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Biobutanol production from C5/C6 carbohydrates integrated with pervaporation: experimental results and conceptual plant design

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Abstract

In this study, a simulated lignocellulosic hydrolysate was used in a continuous two-stage fermentor set-up for production of acetone, butanol and ethanol. An organophilic pervaporation unit was coupled to the second fermentor. The dilution rate in the first fermentor was kept constant at 0.109 h^{-1} while the dilution rate in the second fermentor was gradually decreased from 0.056 h^{-1} to 0.020 h^{-1}. Glucose was completely consumed while 61% of the xylose was consumed at the lowest dilution rate, leading to an overall solvent productivity of 0.65 $g.L^{-1}.h^{-1}$ and a high concentration of 185 $g.kg^{-1}$ solvents in the permeate in the last fermentation zone during 192h. Based on the experimental results, a process integrated with organophilic pervaporation was conceptually designed and compared with a base-case. Chemcad simulations indicate an energy reduction of ~50% when organophilic pervaporation is used. This study also demonstrates significant process flow reductions and energy consumption by the use of organophilic pervaporation as *in situ* product recovery technology.

Keywords: Bioprocess design; Biobutanol; Process Integration; Product inhibition; *In situ* product recovery; Pervaporation

Introduction

While the acetone-butanol-ethanol (ABE) fermentation was an industrial reality in the first half of the twentieth century, it was outcompeted in the 1950s and 1960s by the oxo process followed by catalytic hydrogenation for butanol production and the cumene process for acetone [20]. The main reasons for the decline of the ABE fermentation process were high substrate costs and butanol inhibition leading to low volumetric solvent productivities and high distillation costs [3].

Although the majority of the acetone is used as a solvent, a quarter is used as precursor for methyl methacrylate. In a similar way, *n*-butanol is used as a solvent and as an intermediate for the production of acrylates, ethers and butyl acetate [6].

In a conventional ABE fermentation, solvent concentrations are limited to 2 %(w/w) due to strong product inhibition (especially in the case of butanol) leading to distortion of the integrity of the cell membrane. Consequently, the carbohydrate concentration is limited to only ~6 %(w/w), leading to an undesirably high water consumption per kilogram butanol. An alleviation of product inhibition would allow an increase in carbohydrate concentration and thus a reduction in process flows. This can be achieved by complementing the fermentation process with *in situ* product recovery (ISPR) technologies, such as adsorption, pervaporation, gas stripping or L/L extraction.

In particular, organophilic pervaporation is a unit operation where organics are recovered by selective vaporization through a membrane. In contrast to distillation, pervaporation is not restrained by the normal vapor-liquid equilibrium (VLE), but by the selectivity of the membrane which is dependent on the solubility of target compounds in the membrane and their diffusivity through the membrane. Therefore, organophilic pervaporation is a promising technique for the recovery of low concentrations of volatile organic components from an aqueous phase [9;11;21;22;25]. For this purpose, polymeric pervaporation membranes based on polydimethylsiloxane (PDMS) are of particular interest as they do not seem to suffer from fouling [16;22], a prerequisite for long-term operations. Other membranes offer the promise of increased separation factors but suffer from fouling or lead to a decline in performance when directly in contact with the fermentation broth [2;9].

An interesting feature of the ABE fermentation is that most clostridia are able to convert not only C6, but also C5 carbohydrates. Therefore, agricultural residues, lignocellulosic hydrolysates and hydrolysates from pulp and paper industry can be used as alternative substrates for this fermentation process [8].

Previous investigations of continuous solvent fermentations using C5/C6 carbohydrates limited the total carbohydrate concentration to 6%, even when ISPR was used [1;4]. One of the advantages of ISPR is a reduced process flow by use of a higher substrate concentration [23]. Obviously, the use of ISPR in the case where only 6% carbohydrates is used will not lead to a reduced process flow because the carbohydrates can be completely converted to solvents at this concentration without ISPR.

Furthermore, these studies concerned isopropanol-butanol-ethanol (IBE) fermentations instead of ABE fermentations.

In the present study, we report for the first time the use of a concentrated mixture of 15 %(w/w) C5/C6 carbohydrates mimicking a lignocellulosic hydrolyzate in a two-stage continuous fermentation using organophilic pervaporation as *in situ* product recovery technology. Glucose and xylose were used in a 2:1 ratio as typically found in wheat straw hydrolyzates [13]. This mode of operation leads to significant improvements in the water balance of the process. Due to the expected lower consumption rate of xylose (C5) in comparison to glucose (C6), the dilution rate in the second fermentor is periodically decreased and its effect on the bioconversion kinetics is studied.

Finally, the energy consumption of a base-case scenario consisting of continuous biobutanol production followed by conventional downstream processing is compared with the energy consumption of a continuous biobutanol plant integrated with organophilic pervaporation membranes.

Material and methods

Preparation of culture media

Clostridium acetobutylicum strain ATCC 824 (Belgian co-ordinated collections of micro-organisms, BCCM) was used in all experiments. One liter of medium contains 0.01 g NaCl, 2.2 g ammonium acetate, 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.01 mg biotin, 3 mg *p*-aminobenzoic acid (PABA), 0.2 g $MgSO_4.7H_2O$, 0.01 g $MnSO_4.H_2O$, 11.1 mg NH_4Fe citrate, 100 g glucose, 50 g xylose and 3 g yeast extract. The medium containing all components was prepared and filter-sterilized using a 0.2 μm Supor Membrane (VacuCap Filter, Pall Corporation, Port Washington, NY, USA).

Continuous two-stage fermentor set-up integrated with pervaporation

A two-stage continuous ABE fermentation process was carried out, using a first fermentor of 2 L (working volume 1.1 L) and a second fermentor of 7.5 L (working volume 2.9 – 6.0 L). Both fermentors

were supplied by Infors (Bottmingen, Switzerland). The first fermentor was run at 35°C and the second fermentor was run at a slightly higher temperature of 37°C to provide a higher driving force for pervaporation. The fermentors were sparged with nitrogen prior to inoculation (10 % vol/vol) until the dissolved oxygen tension was close to zero. The in-house developed and assembled pervaporation unit consisted of three rectangular flat membrane modules (Pervatech, Enter, The Netherlands) connected in series with a total membrane surface area of 0.027 m^2. The pervaporation unit is directly coupled to the second fermentor without cell separation. Hence, the entire fermentation broth was constantly recirculated from the second fermentor to the pervaporation unit and back. Figure 1 shows a scheme of the experimental set-up. Thin film composite membranes, consisting of a polydimethylsiloxane (PDMS) separating layer of approximately 1 µm thickness on top of a porous polyimide support (approximately 200 µm), were purchased from Pervatech. An average permeate pressure of 9.6 mbar was established using a membrane vacuum pump (SC920, KNF Neuberger GmbH, Freiburg, Germany). The pH was monitored with a pH sensor InPro 3250 (Mettler-Toledo, Columbus, OH, USA) and left uncontrolled in both fermentors. The pH reached an average of 4.57 in the first fermentor and 4.48 in the second fermentor over the entire course of the fermentation.

Analysis

Xylose (16.0 min retention time) and glucose (14.5 minutes retention time) were determined by high performance anion exchange chromatography using a Dionex CarboPac PA1 column (2.5 m * 4 mm) with pulsed amperometric detection (Dionex ICS-5000 DC, Thermo Fischer Scientific, Waltham, Massachusetts). Column temperature was 25°C while the mobile phase consisted of 92.7 % demineralized water and 7.3% of a 250 mM NaOH solution. Volatile fatty acids and solvents were analyzed as described previously by Van Hecke *et al.* [22].

Calculations

Formulas for the different reported parameters are given below.

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97 It can be shown by deduction that the overall dilution rate D_{ov} can be calculated as:

98 $$D_{ov} = \frac{D_1 * D_2}{D_1 + D_2} \quad (1)$$

99 with D_1 and D_2 the dilution rate in the first and second fermentor respectively.

100 Overall solvent productivity P_{ov} without pervaporation ($g.L^{-1}.h^{-1}$) is:

101 $$P_{ov} = D_{ov} * C_{ABE,2} \quad (2)$$

102 With $C_{ABE,2}$ the total solvent concentration in the second fermentor.

103 To simplify further calculations we calculated the hypothetical "total solvent concentration" as the

104 average solvent concentration from the effluent flow and permeate flow:

105 $$C_{ABE,total} = \frac{C_{ABE,2} * Q_E + C_{ABE,permeate} * Q_P}{Q_E + Q_P} \quad (3)$$

106 With Q_E and Q_P the effluent and pervaporation flow rates respectively.

107 Overall solvent productivity with pervaporation ($g.L^{-1}.h^{-1}$):

108 $$P_{ov} = D_{ov} * C_{ABE,total} \quad (4)$$

109 Solvent productivity in the first fermentor can be calculated as:

110 $$P_1 = D_1 * C_{ABE,1} \quad (5)$$

111 Solvent productivity in the second fermentor can be described by:

112 $$P_2 = D_2 * \left(C_{ABE,total} - C_{ABE,1}\right) \quad (6)$$

113 The overall carbohydrate consumption can be calculated as:

114 $$S_{ov} = D_{ov} * \left(C_{carbohydrate,IN} - C_{\text{carbohydrate},OUT}\right) \quad (7)$$

115 With $C_{carbohydrate,IN}$ and $C_{carbohydrate,OUT}$ the carbohydrate concentration in the feed and in the

116 second fermentor respectively.

117 The solvent yield can then be calculated as the ratio between solvent productivity and glucose

118 consumption:

119 $$Solvent\ yield\ (g.g^{-1}) = Y_{P/S} = \frac{P_{ov}}{S_{ov}} = \frac{C_{ABE,total}}{(C_{glucose,IN} - C_{glucose,OUT})} \quad (8)$$

120 Finally, the glucose and xylose utilization (%) are calculated as:

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$$Glucose\ utilization = \left(1 - \frac{C_{glucose,OUT}}{C_{glucose,IN}}\right) * 100 \quad (9)$$

$$Xylose\ utilization = \left(1 - \frac{C_{xylose,OUT}}{C_{\mathrm{xylose},IN}}\right) * 100 \quad (10)$$

The separation factors obtained during pervaporation for component i to water are defined as:

$$\alpha_{\mathrm{i/water}} = \frac{{}^{\mathrm{y_i}}/_{\mathrm{y_{H_2O}}}}{{}^{\mathrm{x_i}}/_{\mathrm{x_{H_2O}}}} \quad (11)$$

with x_i the mole fraction of component i in the feed and y_i the mole fraction of component i in the permeate.

The enrichment factor for component i, β_i, is defined as [7]:

$$\beta_i = \frac{y_i}{x_i} = \frac{\alpha}{1+(\alpha-1)*x_i} \quad (12)$$

The total flux was calculated as:

$$J = \frac{m}{A*t} \quad (13)$$

with m the weight of the total permeate (g), A the membrane area (m²) and t the time of pervaporation (h).

The flux of component i is calculated as:

$$J_i = c_{i,permeate} * J \quad (14)$$

with $c_{i,permeate}$ the concentration of component i in the permeate.

Simulations

The heteroazeotropic distillation process was simulated using Chemcad 6.3.2 (Chemstations, Houston, TX, USA) chemical process simulation software in which the NRTL (Non-Random Two Liquid model) thermodynamic model and a SCDS (Simultaneous Correction Distillation System) type of column were chosen.

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Results & discussion

Bioconversion kinetics and performance

Table 1 lists the various kinetic parameters of this continuous two-stage ABE fermentation integrated with organophilic pervaporation. The concentrations of carbohydrates, solvents, volatile fatty acids in the first fermentor are plotted in figure 2(a-d) and in the second fermentor in figure 2(e-h). The dilution rates initially applied to the first and second reactor required to avoid wash-out of micro-organisms in the first fermentor and required to ensure a significant solvent production in the second fermentor were determined in preliminary experiments. After inoculation of both reactors and 13.8h of batch operation, the fermentation was changed to continuous conditions (phase 2).

From then, the dilution rate in the first fermentor remained constant between 1.02E-2 h^{-1} and 1.09E-2 h^{-1}. Even though the dilution rate in the first fermentor and initial carbohydrate concentrations were constant throughout the entire course of the fermentation (496.6h equivalent to 52.7 * t_r, more than enough to expect steady state conditions), large fluctuations in acetone, butanol and ethanol titers can be observed in the first fermentor (figure 2c) ranging from 1.8-11.8 $g.L^{-1}$ total solvents (not shown in graph). These fluctuations in the first fermentor might be due to: 1. cell aggregate formation (visually observed in fermentor) and deformation leading to differences between the residence times of cells and liquid in the fermentor; 2. Metabolic oscillations.

The solvent concentration in the second fermentor rises significantly in phase 2 (~35h duration). The organophilic pervaporation module was connected to the second fermentor in the beginning of phase 3 and quickly lead to a decrease in solvent titers (figure 2g). Due to the additional internal volume of the pervaporation unit, the dilution rate in the second fermentor decreased to 3.85E-02 h^{-1}. In phase 4 (160.5h duration equivalent to 3.8 * t_r) the dilution rate of the second fermentor was further decreased to 2.38E-2 h^{-1} by increasing the working volume. The increased working volume combined with the removal of inhibitory solvents by pervaporation allowed complete utilization of glucose (100 $g.L^{-1}$) after

215h of cultivation (figure 2f). To place these results in perspective, in ABE fermentations without *in situ* product recovery only ca. 60 $g.L^{-1}$ of carbohydrates can be converted to solvents [15].

In phase 4, the solvent concentration in the second fermentor gradually increased concomitantly with the solvents in the permeate (figure 2g). In phase 5 (191.7h equivalent to 3.81 * t_r) the working volume in the second fermentor was increased again causing an increase in xylose utilization to 61%. The average solvent concentration in the second fermentor in phase 5 was 13.6 $g.L^{-1}$ with a solvent productivity of 0.65 $g.L^{-1}.h^{-1}$. After 496.6h, the fermentation was halted. The solvent concentration reached an average of 18.5 %(w/w) in the permeate in the last phase of the fermentation which lasted ~8 days by allowing a significant residual solvent concentration in the second fermentor to ensure sufficient solvent transport through the pervaporation membrane.

In this experiment, glucose is preferentially converted and a significant increase in xylose utilization is only observed after 215h of cultivation after depletion of glucose. Hence, carbon catabolite repression on xylose by glucose is observed in accordance with batch fermentations previously conducted with the same strain where xylose uptake was repressed until glucose was completely exhausted [14].

Groot *et al.* [4] studied the continuous conversion of a medium containing glucose and xylose (in total 60 $g.L^{-1}$) to IBE. The fermentation was carried out in a fluid bed reactor at much smaller scale (47 mL) using *Clostridium* cells encapsulated in alginate beads. Also here xylose was consumed only when glucose was completely depleted.

A solvent productivity of 1.13 $g.L^{-1}.h^{-1}$ was obtained when using a feedstock containing 150 $g.L^{-1}$ glucose as sole carbon source [22]. Therefore, at this stage, the use of lignocellulosic hydrolysates will certainly lead to increased capital expenditures since productivity is decreased due to the slower conversion of xylose. It is assumed that xylose utilization could be increased further in this configuration by decreasing the dilution rate in the second fermentor, but this will lead to an additional decrease in solvent productivity. Evidently, solvent productivity would increase if simultaneous consumption of glucose and xylose occurred. Therefore, the use of strains that simultaneously convert glucose and xylose would overcome this major bottleneck. Gu *et al.* (2009) [5] demonstrated an improved xylose utilization by

overexpression of a transaldolase in *C. acetobutylicum 824*. Despite this, catabolite repression on xylose by glucose could not be avoided [5]. Eventhough the consumption rate of xylose is still significantly lower than the consumption rate of glucose, concomitant consumption of glucose and xylose was demonstrated in a recent study by a promising newly reported *Clostridium* sp. strain BOH3 [26]. Therefore, a combinatorial approach involving both process development and metabolic engineering/strain selection for increased xylose consumption would definitely have a significant impact on the test results.

To the best of our knowledge, no continuous fermentations using lignocellulosic hydrolyzates or mixtures of glucose/xylose have been described for ABE production, involving ISPR. However, several studies where hydrolyzates were used as feedstock in (fed)-batch mode for production of ABE were performed and are summarized in table 2. Lu *et al.* (2013) [10] and Qureshi *et al.* (2006) [18] respectively used wood pulping hydrolyzate and corn fiber as feedstock at low carbohydrate concentrations. Wheat straw hydrolyzate and corn stover were used as feedstock in batch mode at a concentration of respectively 128 and 86 $g.L^{-1}$ [17;19]. Furthermore, De Vrije *et al.* [1] applied gas stripping for removal of Isopropanol-Butanol-Ethanol (IBE) during a continuous fermentation using a medium containing 40 $g.L^{-1}$ glucose and 20 $g.L^{-1}$ xylose. These carbohydrate concentrations are significantly lower than the 150 $g.L^{-1}$ total carbohydrates used in this manuscript and do not lead to decreased process flows, a desirable feature in any (bio)chemical process. *C. beijerinckii* NRRl B593 was used as micro-organism. At a dilution rate of 0.06 h^{-1} the glucose was completely converted while ~ 57% xylose was converted. Unfortunately, the solvent concentration in the condensate is unreported.

Pervaporation performance

The solvent concentration in the permeate was strongly correlated with the solvent concentration in the second fermentor and reached 18.5 %(w/w) in the last phase (table 1, figure 2g). The total permeate flux as well as the component fluxes of the individual solvents depend on the corresponding

solvent concentration in the feed, as graphically shown in figure 3. Table 3 lists the total fluxes, separation and enrichment factors obtained in the different phases of the continuous fermentation. No irreversible decline in flux was observed during the course of this experiment, similar to previously reported continuous fermentations on glucose with *in situ* solvent removal using the same kind of PDMS membranes [22;24].

Energy consumption

N-butanol as well as its isomer isobutanol are considered as second-generation drop-in biofuels. When this market is targeted, it is recommendable to attempt to decrease the energy consumption during production and recovery. Even when *n*-butanol is intended to be used as a chemical intermediate, it is desirable to decrease the energy consumption per kg *n*-butanol. In this section, the energy consumption is calculated for a 100 000 ton per annum *n*-butanol facility (operating 8 400h per year and producing 11 900 kg butanol per hour). A base-case considering a continuous multi-stage fermentation with a conventional downstream process is compared to an alternative where a continuous multi-stage fermentation is integrated with organophilic pervaporation followed by downstream processing. Cooling and electricity costs were neglected in both the base-case and alternative process. The energy required for feedstock preparation is not taken into account, but is assumed to be identical for base-case and process alternative. In both cases, butanol is obtained at a purity of 99.75 %(w/w), acetone at a purity of 99.03 %(w/w), and ethanol is purified to 86.96 %(w/w) with the remainder being acetone (2.65 %(w/w)) and water (10.4 %(w/w)).

The solvent-depleted fermentation broth is concentrated in multiple effect evaporators to 20% of its original volume in base-case and process alternative. It is assumed that this stream can be valorized as fertilizer or animal feed.

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Conventional downstream processing

Figure 4 shows a process flow diagram of a conventional downstream processing unit. Biobutanol is produced continuously in a multi-stage fermentation set-up. The carbohydrate concentration in the feed is 60 $g.L^{-1}$. Full carbohydrate conversion is assumed in the final fermentor. The effluent from the final fermentor containing high solvent titers (20 $g.L^{-1}$) is introduced as feed in a beer stripper. The top stream of the beer stripper (stream 3) containing 18.7 %(w/w) solvents is introduced on the 9^{th} tray of a 30 stage distillation column (b) to separate acetone and ethanol (stream 8) from butanol and the bulk of the remaining water (stream 7). Acetone is purified to 99.03 %(w/w) in distillation column c while ethanol (86.96 %(w/w)) can be found in the bottom (stream 10) of this distillation tower. The butanol-water mixture (stream 7) is sent to a decanter where the solvent-rich phase (stream 11) is distilled to 99.76 %(w/w) purity. The top stream 12 of the distillation column d is recycled back to the decanter. The aqueous phase of the decanter (stream 13) is brought to distillation column e with the top stream 14 being recycled to the decanter and with discharge of the bottom stream 15 depleted of n-butanol. Countercurrent heat exchangers recover the heat from the spent and stripped fermentation broth to heat the incoming fermentation broth (stream 1) from 35 to 80°C. An additional heat exchanger heats the fermentation broth to 93°C (slightly below boiling point of stream 3) before introduction to the steam stripper. Table 4 shows the weight percentages and temperatures of all streams in the downstream processing section. The solvent-depleted stream 6 is sent to a 5-effect evaporator (with an assumed steam-economy of 4.5 kg water / kg steam; not drawn in figure 4) to further concentrate this solvent-depleted stream to 20 %(w/w) of the original flow.

The design parameters for the different distillation towers are summarized in table 5. The duty of the heat exchanger (before the beer stripper) is calculated to be 54 367 MJ/hr. Hence, this purification scheme leads to an energy consumption of 23.7 MJ/kg solvents or 39.3 MJ/kg *n*-butanol if all energy consumed is allocated to n-butanol.

The solvent-depleted stream from the beer strippers (894 970 kg/hr) is sent to 2 parallel 5-effect evaporation trains (with an assumed steam economy of 4.5) where 80% of the water is evaporated. This leads to an additional energy consumption of 18.2 MJ/kg solvent or 30.2 MJ/kg *n*-butanol if all the consumed energy is allocated to *n*-butanol. Hence, the total energy consumption in this scheme is 41.8 MJ/kg solvents or 69.5 MJ/kg *n*-butanol.

To compare these calculations with historical data of ABE fermentations (production data from 2008), a steam consumption of 13–25 tons per ton of ABE produced was mentioned by Ni and Sun (2009) [12]. This corresponds to 29.4–56.5 MJ per kg of ABE produced assuming an evaporation enthalpy of 2.26 MJ/kg steam.

Integrated production of biobutanol

Biobutanol is produced continuously in a multi-stage fermentation set-up (as shown in figure 5). The carbohydrate concentration in the feed is 150 $g.L^{-1}$ (2.5 fold higher as compared to the base-case). If only the solvents in the permeate from the organophilic pervaporation unit are further purified, a substantial amount of solvents and residual xylose (figure 2f and 2g) would be lost leading to an economically unacceptable situation. Also, the solvent concentration in the second fermentor (13.6 g ABE.L^{-1} in phase 5) is lower than expected from a batch or continuous fermentation without ISPR (20 $g.L^{-1}$). Hence, sending this stream to a conventional end-of-pipe treatment would only increase the energy consumption per kg of solvents in that particular stream. Hence, in the knowledge that some clostridial strains have been proven to consume xylose completely [26], it is proposed to send the effluent from the second fermentor to a third fermentor where the residual xylose can be further converted to solvents using the same strain as used in the first fermentors. In this fashion, full glucose and xylose utilization are obtained, while guaranteeing a similar energy consumption for recovery of the residual solvents in the effluent from the third fermentor as compared to the base-case. Hence, this process configuration allows a 2.5 fold decrease in water consumption per kg solvent due to the increased carbohydrate concentration.

Figure 5 shows the alternative with ISPR where the second fermentor is connected with the organophilic pervaporation unit. The permeate from the pervaporation unit (containing 18.4 %(w/w) solvents) is combined with the top stream of the beer stripper and sent to the subsequent distillation columns. Sixty percent of all generated solvents are removed by the organophilic pervaporation unit. The design parameters for the different distillation towers are summarized in table 6. Due to the decreased total flow, the reboiler duty of the steam stripper is significantly decreased in comparison with the base-case. Since the composition of the permeate is equal to the composition of the top stream of the beer stripper, the reboiler duty of the other distillation towers remains unchanged. The duty of the heat exchanger (before the beer stripper) is calculated to be 21 617 MJ/hr. Hence, this purification scheme leads to an energy consumption of 14.8 MJ/kg solvents or 24.6 MJ/kg *n*-butanol if all energy consumed is allocated to *n*-butanol.

Pervaporation involves phase transfer and therefore requires energy to supply the heat of vaporization. This was simulated in Chemcad using a simple flash operation. A permeate containing 18.4 %(w/w) (4.9 %(w/w) acetone; 12.6 %(w/w) butanol; 0.9 %(w/w) ethanol) was assumed (as experimentally obtained) in this calculation. The heat of vaporization was 2.08 MJ/kg permeate and corresponds to 11.33 MJ/kg solvents or 16.53 MJ/kg *n*-butanol (118 311 MJ/hr in the plant of 100 000 ton *n*-butanol per annum where 60% of the generated *n*-butanol was assumed to be recovered through pervaporation). Since the organophilic pervaporation is operated at 37°C, the heat of condensation from the distillation towers in the downstream process (operated at significantly higher temperatures) can be recovered to provide the energy demand of the organophilic pervaporation. Therefore, the energy demand of the organophilic pervaporation unit is not taken into account in further calculations.

The solvent-depleted bottom stream 6 (355 849 kg/h) from the beer stripper is sent to a 5-effect evaporator system (with a steam-economy of 4.5 kg water/kg steam; not shown in figure 5) to further concentrate this solvent-depleted stream to 20 %(w/w) of the original flow. This leads to an additional energy consumption of 7.2 MJ/kg solvent or 12.0 MJ/kg *n*-butanol if all the consumed energy is

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allocated to *n*-butanol. Hence, the total energy consumption in this scheme is 22.0 MJ/kg solvents or 36.6 MJ/kg *n*-butanol, leading to a 47.4% lower energy demand compared to the base-case. Higher solvent concentrations in the permeate of the organophilic pervaporation unit will lower the required energy consumption per kg permeate in the pervaporation unit and in the subsequent distillation towers. To achieve this goal with the membranes used in this study, the system has to be operated at higher residual solvent titers in the second fermentor. With the clostridial strain used in this study, this will lead to an increased solvent inhibition and consequently a decreased solvent productivity. Therefore, key factors in advancing this technology further are: 1. selection/development of strains with an increased solvent tolerance; 2. Development of membranes with high(er) separation factors and high (and stable) fluxes. Estimations of bare module costs in order to allow economic success are part of a follow-up study.

Conclusions

A feedstock containing 100 $g.L^{-1}$ glucose and 50 $g.L^{-1}$ xylose was continuously converted to ABE in a two-stage fermentation process. Glucose was fully converted, while 61% of the xylose was converted at the lowest tested dilution rate. Carbon catabolite repression was observed, *i.e.* glucose was consumed completely before a significant xylose consumption was observed. Introduction of an organophilic pervaporation unit leads to significant improvements in the water balance and energy consumption of the process. Based on the obtained experimental results and rigorous calculations, it was estimated that the energy consumption of a plant integrated with organophilic pervaporation was 47.4% lower than a conventional biobutanol plant.

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List of abbreviations

ABE	Acetone-Butanol-Ethanol
A	acetone
B	butanol
D	dilution rate [h^{-1}]
E	ethanol
J	flux [$g.m^{-2}.h^{-1}$]
LHV	Lower Heating Value
MEE	Multiple-Effect Evaporator
P	solvent productivity [$g.L^{-1}.h^{-1}$]
PDMS	polydimethylsiloxane
S	glucose consumption [$g.L^{-1}.h^{-1}$]
t_r	residence time [h]
VFA	volatile fatty acids [$g.L^{-1}$]
VLE	Vapor-Liquid Equilibrium
x	mole fraction in feed [-]
Q_E	effluent flow rate [$kg.h^{-1}$]
Q_P	pervaporation flow rate [$kg.h^{-1}$]
y	mole fraction in vapour [-]
$Y_{P/S}$	solvent yield [$g_{solvents}.\ g_{glucose}^{-1}$]

Greek symbols:

α	separation factor (-)
β	enrichment factor (-)

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Conflict of interest

The authors declare that they have no conflicting interests.

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Figure captions:

Figure 1: Laboratory set-up consisting of a two-stage fermentation integrated with organophilic pervaporation

Figure 2: Evolution of pH and concentrations of carbohydrates, solvents and VFAs in first (left; a-d) and second (right;e-h) fermentor. The pervaporation unit was coupled to the second fermentor at the beginning of phase 3 until the end of the fermentation. Symbols: (-) pH, (×) glucose, (■) xylose, (●) acetone, (■) butanol, (■) ethanol, (✳) acetone in permeate, (▪) butanol in permeate ,(◆) ethanol in permeate, (◆) total solvents in permeate, (✳) acetate, (●) butyrate.

Figure 3: Component fluxes in function of time: (■) acetone flux, (◆) butanol flux, (▲) ethanol flux, (✳) water flux

Figure 4: Conventional downstream process for purification of 2 %(w/w) ABE. a: beer stripper; b-e: distillation columns; f: decanter.

Figure 5: Process flow diagram for production of biobutanol integrated with pervaporation. a: beer stripper; b-e: distillation columns; f: decanter; g: fermentor; h: pervaporation unit.

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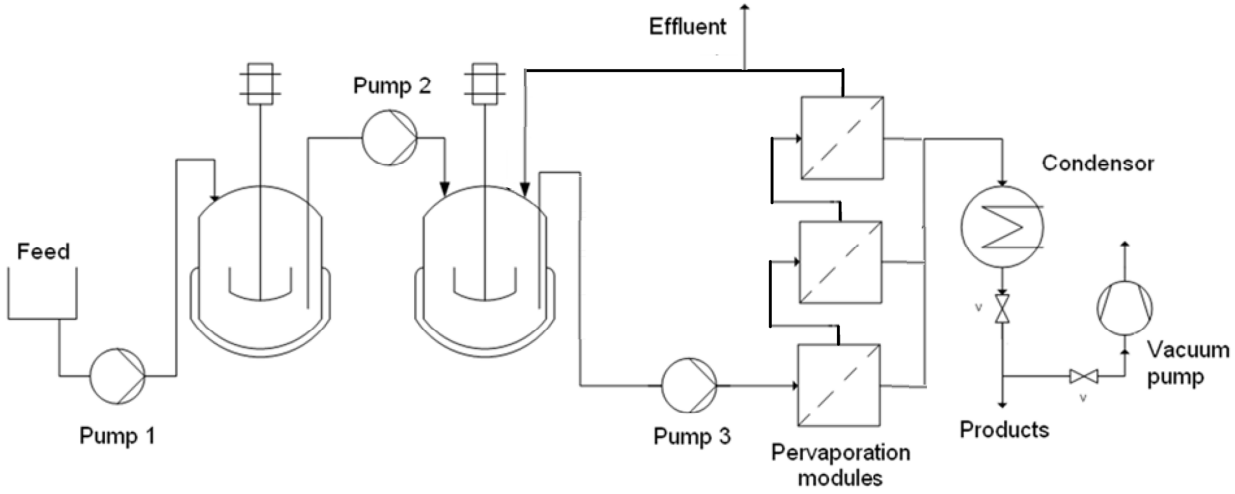

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447 Figure 1

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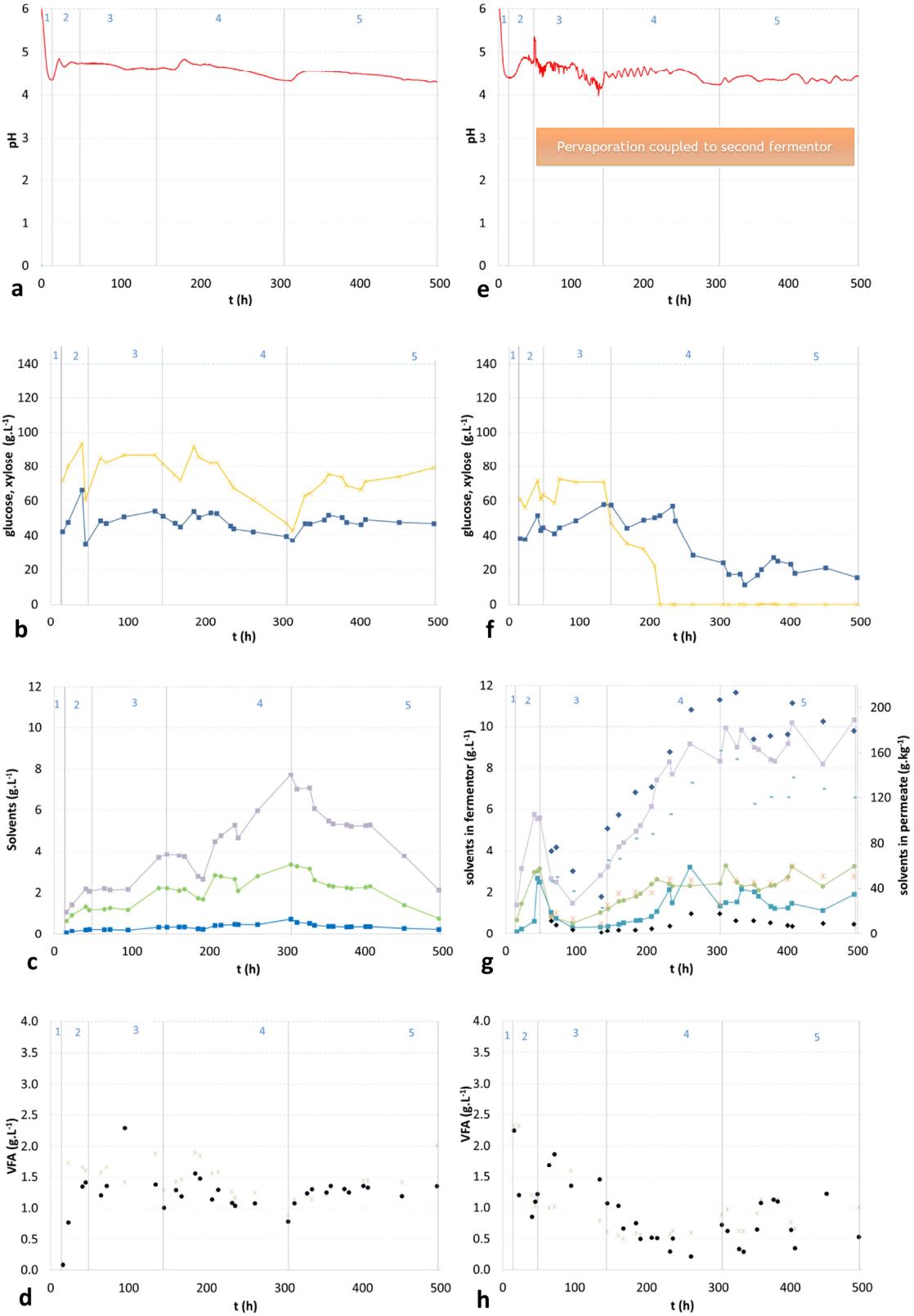

Figure 2

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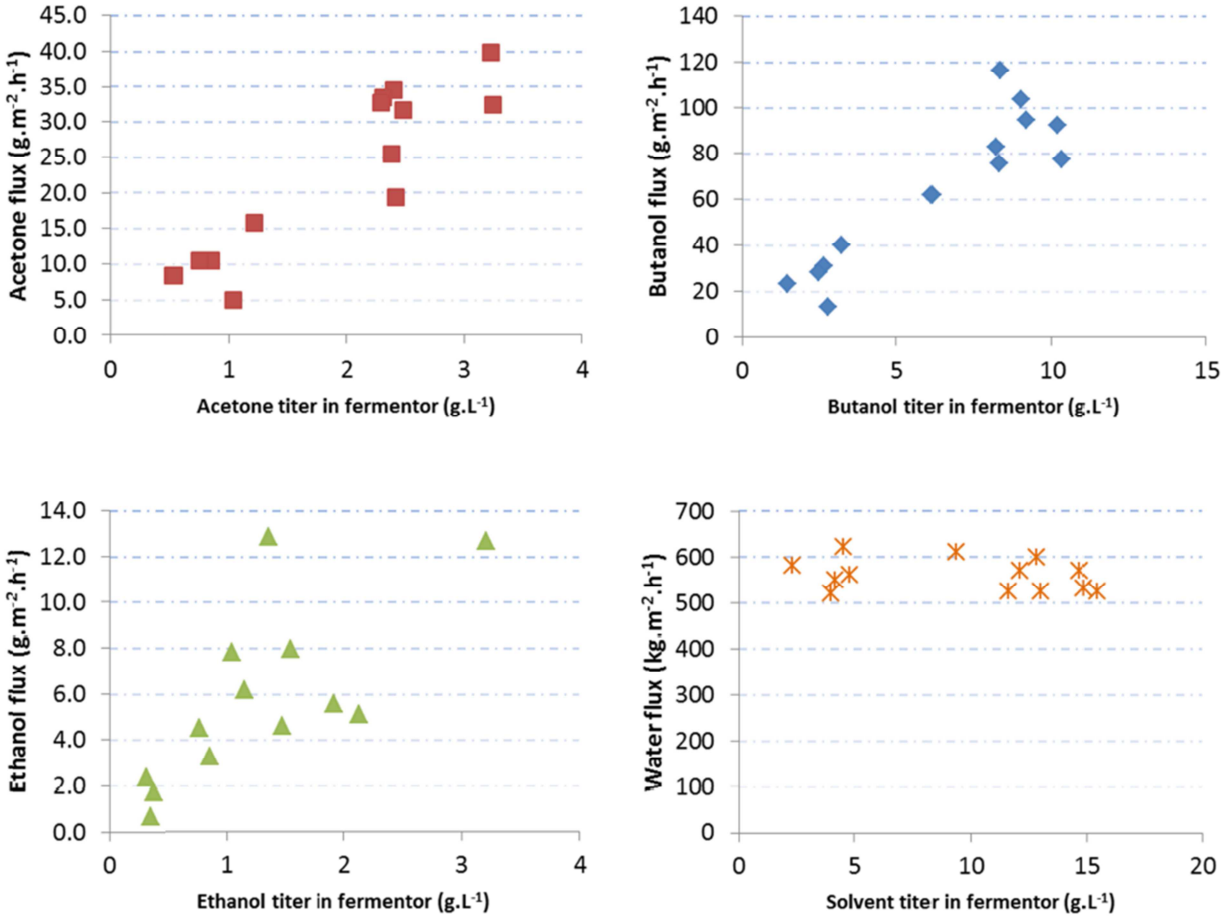

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453 Figure 3

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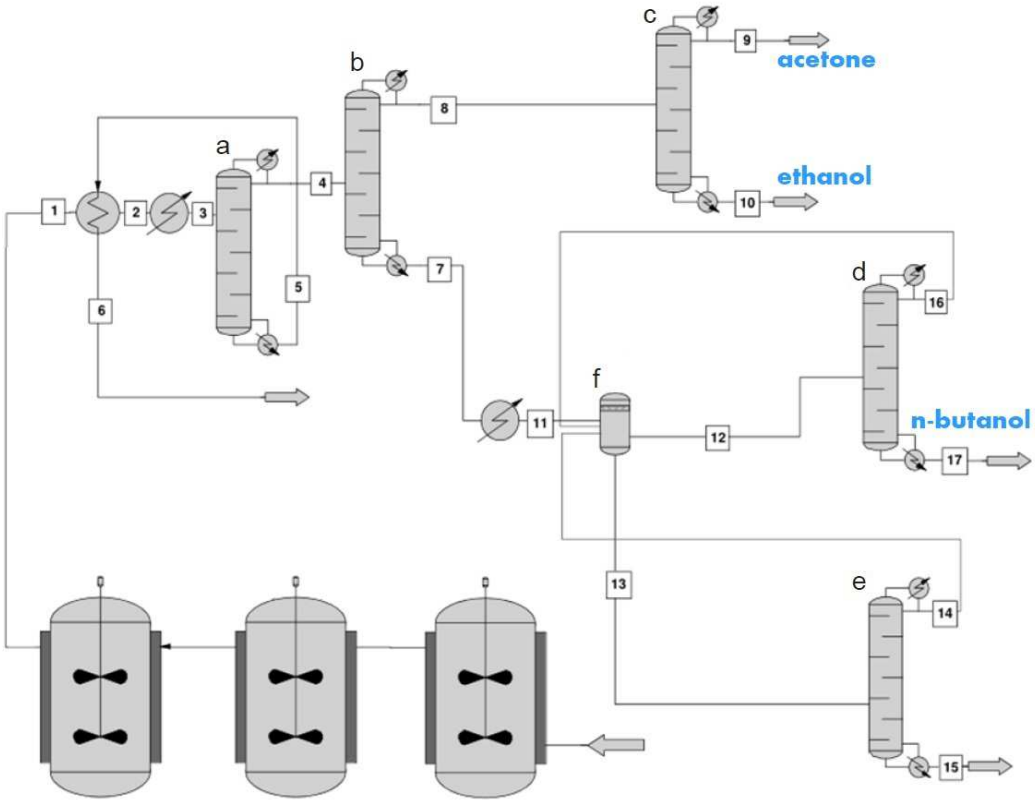

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456 Figure 4

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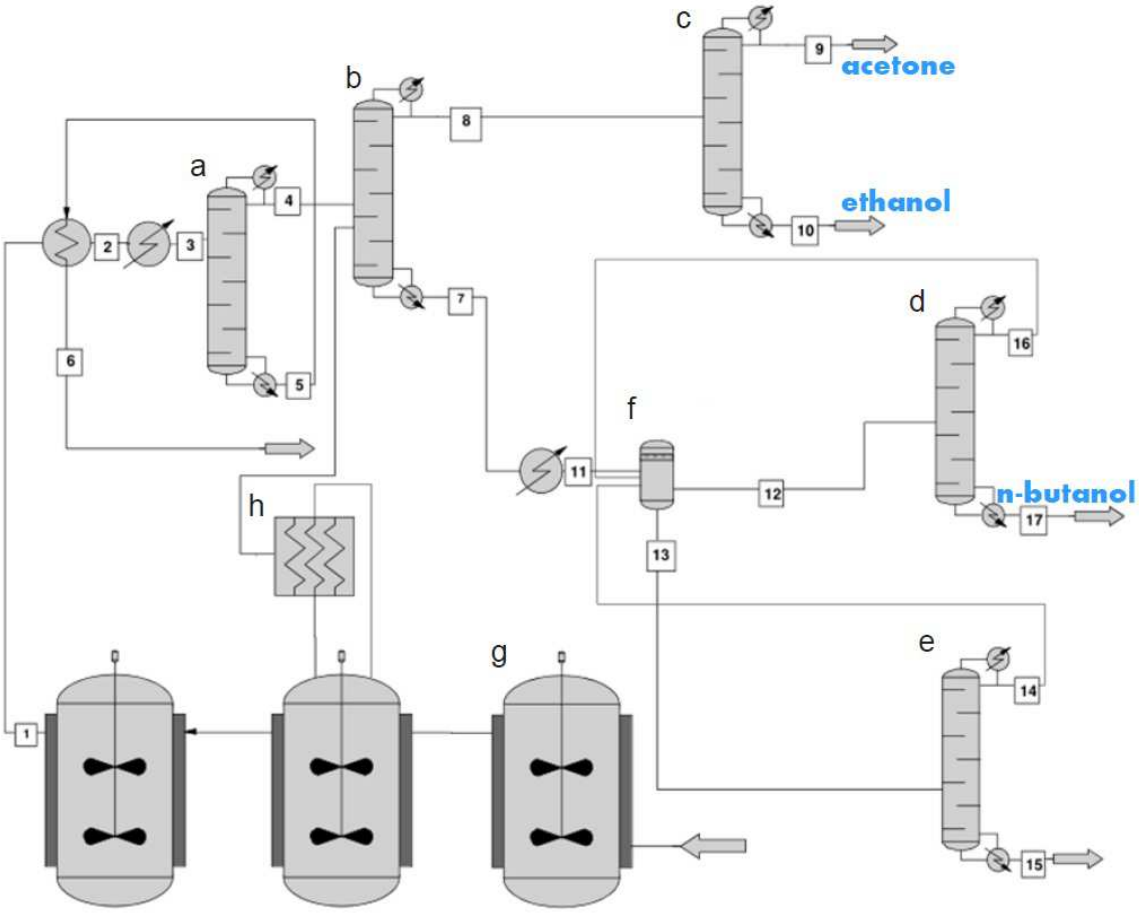

Figure 5

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471 Table 1: Average of the kinetic parameters for the different zones in the continuous bioconversion

	2	3	4	5
from start to end	13,8-48,5	48,5-144,3	144,3-304,9	304,9-496,6
Duration (h)	34.8	95.8	160.5	191.7
Q_E ($kg.h^{-1}$)	0.120	0.096	0.101	0.102
Q_P ($kg.h^{-1}$)	-	0.016	0.019	0.018
Q_{tot} ($kg.h^{-1}$)	0.120	0.112	0.120	0.120
D_1 (h^{-1})	1.09E-01	1.02E-01	1.09E-01	1.09E-01
D_2 (h^{-1})	5.60E-02	3.85E-02	2.38E-02	1.99E-02
D_{ov} (h^{-1})	3.70E-02	2.80E-02	1.95E-02	1.68E-02
solvents in first fermentor ($g.L^{-1}$)	2.9	4.3	7.5	7.9
solvents in second fermentor ($g.L^{-1}$)	7.8	3.8	10.1	13.6
solvents in permeate ($g.kg^{-1}$)	-	50.8	161.4	184.6
Total solvents ($g.L^{-1}$)	7.8	10.6	34.0	38.6
Solvent yield ($g.g^{-1}$)	0.175	0.315	0.381	0.295
Overall solvent productivity ($g.L^{-1}.h^{-1}$)	0.287	0.296	0.663	0.649
Solvent productivity in acidogenic fermentor($g.L^{-1}.h^{-1}$)	0.316	0.438	0.818	0.862
Solvent productivity in solventogenic fermentor ($g.L^{-1}.h^{-1}$)	0.273	0.242	0.629	0.610
Initial glucose ($g.L^{-1}$)	100	100	100	100
Initial xylose ($g.L^{-1}$)	50	50	50	50
Final glucose ($g.L^{-1}$)	62.7	68.3	15.2	0.1
Final xylose ($g.L^{-1}$)	43.0	48.1	45.6	19.4
Glucose utilization	0.37	0.32	0.85	1.00
Xylose utilization	0.14	0.04	0.09	0.61
Glucose utilization rate ($g.L^{-1}.h^{-1}$)	1.38	0.89	1.66	1.68
Xylose utilization rate ($g.L^{-1}.h^{-1}$)	0.26	0.05	0.09	0.52
Carbohydrate utilization rate ($g.L^{-1}.h^{-1}$)	1.64	0.94	1.74	2.20

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Table 2: Studies where hydrolyzates were used as feedstocks for ABE fermentations coupled to in situ product recovery technique for solvent recovery.

References	Qureshi et al. (2007) [18]	Qureshi et al. (2014) [16]	Lu et al. (2013) [10]	Qureshi et al. (2006) [17]	This study (zone 5)
Products	ABE	ABE	ABE	ABE	ABE
Cultivation Mode	batch	batch	batch	Fed-batch	Continuous
ISPR technique					
Gas-stripping	√		√	√	
Vacuum process		√			
Pervaporation					√
Total fermentation time (h)	131	60	85	60	192
Product in concentrate ($g.L^{-1}$)	-	-	40-80	-	185
Initial total carbohydrate concentration ($g.L^{-1}$)	128	86	55	65	150
Substrate	Wheat straw hydrolyzate	Corn stover	Wood pulping hydrolyzate	Corn fiber	Glucose/xylose
Product in reactor ($g.L^{-1}$)	6	13.8	9	0-5.8	13.6
Overall product ($g.L^{-1}$)	-	-	17.7	24.7	38.6
Yield ($g.g^{-1}$)	0.37	0.39	0.44	0.44	0.30
Productivity ($g.L^{-1}.h^{-1}$)	0.36	0.34	0.25	0.47	0.65

Table 3: Average of parameters related to the performance of the organophilic pervaporation membrane in the different phases of the continuous fermentation

Phase	3	4	5
Separation factor			
$\alpha_{Acetone/water}$	16.0	21.0	21.8
$\alpha_{BuOH/water}$	14.9	20.0	16.4
$\alpha_{EtOHwater}$	8.1	9.4	7.3
Enrichment factor			
$\beta_{Acetone/water}$	16.0	20.7	21.5
$\beta_{BuOH/water}$	14.7	19.4	15.9
$\beta_{EtOHwater}$	8.1	9.4	7.3
Total flux ($g.m^{-2}.h^{-1}$)	601	702	649

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491 Table 4: Composition and temperature of streams in simulation of downstream processing shown in

492 figure 4

Stream	Water (wt%)	Ethanol (wt%)	Acetone (wt%)	Butanol (wt%)	T(°C)
1	98.0	0.2	0.6	1.2	35.0
2	98.0	0.2	0.6	1.2	80.0
3	98.0	0.2	0.6	1.2	93.0
4	81.3	1.9	5.7	11.2	86.7
5	100.0	0.0	0.0	0.0	99.6
6	100.0	0.0	0.0	0.0	49.8
7	87.8	0.0	0.0	12.2	95.7
8	3.0	23.8	73.1	0.0	59.4
9	0.3	0.6	99.0	0.0	55.9
10	10.4	87.0	2.6	0.0	76.8
11	87.8	0.0	0.0	12.2	40.0
12	23.4	3.5	0.0	73.1	50.7
13	92.0	1.6	0.0	6.4	50.7
14	55.4	8.7	0.0	35.9	92.8
15	100.0	0.0	0.0	0.0	99.6
16	55.2	8.3	0.0	36.5	92.9
17	0.2	0.0	0.0	99.8	117.4

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494 Table 5: Design parameters for the different distillation towers for a 100 000 ton per annum n-butanol

495 facility (base-case)

	Beer stripper (a)	Distillation tower (b)	Distillation tower (c)	Distillation tower (d)	Distillation tower (e)
Diameter (m)	3	3.2	1.5	2.9	2.6
# trays	20	30	30	17	10
# columns	4	1	1	1	1
Reboiler duty (MJ/h)	237003	67001	11045	48335	50000

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498 Table 6: Design parameters for the different distillation towers for a 100 000 ton per annum n-butanol
499 facility (alternative)

	Beer stripper (a)	**Distillation tower (b)**	**Distillation tower (c)**	**Distillation tower (d)**	**Distillation tower (e)**
Diameter (m)	2.6	3.2	1.5	2.9	2.6
# trays	20	30	30	17	10
# columns	2	1	1	1	1
Reboiler duty (MJ/h)	94249	67001	11045	48335	50000

500