
127 0.061 g/L, niacinamide 0.61 g/L, and excipient qs. The bioreactor was inoculated with
128 8% (v/v) highly active, motile cells and the fermentation was carried out at 150 rpm and
129 35 °C. Samples were withdrawn at regular intervals for sugars, products, and optical
130 density analysis.

131 The fermentation with *in situ* gas stripping was conducted in the bioreactor
132 containing 1.5 L of the medium. The experimental set-up is detailed by Rochón et al. [41].

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134 *2.1.3. Repeated-batch fermentations*

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136 Repeated-batch IBE fermentations of the industrial juices were performed in
137 bottles of 250 mL with 100 mL of medium. The industrial juice mixture was diluted to
138 reach a total sugar concentration of 55-60 g/L and supplemented with yeast extract (1
139 g/L). The pH was adjusted initially to 6.0 ± 0.1. The medium was swept with O_2-free N_2
140 over the headspace of the bottles. It was sterilized at 121 °C during 15 min. On cooling
141 to room temperature, 1% (v/v) of filter-sterilized P2 stock solutions and vitamin complex
142 Dispert® were added, followed by inoculation with 8% (v/v) highly motile cells. The
143 bottles were incubated in an orbital shaker (Infors HT Ecotron, Switzerland) at 150 rpm
144 and 35°C. At the end of each batch fermentation (48 h), 8 mL of the culture were taken
145 and inoculated into a bottle containing 92 mL of fresh medium (8% v/v). Two sets of
146 repeated-batch fermentations were carried out.

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148 *2.1.4. Pervaporation assays*

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Pervaporation assays were done with a polydimethylsiloxane (PDMS) membrane with a total surface area of 50 cm^2 (Pervatech BV, the Netherlands). The feed solution was heated to 70 °C and circulated at a flow rate of 50 mL/min. The pressure on the permeate side was maintained at ~ 2 kPa by a vacuum pump IDP-3 (Agilent Technologies, USA) monitored by a vacuum gauge. The permeated vapor was condensed at -6 °C in vacuum traps immersed in a refrigerated circulating bath.

An IBE aqueous solution with the same condensate composition as that obtained from a batch fermentation of sugarcane-sweet sorghum juices coupled with *in situ* gas stripping using *C. beijerinckii* DSM 6423, was used as the feed solution. A schematic diagram of the integrated reactor set up is shown in Figure 1. Samples of both retentate and permeate were withdrawn every 3 h until 20 h and every 48 h until 38 h for solvent analysis.

2.1.5. Analytical methods

Isopropanol, butanol and ethanol from the gas stripping assays, batch, repeated-batch fermentation and fermentation with *in situ* gas stripping, both in the fermentation broth and in the gas stripping condensate, were measured with a gas chromatograph (GC, Shimadzu GC-2010) equipped with a flame ionization detector and a fused silica column (RTX®-Wax, 30 m long, 0.5 μm film thickness and 0.32 mm ID,Restek). Sugars were determined by HPLC (Shimadzu, Kyoto, Japan) using an Aminex 87-H column (Bio-Rad Europe GmbH) at 45 °C, 0.01 N sulfuric acid as eluent at a flow rate of 0.3 mL/min and a refractive index detector (RID).

Isopropanol, butanol and ethanol concentrations from pervaporation assays were determined by HPLC using an Aminex 87-H column (Bio-Rad Europe GmbH) at 30 °C,

0.01 N sulfuric acid as eluent at a flow rate of 0.6 mL/min and a refractive index detector (RID, Waters 2414).

In each sample, the total permeate mass was measured. Since all the permeates presented phase separation, the mass of each of the phases was also measured using an analytical balance.

To evaluate the pervaporation performances, the partial permeation flux of a given component (J_i) and the separation factor were defined and calculated according to the following equations [42]:

$$J_i = \frac{W}{A*t} \quad (1)$$

$$Separation\ factor\ = \frac{\left(\frac{y}{1-y}\right)}{\left(\frac{x}{1-x}\right)} \quad (2)$$

where W is the weight of the permeated condensate (g), A is the PDMS membrane area (m^2) and t is the operating time (h). x and y are the mass fractions of a given component (isopropanol, butanol or ethanol) in the retentate and permeate samples of the pervaporation system, respectively.

2.2. Process models

The modified Monod kinetic model with terms of product inhibition and bacterial death [14] was used in this study to describe microbial growth, substrate consumption and butanol production of an IBE fermentation. Therefore, the equations below were developed as follows:

$$\frac{dX}{dt} = \frac{\mu_m\ S}{K_s + S}\ X\left(1 - \frac{P}{K_p}\right)^a - k_d\ X \quad (3)$$

$$-\frac{dS}{dt} = \frac{\mu\, X}{Y_{X/S}} = \frac{\mu_m\, S}{K_s + S}\left(1 - \frac{P}{K_p}\right)^a \frac{X}{Y_{X/S}} \quad (4)$$

$$\frac{dP}{dt} = \frac{\mu\, X\, Y_{P/S}}{Y_{X/S}} = \frac{\mu_m\, S}{K_s + S}\left(1 - \frac{P}{K_p}\right)^a X\, \frac{Y_{P/S}}{Y_{X/S}} \quad (5)$$

where X is the dry cell weight (g/L), μ is the specific growth rate (h^{-1}), μ_m is the maximum specific growth rate (h^{-1}), S is the growth-limiting substrate concentration (g/L), K_s is the substrate saturation constant (g/L), k_d is the specific cell death rate (h^{-1}), P is the butanol concentration (g/L), K_p is the product concentration at which no cell growth occurs (g/L), a is the degree of product inhibition (-), $Y_{X/S}$ is the biomass yield coefficient (g/g) and $Y_{P/S}$ is the butanol yield coefficient (g/g).

Parameter estimation was carried out using global optimization tools presented in MATLAB® software (MathWorks, Nattick, MA, USA). The objective function was defined with the method of maximum-likelihood to minimize the differences between the experimental data obtained and the results of the model predictions. Fitting accuracy of the models was evaluated through analysis of coefficient of determination, R^2.

2.3. Simulation methodology

2.3.1 Process Description

The facility processes 490 000 t of sugar cane and sweet sorghum per year (annual production in Uruguay) and works 180 days (24 h per day) per year since the crop is seasonally available. Isopropanol, butanol and ethanol purities were defined as 99.5% (w/w), 99.8% (w/w), and 88.4% (w/w), respectively. The solvent mixture presents a water concentration of ~ 0.5% which, according to literature, could be directly used as a fuel

[6]. In this way, the process could be evaluated as either butanol or IBE production process. The simulated process can be grouped into juice treatment, fermentation with *in situ* gas stripping, butanol or IBE recovery, and wastewater treatment. A detailed description of juice treatment, inoculum development and wastewater treatment stages was already done for ABE fermentation in a previous work [43].

Both sugarcane and sweet sorghum are sent to the industrial plant in trucks. The transport energy consumption was estimated as 21 MJ/t from data reported for a sugarcane ethanol production facility in Uruguay (average distance 20 km) [44]. The material is transported to the mill by a conveyor belt. Water is added, the bagasse is separated from the juice and sent to the boiler for steam generation. The pH is adjusted to 7 by adding lime. The juice is heated at 105 °C by using two heat exchangers. Then the juice is clarified in another tank where flocculant is added. The clarifies juice is sent to the fermenters which are subsequently inoculated by a direct transfer of a culture of *C. beijerinckii* DSM 6423. Each seed train consists of bioreactors operating in batch mode for 24 h at 35 ºC. In the IBE fermentation stage, the inoculated cells are reutilized for a period of no more than 288 h, in accordance to the results found in the repeated-batch fermentations (section 3.2). Fermenters of 1700 m^3 are used, a typical size of Uruguay facility. RYield reactor type was used in the simulation. The fermenter temperature is kept constant at 35 °C by pumping 2% of the medium through an external heat exchanger [13]. Initial sugar concentration is fixed in 60 g/L to avoid substrate inhibition. Each fermentation presents a duration of 84 h achieving a sugar consumption of 95% and a biomass, isopropanol, butanol and ethanol concentrations of 5, 4, 15 and 1 g/L, respectively. Gas stripping starts at 24 h when butanol concentration is approximately 5 g/L (butanol separator factor: 9). The off gasses (CO_2 /H_2) are then recycled at a flow rate of 0.4 vvm (volume of gas/volume of medium min) and pass through the culture broth

until the fermentation is completed. Gas stripping is continued after the fermentation is finished to recover butanol remaining in the fermentation broth. The fermented broth is centrifuged to separate bacterial cells. Cells are reused in the next batch.

Regarding IBE purification section, it consists of a holding tank to store the recovered condensate containing the IBE products which is then concentrated by a pervaporation stage (butanol separator factor: 50). The energy consumption of the pervaporation was calculated as reported by Vane [45]. The remaining water is removed by a series of five distillation columns and a decanter. The first distillation column separates an ethanol/isopropanol/water mixture from a butanol-water mixture. The ethanol/isopropanol/water mixture is sent to another distillation column where ethanol is separated from the top of the column. The isopropanol/water mixture is sent to a third distillation column which separates isopropanol. Other two distillation columns and a decanter separate the butanol/water mixture into butanol and water.

It is widely known that the application of many of the recovery technologies allows only part of the desired product to be recovered. The separation efficiencies of the recovery section, both for gas stripping during and post-fermentation and pervaporation are detailed in sections 3.3. and 3.4. The amount of product remaining in the bioreactor, not recovered by gas stripping after fermentation or by pervaporation, results in product loss. The economic justification for incorporating a specific stage for its recovery could depend on the scale of industrial plant. If it is not recovered, more substrate will be needed to reach the determined production. For this reason, the *in-situ* recovery processes can be complemented by incorporating the conventional process known as end of pipe [46]. In some works, in which various *in-situ* removal methods are compared, it is assumed that all processes have the same annual production and substrate consumption, but the production will vary depending on the recovery efficiency of the process used. However,

to achieve a good economy, it should be considered that all products are recovered at some stage of the process [47].

Based on the separation efficiencies obtained experimentally in this work, not all the butanol, nor the rest of the solvents, are recovered after gas stripping and pervaporation. To solve this, it was considered they were sent to another distillation column to remove most of the water and other components present in the fermentation medium. It then goes through various stages of distillation to achieve the desired purity of butanol. For these stages, an estimated energy consumption was considered from the data reported by Mariano et al. [17] and Vane and Alvarez [48].

2.3.2 Process simulation

The process was simulated using Aspen Plus® software (Aspen Technologies Inc., Cambridge, MA version V8.8). The Aspen Plus model of the butanol/IBE production plant was developed based on the results obtained in our laboratory for fermentation, gas stripping and pervaporation stages presented in this work. Besides, values from expert consultations were utilized in the clarification stage. Butanol and IBE production scenarios were compared. Figure 2 shows a simplified flow diagram of the process.

Due to the complexity of the process, two Aspen Plus® methods were used to simulate the thermodynamic properties of the components. The non-random two liquid method, Haiden O´Conell (NRTL-HOC) was used in most of the process as it is the most suitable to evaluate the components properties (help from Aspen Plus® V 8.8; [37]). To model the decanter used in the butanol purification stages, a variable of the universal

quasi-chemical method (UNIQUAC) called UNIQ2 was used as it is adequate to predict liquid-liquid separations (help from Aspen Plus® V8.8; [48]).

3. Results and discussion

3.1. Fermentation model

Batch fermentation studies of *C. beijerinckii* DSM 6423 were performed with the industrial juices. The Eqs. (1)-(3) fitted well to the experimental data (Figure 3). The model allowed to describe biomass production, sugar consumption and butanol production appropriately (R^2_X = 0.97, R^2_S = 0.99, R^2_P = 0.99). The model parameters and coefficients of determination are presented in Table 1.

The maximum specific growth rate (μ_m) and biomass yield ($Y_{X/S}$) values determined by the model were similar to those obtained for *C. acetobutylicum* DSM 792 in a glucose-based medium (0.23 h^{-1} and 0.09 g/g, and 0.22 h^{-1} and 0.11 g/g, for *C. beijerinckii* DSM 6423 and *C. acetobutylicum* DSM 792, respectively) [14]. However, a higher butanol yield ($Y_{P/S}$) was found, 0.22 and 0.19 g/g for *C. beijerinckii* DSM 6423 and *C. acetobutylicum* DSM 792, respectively. To the authors' knowledge, there are no kinetic parameters for butanol production from an IBE fermentation using *C. beijerinckii* DSM 6423 reported in literature for further comparison. These values were used in the calculations corresponding to the design and operation of the bioprocess in the fermentation section of the sugarcane-sweet sorghum juices based biobutanol plant model performed with Aspen Plus.

3.2. Repeated-batch fermentations

The capacity of *C. beijerinckii* DSM 6423 to be reused in repeated-batch IBE fermentations of a mixture of industrial juices of sugarcane and sweet sorghum was evaluated to determine if the cells could be reused after the end of a batch fermentation or if they degenerate due to long exposure to butanol. An initial batch fermentation showed that the process finished at 48 h, when the total solvent concentration was 11.8 g/L and sugar conversion 72%. Solvents yield and productivity were 0.21 g/g and 0.21 g/Lh, respectively. Therefore, repeated-batch fermentations were performed every 48 h. Final acids and solvents concentrations obtained for each of the fermentation sets are shown in Figure 4. Table 2 shows the biomass concentration and the butanol and IBE productivities obtained for each of the batches.

A total solvents concentration in the range 7.4-16.7 g/L (4.1-10.5 g/L of butanol) was observed until the seventh batch fermentation. IBE productivities were in the range 0.12-0.32 g/Lh. Acetic and butyric acids were also produced (1.6-2.2 and 0.2-0.5 g/L, respectively). Biomass concentration varied between 1.0 and 3.3 g/L. As expected, low cell motility was observed after 48 h.

In the second batch of the set 1, very low solvents concentration was observed (< 1.5 g/L) possibly due to “acid crash” phenomenon. Acetic and butyric acids concentrations were higher (2.3 and 1.8 g/L, respectively). From the seventh batch onwards, solvent production decreased significantly. Acetic acid concentrations were higher (2.1-2.2 g/L) and no biomass growth was observed (< 0.3 g/L).

Repeated-batch fermentations from a glucose-based medium (60 g/L) using *C. beijerinckii* DSM 6423 immobilized on natural sugarcane bagasse was recently reported by Vieira et al. [49]. They found that IBE production was not stable in repeated batches

and that IBE yield generally decreased throughout batches. Butanol concentrations decreased from 5.4-6.2 g/L to 1.1-2.6 g/L after three batches for fermentations of 55 h. This behavior was attributed to cell degeneration due to long exposure to butanol. For this reason, they reduced the fermentation time from 55 to 36 h and carried out seven repeated batches (257 h). Butanol concentrations in the range 1.5-8.6 g/L, IBE concentrations in the range 3.9-14.3 g/L, and IBE productivities in the range 0.11-0.27 g/Lh were reached, which were similar to those obtained in this work. In the present work, higher butanol and IBE concentrations were found in some batches using an industrial medium (10.5 and 16.7 g/L respectively).

Although more studies are needed to understand the changes in the metabolism of *C. beijerinckii* DSM 6423, the results showed that the cells could be reused for a period of approximately 288 h (6 cycles of 48 h), saving operational costs due to the development of inoculum. Results were incorporated in the butanol plant model for the energy consumption estimation.

3.3. Fermentation with in situ gas stripping

Batch fermentation coupled with butanol extraction by *in situ* gas stripping was performed to alleviate butanol inhibition. The average solvent concentration obtained in the condensate after the use of gas stripping was: isopropanol 47 g/L, butanol 33 g/L, and ethanol 5 g/L. Neither acetic nor butyric acids were detected in the condensate.

The separation efficiency of gas stripping for isopropanol, butanol and ethanol was 53, 49 and 41% during the fermentation and 21, 32 and 21% during 40 h of gas stripping post-fermentation, respectively. The overall gas stripping separation efficiency for isopropanol, butanol and ethanol was 63, 60 and 60%, respectively.

Other results of batch fermentations with *in situ* gas stripping have been reported by Rochón et al. [41].

3.4. Pervaporation assays

Since the one-stage butanol recovery process by *in situ* gas stripping is not efficient enough to concentrate butanol at a high level [14], in this study it is proposed to use a second stage of recovery by pervaporation for further purification.

Figures 5a and 5b show the solvent concentration profiles on the feed side (gas stripping condensate as feedstock) of PDMS membrane and solvents flux *vs* its retentate concentrations, respectively. Butanol concentration on the feed side decreased significantly from 36 to 13 g/L, isopropanol decreased from 46 to 31 g/L and ethanol scarcely permeated. This behavior was expected because of the selective separation of volatile organic compounds by the PDMS membrane [39].

At the beginning of the pervaporation, butanol and IBE fluxes were 100 and 134 g/hm^2, respectively, which decreased to 39 and 52 g/hm^2 after 38 h due to the decrease in their retentate concentrations. Isopropanol and ethanol fluxes were lower (9-32 and 1-2 g/hm^2, respectively). Separation factor for butanol varied in the range 50-78, while isopropanol and ethanol values were stable at less than 6. The hydrophobic characteristic of the PDMS contributed to the high selectivity for butanol and the low selectivity for isopropanol and ethanol. Kieblich et al. [32] have studied *in situ* butanol removal from PBE (1,3-propanediol-butanol-ethanol) fermentation process by pervaporation obtaining a separation factor of 40 with a PDMS membrane (Pervap 4060) at 50 °C. They also reported a butanol flux of 517.3 g/m^{2}h and a butanol concentration of 328 g/L when

393 operated at 50 °C and a feed flow rate of 4 L/min at a feed butanol concentration of 11
394 g/L demonstrating the potential of butanol removal by pervaporation.

395 Xue et al. [18] have studied an integrated ABE fermentation-gas stripping-
396 pervaporation process. They reported that the performance and efficiency of the
397 membrane were greatly affected by the solvent concentrations in the retentate. However,
398 a clear correlation between butanol concentration in the retentate and permeate was not
399 observed (Figure 5c). This could be due to adsorption of butanol into the tube and
400 membrane, and possibly desorption in different time periods. Permeate average
401 concentrations obtained were 140, 559 and 10 g/L of isopropanol, butanol and ethanol,
402 respectively. Butanol and ethanol concentrations were similar to those reported by Xue
403 et al. [18] for a similar process using ABE as feed solution. The results showed that the
404 membrane was effective in recovering butanol if a high butanol concentration feed was
405 used.

406 Table 3 presents the solvents concentration obtained by different authors. The
407 experimental results are compared with those obtained for ABE fermentation, since to
408 authors´ knowledge there is no data in the literature for IBE fermentation using a two-
409 stage *in situ* recovery process. The butanol concentration reached in this study (559 g/L)
410 was the highest and total solvent concentration was relatively high compared to those
411 obtained by the other authors for ABE fermentation. Furthermore, to the author´s
412 knowledge, total IBE concentration obtained (712 g/L) was the highest reported in the
413 literature. The two-stage gas stripping-pervaporation separation process provides a high
414 IBE concentration and, therefore could be a more efficient promising system than
415 conventional systems.

416 The separation efficiency (solvent in permeate-solvent in retentate ratio) were 16,
417 82 and 8% for isopropanol, butanol and ethanol, respectively. The losses of products

could be mainly attributed to sampling and solvent adsorption on tubes and membrane. In addition, it should be noted that there are solvents present in the feed solution (31, 13 and 5 g/L of isopropanol, butanol and ethanol, respectively) at the end of the pervaporation process (38 h). Longer times are required for pervaporation assays in these conditions to achieve complete removal of solvents.

3.5. Energy consumption

The energy consumption of an industrial plant that produces IBE from the industrial sugarcane-sweet sorghum juices through a batch fermentation strategy was evaluated. Gas stripping was coupled to the fermentation as an *in-situ* recovery technique followed by pervaporation for further product purification. As already mentioned, experimental results presented above were used throughout the simulation (kinetic parameters, batch and repeated batch fermentation, *in situ* gas stripping and pervaporation results). Since the kinetic model did not consider neither isopropanol nor ethanol production, experimental yield values obtained in the batch fermentation were used ($Y_{isopropanol/S}$ = 0.07 g/g, $Y_{butanol/S}$ = 0.26 g/g, $Y_{ethanol/S}$ = 0.01 g/g) [8].

The energy required by the process was covered by the energy generated by burning the bagasse. Butanol and IBE recovery stages presented the higher energy consumption of the process (Table 4). They presented an energy consumption of 29.63 and 22.66 GJ/m^3, for butanol and IBE production process, respectively, which are higher than the estimated value reported by Cai et al. [39] (20.1 GJ/$m^3_{butanol}$) for ABE production with a similar recovery process (gas stripping-pervaporation-distillation). Pyrgakis et al. [50] evaluated different scenarios for butanol production through IBE fermentation with gas stripping coupled to adsorption/desorption and condensation methods. The scenarios

consisted in three different product portfolios with adsorption as the recovery method and one portfolio for IBE production with condensation as recovery method. They concluded that condensation was not sustainable due to the high energy cost that is required for the recovery of alcohols. Grisales-Diaz and Tost [51] have recently reported an alternative distillation system for IBE recovery with an energy requirement between 5.3 and 6.6 GJ/m^3_{IBE}, which is approximately half of that obtained in this work (11.8 GJ/m^3_{IBE}). This could probably be due to the alternative efficient distillation system proposed in their work, which is a combination of azeotropic and extractive distillation.

Butanol production by ABE fermentation from sugarcane-sweet sorghum juices in a similar plant and process configuration was evaluated previously [43]. The total energy consumption of the butanol plant by IBE fermentation was 15% higher than that through ABE fermentation. One reason could be the higher energy consumption in the distillation, since it involves more distillation columns. However, if the IBE mixture is considered as the final product, the energy consumption was lower (12%).

A mass balance of the overall process for biobutanol production from sugarcane and sweet sorghum juices was performed. Isopropanol, butanol and ethanol production were 2670, 9920 and 380 ton/year. Butanol and solvents yield of 25 and 32 g per kg of juices, respectively, were reached by IBE fermentation whereas 19 g butanol per kg of juices was obtained by ABE fermentation.

Regarding the two scenarios evaluated, as it was expected, the energy consumption was lower (23%) when the IBE mixture was considered as the final product (Table 4). Calorific value (lower heating value) of the IBE mixture was calculated as 26.1 GJ/m^3 based on data reported by Yanowitz et al. [52] for an I:B:E mass solvent relation produced of 7:26:1. Unfortunately, both scenarios presented an energy consumption higher than their calorific value, which suggests that improvements should be made in the

IBE production process from sugarcane-sweet sorghum juices either by genetic engineering of the strain or by improvements in the fermentation and purification processes.

4. Conclusions

The integrated gas stripping-pervaporation process utilized was successful in terms of condensate concentrations obtained (140, 560, and 10 g/L for isopropanol, butanol and ethanol, respectively). A modified Monod kinetic model with terms of product inhibition and bacterial death showed satisfactory agreement with the experimental data obtained with *C. beijerinckii* DSM 6423 in terms of cell growth, sugar consumption, and butanol production which could be used in models for the design and control of an IBE fermentation. *C. beijerinckii* DSM 6423 could be used in repeated-batch fermentations, saving operational costs due to inoculum development although more in-depth studies are required in order to have a more predictable performance. Kinetic parameters and experimental data were used to estimate the energy consumption of the sugarcane-sweet sorghum IBE production process. It was found that although the IBE production process showed less energy consumption than the butanol production process by ABE fermentation, a substantial improvement is still necessary for the process to be energetically/economically attractive.

CRediT authorship contribution statement

Eloísa Rochón: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing-original draft., Visualization. **Gastón Cortizo:** Validation,

Investigation. **María Inés Cabot:** Validation, Investigation. **María Teresa García Cubero**: Resources, Visualization, Supervision, Writing-review & editing. **Daniel Ferrari**: Conceptualization, Methodology, Validation, Visualization, Writing-review & editing. **Mónica Coca**: Visualization, Supervision, Writing-review & editing. **Claudia Lareo**: Conceptualization, Methodology, Validation, Formal analysis, Resources, Visualization, Supervision, Project administration, Funding acquisition, Writing-review & editing.

Declaration of interests

The authors declare that they have no known competing financial interests of personal relationships that could have appeared to influence the work reported in this paper.

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702
703

Figure captions

Figure 1. Schematic diagram of the integrated process: batch fermentation-gas stripping-pervaporation.

Figure 2. Simplified flow diagram of the isopropanol, butanol and ethanol production from sugarcane and sweet sorghum juices in Aspen Plus®.

Figure 3. Glucose, biomass and butanol concentration profiles during a batch fermentation of the industrial juices. Experimental (symbols); simulated (lines).

Figure 4. Solvents and acetic and butyric acid concentrations for repeated-batch fermentations of *C. beijerinckii* DSM 6423 at 48 h using a mixture of industrial juices of sugarcane and sweet sorghum. a) set 1: b) set 2.

Figure 5. Performance of the pervaporation process. a) solvent concentration profile in the feed; b) solvent flux as a function of their concentration in the retentate; c) solvent concentration on the permeate side.

Tables

Table 1. Kinetic model parameters.

Parameter	Unit	Value
μ_m	h^{-1}	0.23
K_s	g/L	2.0
$Y_{X/S}$	g/g	0.09
$Y_{P/S}$	g/g	0.22
K_p	g/L	9.7
k_d	h^{-1}	0.03
a		2.1
R^2_X	-	0.97
R^2_S	-	0.99
R^2_P	-	0.99

R^2_X, R^2_S, R^2_P are coefficient of determination for Eq. (1), Eq. (2) and Eq. (3), respectively [14].

Table 2. Repeated-batch fermentation parameters of *C. beijerinckii* DSM 6423 at 48 h.

Set 1				Set 2			
Batch number	Butanol productivity (g/Lh)	IBE productivity (g/Lh)	X (g/L)	Batch number	Butanol productivity (g/Lh)	IBE productivity (g/Lh)	X (g/L)
1	0.13	0.19	1.7	1	*	*	1.0
2	*	*	**	2	0.18	0.27	2.5
3	0.17	0.25	3.3	3	0.08	0.12	2.7
4	0.18	0.25	1.9	4	0.15	0.21	2.1
5	0.13	0.29	**	5	0.18	0.26	**
6	0.21	0.32	2.8	6	0.15	0.26	1.4
7	0.20	0.28	3.2	7	*	*	**
8	*	*	**	8	*	*	**

(*) not calculated. Butanol and IBE concentration produced at the end of the batch was less than 0.05 g/L, and 0.15 g/L, respectively.

(**) not measured.

Table 3. Comparison of the solvent concentration obtained in the condensate by ABE and IBE fermentations using different two-stage separation processes.

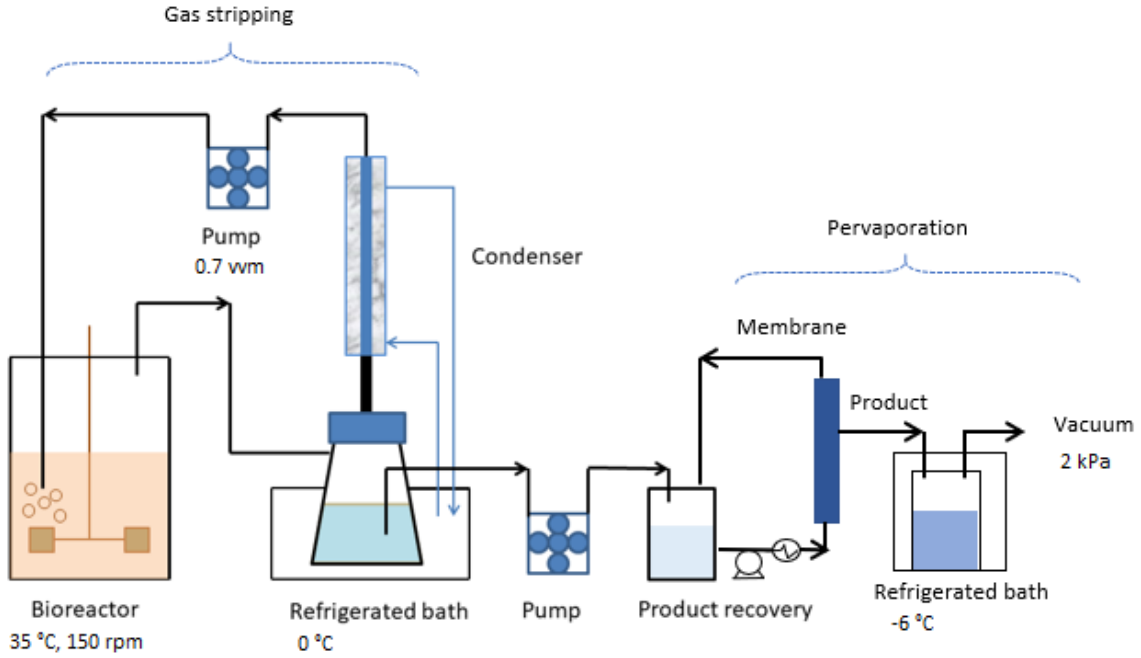
Strain	Substrate	Fermentation strategy	Strategy	Acetone (g/L)	Isopropanol (g/L)	Butanol (g/L)	Ethanol (g/L)	Total solvents (g/L)	Reference
C. acetobutylicum JB200	Glucose	Batch with immobilized cells	Two stage gas stripping	94.0	na	420.3	18.0	532.3	[9]
C. acetobutylicum JB200	Glucose	Fed batch with immobilized cells	Gas stripping-pervaporation	91.5	na	521.3	10.1	622.9	[18]
C. acetobutylicum ABE 1401	Glucose	Fed batch with immobilized cells	Gas stripping-pervaporation	169.9	na	482.5	54.2	706.7	[39]
C. acetobutylicum ABE 1201	Glucose	Continuous	Two stage pervaporation	304.6	na	451.9	26.0	782.5	[40]
C. acetobutylicum ABE 1201	Sweet sorghum bagasse	Batch	Gas stripping-salting out	203.5	na	520.3	23.8	747.6	[53]
C. beijerinckii DSM 6423	Sugarcane-sweet sorghum	Batch	Gas stripping-pervaporation	na	140.0	558.9	10.0	712.4	This study

na: not applicable

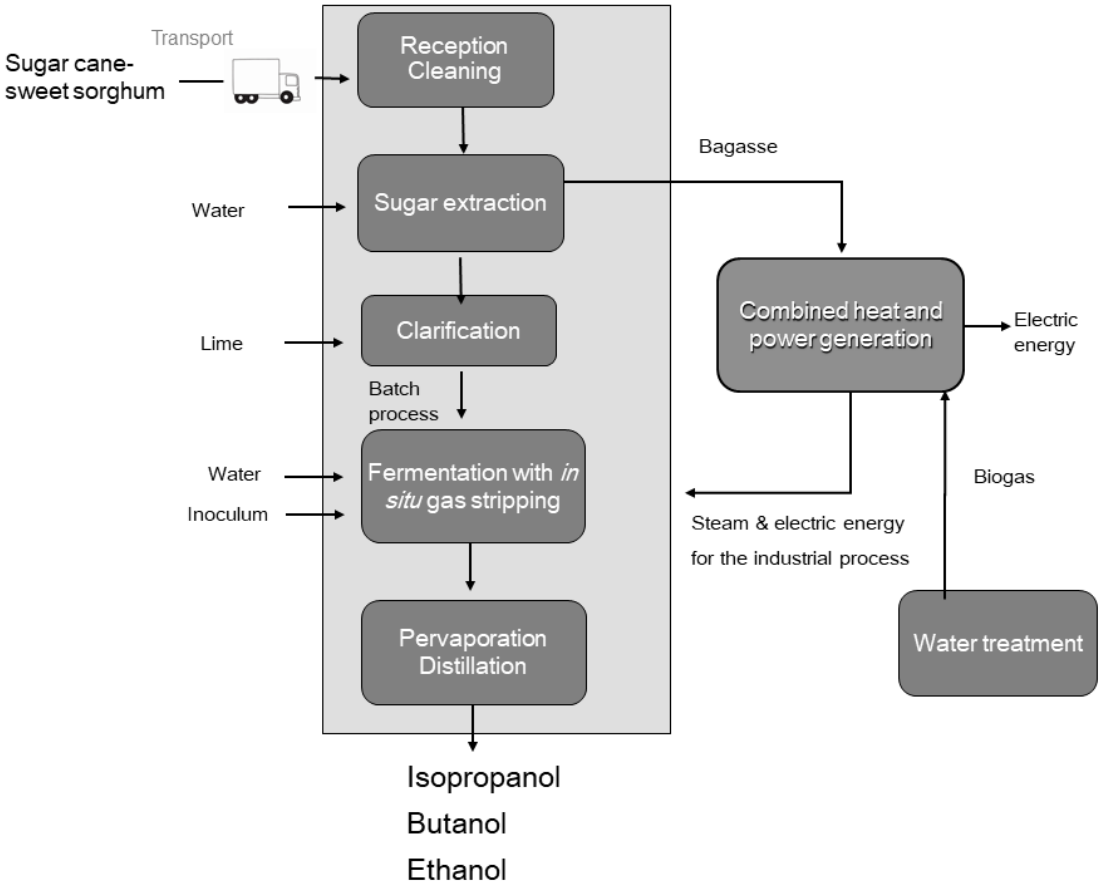
Table 4. Energy consumption for butanol and IBE production from sugarcane-sweet sorghum juices.

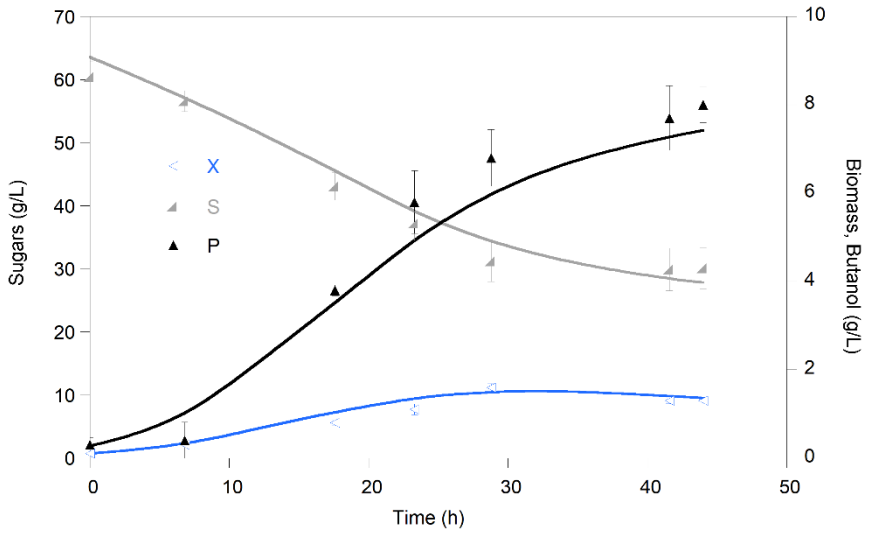
Stages of the process	**Energy consumption**	
	Butanol production ($GJ/m^3_{butanol}$)	**IBE production** (GJ/m^3_{IBE})
Transport	0.85	0.65
Milling	1.26	0.97
Clarification	9.91	7.58
Inoculum development and fermentation	0.41	0.31
Recovery	29.63	22.66
Water treatment	0.32	0.25
Total	42.38	32.41

11


Gas stripping
Pump
0.7 vvm
Condenser
Pervaporation
Membrane
Product
Vacuum
2 kPa
Bioreactor
35 °C, 150 rpm
Refrigerated bath
0 °C
Pump
Product recovery
Refrigerated bath
-6 °C

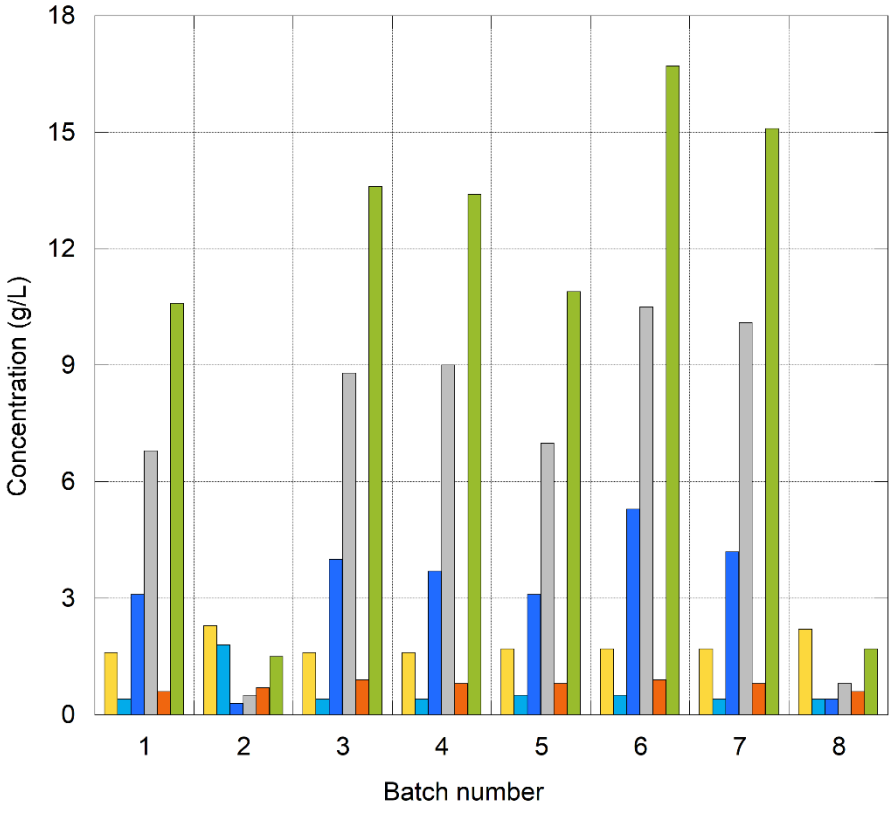
12


Transport
Sugar cane-
sweet sorghum
Reception
Cleaning
Water
Sugar extraction
Lime
Clarification
Batch
process
Water
Inoculum
Fermentation with in situ gas stripping
Pervaporation
Distillation
Isopropanol
Butanol
Ethanol
Bagasse
Combined heat and power generation
Electric energy
Biogas
Steam & electric energy
for the industrial process
Water treatment


Sugars (g/L)
Biomass, Butanol (g/L)
Time (h)
X
S
P
0
10
20
30
40
50
60
70
0
2
4
6
8
10
0
10
20
30
40
50

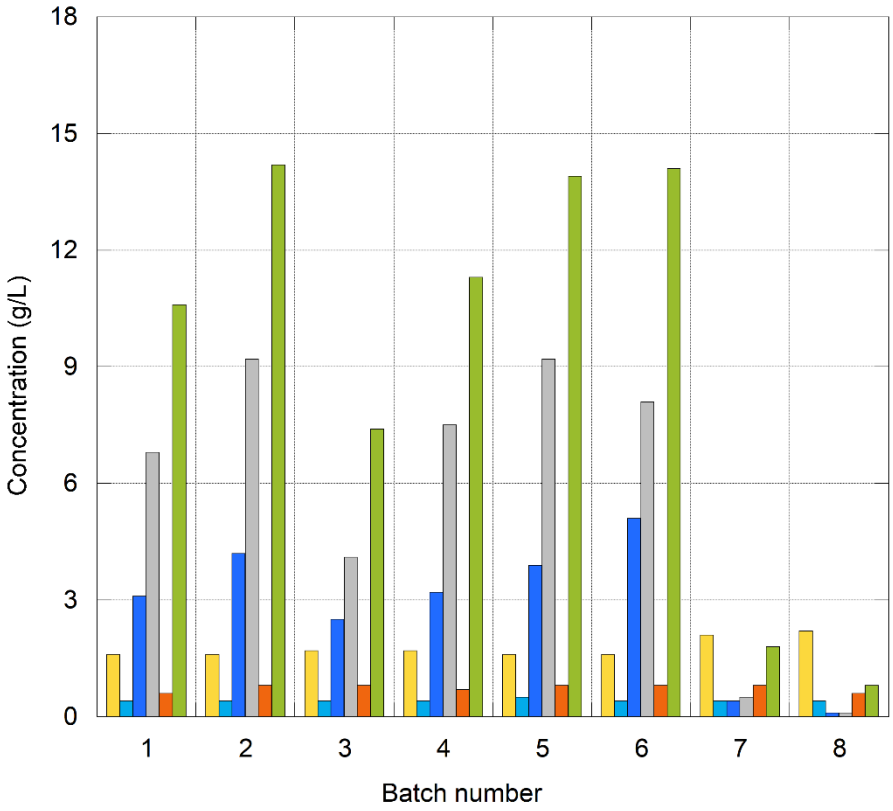
13

A)


Concentration (g/L)
Batch number
0
3
6
9
12
15
18
1
2
3
4
5
6
7
8

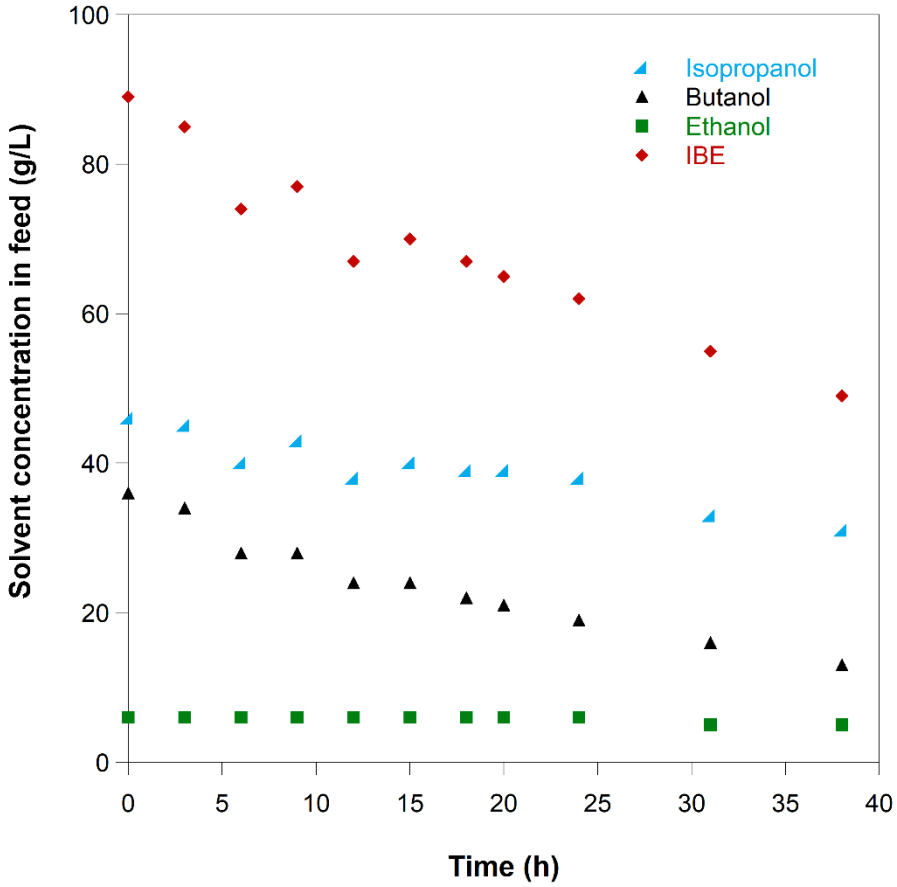
14

B)



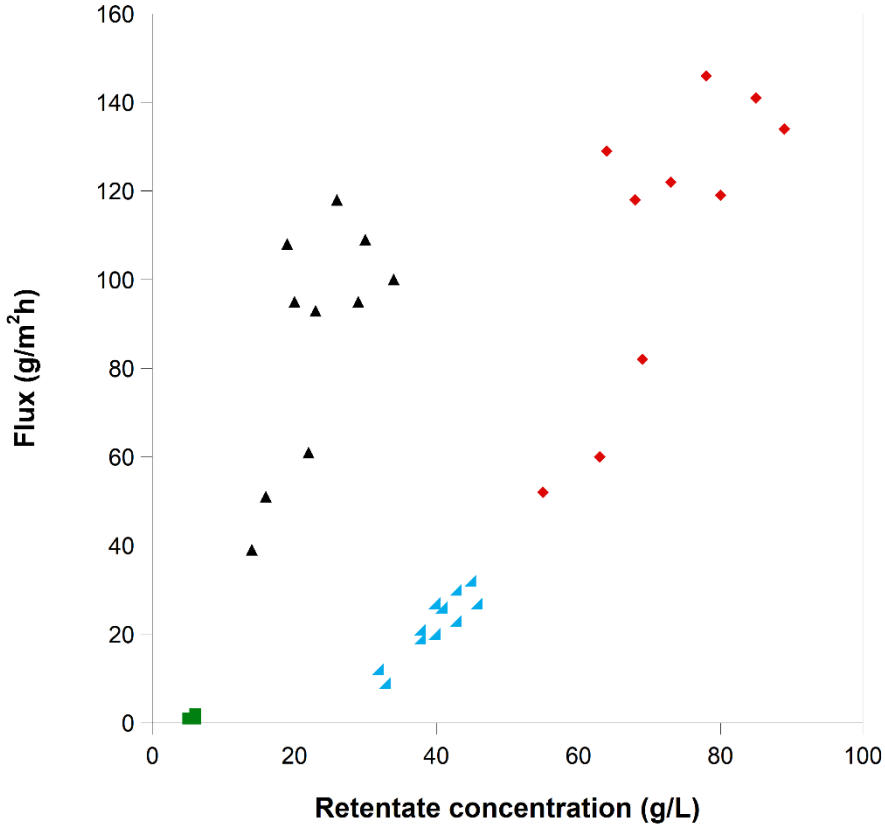
15

A)


Solvent concentration in feed (g/L)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Time (h)
Isopropanol
Butanol
Ethanol
IBE

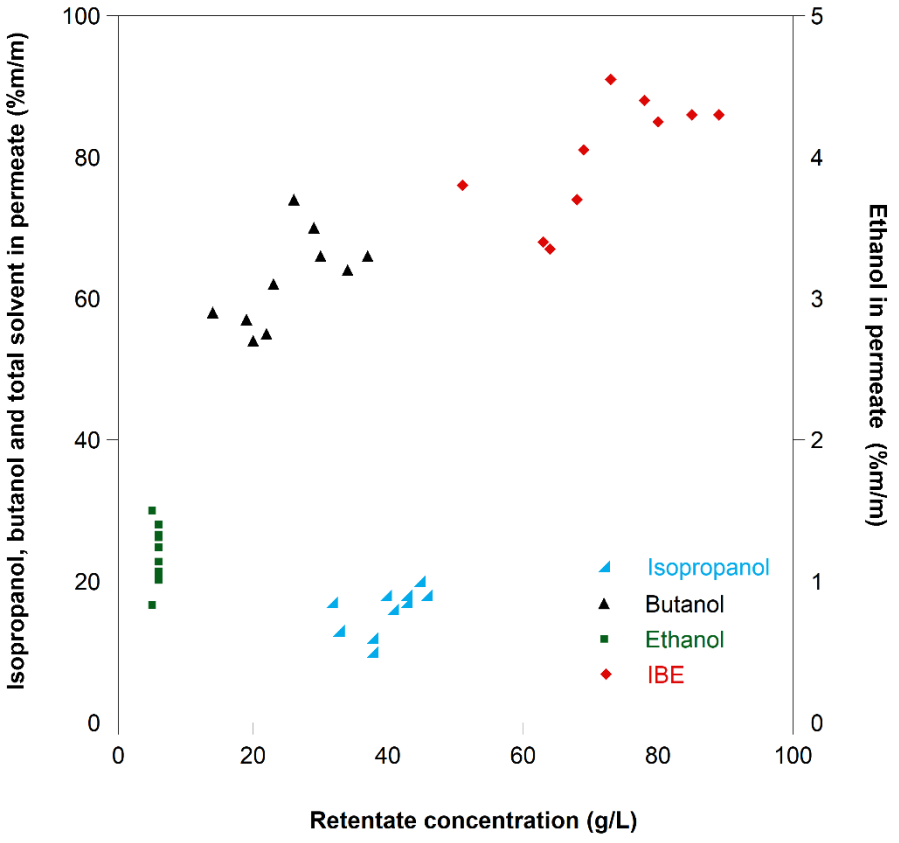
16

B)


Flux (g/m²h)
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Retentate concentration (g/L)

17

C)


Isopropanol, butanol and total solvent in permeate (%m/m)
Ethanol in permeate (%m/m)
100
80
60
40
20
0
5
4
3
2
1
0
0
20
40
60
80
100
Retentate concentration (g/L)
Isopropanol
Butanol
Ethanol
IBE

18

19