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In Situ Two-Stage Gas Stripping for the Recovery of Butanol from Acetone-Butanol-Ethanol (ABE) Fermentation Broths

Rebeca Díez-Antolínez[*a,b], María Hijosa-Valsero[a], Ana I. Paniagua-García[a,b], Xiomar Gómez[b]

[a]Centre of Biofuels and Bioproducts. Instituto Tecnológico Agrario de Castilla y León (ITACyL), Villarejo de Órbigo, E-24358, León, Spain
[b]Chemical and Environmental Bioprocess Engineering Group. Natural Resources Institute (IRENA), Universidad de León, Avenida de Portugal 42, E-24071, León, Spain
dieantre@itacyl.es

Separation and purification of butanol from Acetone-Butanol-Ethanol (ABE) fermentation broths is one of the main challenges related to the implementation of butanol biorefineries from agrofood wastes. Due to the great energy consumption of conventional solvent recovery technologies, it is essential to find more environmentally friendly and economically viable techniques. In this work, two-stage gas stripping was assessed as an ecoefficient butanol recovery technique. Firstly, in a one-stage stripping unit, three working variables (feed temperature, gas flow and refrigeration temperature) were optimized via response surface methodology (RSM) to increase simultaneously butanol selectivity (α_B) and butanol recovery efficiency (η_B). After one-stage simple gas stripping optimization (T feed = 60 °C, Gas flow = 1.34 L/min, T refrigeration = 5 °C and t = 4 h), values of α_B = 11.08-13.95 and η_B = 59-67% were reached, with a butanol concentration in the condensate of 119 g/L (141 g/L ABE). These working conditions were applied to two-stage gas stripping, and a butanol concentration of 300-360 g/L was attained in the condensate of the second stripping, reaching a butanol selectivity (α_B) of 7-8 and a butanol recovery (η_B) of 70-80 %. This process could significantly reduce energy consumption in the final butanol purification process. In addition, the resistance of the ABE fermentation strain *Clostridium beijerinckii* CECT 508 to this technique was assessed by subjecting the microorganism to various *in situ* gas stripping processes under fed-batch conditions employing cheese whey as a substrate. Bacteria were negatively affected by the stripping process, due to the high T feed (60 ºC) but they were able to recover and produce butanol again.

1. Introduction

Biobutanol is not only an important commodity with capacity as a platform chemical. Moreover, butanol is an advanced biofuel which overcomes most of the limitations of ethanol as a biofuel. In this line, butanol has higher energy content, lower volatility, lower hygroscopicity and it is less corrosive than ethanol (Taconi et al., 2009). It could be even directly used in current internal combustion engines without any modification. However, until now, ABE fermentation, the most common process to produce biobutanol, is not economically competitive with petrochemical processes (Kumar and Gayen, 2011). The high cost of traditional food feedstocks, together with the low titer, yield and volumetric productivity of butanol in fermented broths, solvent toxicity and the high cost of solvent recovery processes compromise its economic feasibility (Green, 2011).
To decrease the feedstock cost, the utilization of agrofood wastes with high polluting potential is of great relevance. Interesting substrates for ABE fermentation are cheese whey and cheese whey permeates (CWP) (Diez-Antolinez et al., 2016). On the other hand, integrated fermentation with *in situ* product recovery is an effective way to reduce ABE fermentation energy costs (Xue et al., 2012; 2014a). Among butanol recovery methods, gas stripping has some advantages such as an easy operation, simple scale-up, low capital investment and low energy cost (Kumar and Gayen, 2011; Xue et al., 2013, 2014a). However, gas stripping typically removes large amounts of water in the butanol stream and requires a high energy input due to its lower selectivity in comparison with other recovery techniques (Qureshi et al., 2005). To improve gas stripping

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efficiency, coupling gas stripping to broths with butanol concentrations higher than 8 g/L - concentration at which the condensate vapor has a butanol concentration higher than its solubility in water (7.7 %, w/w) - increases butanol concentration in the organic phase of the condensate (Chen et al., 2014).
In this study, a central composite design with three independent variables (feed temperature, gas flow and refrigeration temperature) was used to determine the optimum combination of these working conditions to maximize the response variables (butanol selectivity (α_B) and butanol recovery efficiency (η_B)). Moreover, a two-stage gas stripping was performed increasing significantly butanol concentration in the condensate to reduce dewatering energy consumption and improving the energetic efficiency of the overall recovery process. Besides, a simplified gas stripping unit was coupled with fermentation of cheese whey by *Clostridium beijerinckii* CECT 508 to enhance the fermentability and assess the effect of gas stripping conditions on bacterial development by performing two consecutive stripping processes on the same fermentation broth.

2. Materials and Methods

2.1. Media and culture

Two different feed media solutions were tested: a) a synthetic aqueous solution containing 5 g/L acetone, 10 g/L butanol and 1.67 g/L ethanol (A:B:E, 3:6:1) and b) a real fermentation broth containing bacteria. These broths were obtained by fermenting sheep cheese whey with an initial lactose concentration of 40 g/L, using *Clostridium beijerinckii* CECT 508, as described by Díez-Antolínez et al. (2016).

2.2. Gas stripping optimization

The gas stripping system including a double-cooling coil Dimroth condenser (400 mm length and 1135 cm^2 surface area (Pobel, Madrid, Spain)) for vapour condensation, pumps and storage vessels was connected to a screw-capped bottle (1 L) (Figure 1). The gas flow was controlled by a manually adjustable rotameter (Key Instruments, Brooks Instruments, Hatfield, PA, USA). The feed solution consisted of a 500-mL aqueous solution (5 g/L acetone, 10 g/L butanol, 1.67 g/L ethanol) placed in a 1-L glass bottle containing a magnetic stirrer. The bottle was closed with a special cap which enabled the entrance of a temperature probe, a submerged metal stone diffuser for the entrance of the stripping gas and a stripping gas exit over the surface of the solution. The bottle was placed on a heating-stirring plate with controllable temperature. The whole gas circuit was purged during 2 min with industrial N_2 to replace atmospheric air. Then, the gas flow was fixed with a rotameter and the internal N_2 was continually recirculated with a pump. The refrigeration temperature of the condensation system was controlled with a thermostat LAUDA Integral T 2200 using a monoethylene glycol/water mixture (LAUDA, Lauda-Königshofer, Germany). After 15 min of stabilization, the gas stripping experiment was started. For each experimental run, the duration of the stripping was initially fixed in 18 h.

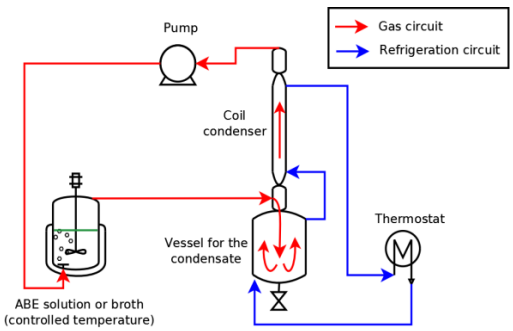


Figure 1: Setup diagram of the gas stripping system used in the experiments

A central composite design with three independent variables (feed temperature, gas flow and refrigeration temperature) was used to determine the optimum combination of these working conditions to maximize the response variables (butanol selectivity (α_B) and butanol recovery efficiency (η_B)). The RSM design had 20 experiments and included 8 cube points, 6 central points and 6 axial points (α=1.68179). The responses measured for each trial were introduced in the model to obtain three-variable quadratic polynomial regression equations to estimate the response variables. These equations were used to mathematically determine the values of the three independent variables (T feed, Gas flow and T refrigeration) which maximized the response values (butanol selectivity and butanol recovery). The response variables were calculated according to Equations (1) (Lu et al., 2016) and (2), respectively:

$$\alpha_i = \frac{y_i/(1-y_i)}{x_i/(1-x_i)} \quad (1)$$

$$\eta_i = \frac{m_i C}{m_i F} \times 100 \quad (2)$$

where α_i is the selectivity for metabolite i, x_i is the mass ratio of metabolite i in the feed solution, y_i is the mass ratio of metabolite i in the condensate, η_i is the percentage recovery efficiency for metabolite i, m_{iC} is the mass of metabolite i in the condensate (expressed in g) and m_{iF} is the mass of metabolite i in the feed solution.
The final output of the RSM is an equation for each response variable, which is used to estimate the most adequate values for the independent variables to maximize α_B and η_B. The estimated optimal working conditions were experimentally validated with a synthetic aqueous solution and with a real fermentation broth. Moreover, the effect of gas stripping on bacteria was assessed by performing two consecutive stripping processes separated by a resting fermentation period where bacteria were kept at fed-batch conditions. The optimal working conditions calculated with the RSM equations were experimentally validated at various operation times (4-18 h). Besides, alternative feed temperatures (35 °C) suitable for bacteria were also tested.

2.3. Two-stage gas stripping

A second-stage gas stripping was applied to further concentrate butanol from the aqueous phase formed in the condensate collected from the first-stage gas stripping. The condensate obtained in the first-stage, with an organic phase consisting of ~ 60% (w/w) butanol was stored and the aqueous phase with ~ 8% (w/w) butanol was subjected to gas stripping again under the working conditions optimized according to section 2.2.

2.4. Fed-batch fermentation system coupled with gas stripping

The best working conditions obtained with the synthetic aqueous medium were tested with a fermentation broth. The feed solution was a 500-mL fermented cheese whey sample containing 0.40 g/L residual lactose, 3.02 g/L acetone, 12.04 g/L butanol, 0.23 g/L ethanol, 0.99 g/L acetic acid and 1.15 g/L butyric acid, with a bacterial density of $7 \cdot 10^8$ cell/mL. The gas stripping started as explained in 2.2. A stripping time of 4 h was the most suitable from preliminary tests. Additionally, the effect of gas stripping conditions on *C. beijerinckii* CECT 508 was assessed. After a first stripping cycle, distilled water and lactose were added to the fermentation to guarantee a total volume of 500 mL and a lactose concentration of ~40 g/L; yeast extract (0.5 g) was also supplemented. When bacteria had reached again their stationary phase and produced ~10 g/L butanol, a second stripping cycle was performed employing the same working conditions that were used in the first cycle.

2.5. Analytical methods

Aqueous samples were taken from the feed bottle before and after the gas stripping process, and from the condensate after the stripping process. All volumes were measured with a graduated cylinder. Synthetic aqueous solutions and the condensate were directly analysed for ABE metabolite concentration. Samples from the real fermentation broth were centrifuged at 13000 rpm for 3 min in a microcentrifuge (Minispin, Eppendorf, Hamburg, Germany) and their supernatant was filtered through a 0.20 µm nylon filter. Analyses by GC were carried out as described by Díez-Antolínez et al. (2016).

2.6. Statistical analyses

For the optimization step, experimental designs following the Response Surface Methodology (RSM) were generated and interpreted with the software Minitab 16 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Gas stripping optimization

The results of the RSM model showed that the optimal working conditions to maximize butanol selectivity (α_B) and recovery efficiency (η_B) were T feed = 60 °C, gas flow = 1.34 L/min, T refrigeration = 5 °C. The estimated responses for an 18-h gas stripping were α_B = 6.29; η_B = 94.56 %. The model was experimentally validated for various stripping times (Table 1). The results were in accordance with literature, where feed temperatures for gas stripping of 30-67 °C, refrigeration temperatures from -60 to 4 °C, and gas flow rates of 1.25-15 L/min have been reported (Yang and Lu, 2013; Abdehagh *et al*., 2014). It was observed that shorter stripping times increased selectivity (α_B) without drastically reducing recovery efficiency (η_B) (Table 1). It must be pointed out that the shortest stripping time (4 h) produced a condensate with a butanol concentration of 92 g/L, which is

above its solubility value in water, which implies the formation of two phases (an aqueous and a solvent phase). The upper solvent phase represents about 10% of the total volume and it usually contains 500-680 g/L butanol; and it can be easily separated from the bottom aqueous phase, which contains 77-79 g/L butanol.

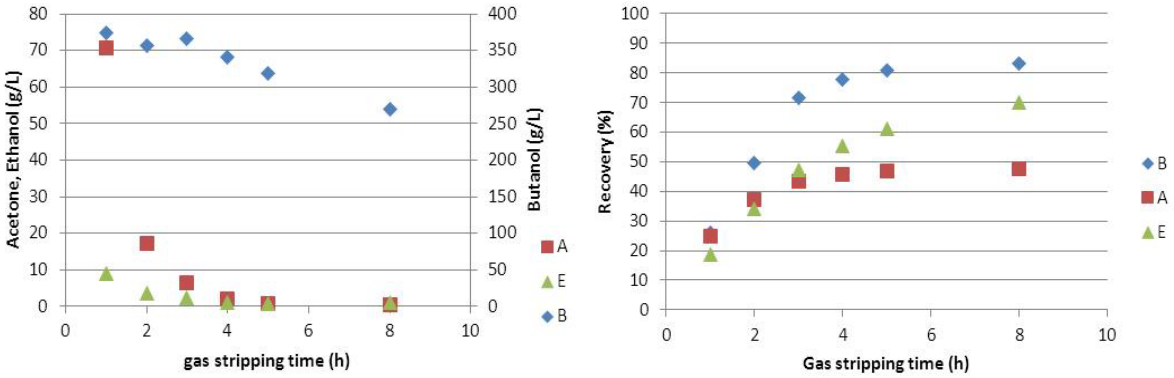
Table 1: Gas stripping performance indicators for a synthetic aqueous medium (5 g/L A; 10 g/L B; 1.67 g/L E) under optimal RSM conditions. Operation conditions: T feed = 60 °C, Gas flow = 1.34 L/min, T refrigeration = 5 °C. A: Acetone, B: Butanol, E: Ethanol.

	Selectivity, α			Recovery efficiency, η (%)			Concentration in condensate, (g/L)		
Stripping time (h)	A	B	E	A	B	E	A	B	E
18	1.74	3.61	3.64	41.6	84.6	87.0	9.41	34.71	6.34
10	3.66	5.52	5.32	59.2	86.8	86.6	19.23	51.32	9.46
4	6.28	10.36	7.74	51.3	79.8	64.2	32.55	92.14	13.69

It is worth mentioning that a feed temperature of 60 °C differs from the standard fermentation temperature of solventogenic *Clostridium* species (35-37 °C). Therefore, performing the gas stripping at T feed = 35 °C, keeping the other independent variables fixed (Gas flow = 1.34 L/min, T refrigeration = 5 °C), was tested with synthetic aqueous medium. However, butanol selectivity (α_B) of 4.21-5.71 and butanol recovery efficiencies (η_B) of 15.29-35.90 %, depending on the stripping time, were obtained. As a result, it was decided to work at T feed = 60 °C and experimentally assess the resistance of bacteria to these conditions (see section 3.3).

3.2. Two-stage gas stripping

To further concentrate butanol from the aqueous phase generated in the condensate collected from the first-stage gas stripping, a second gas stripping stage was applied to that phase after separation. The dynamic variations of ABE concentration in the condensate and the ABE recovery percentage are shown in Figure 2. After 4-5 h, the second-stage gas stripping produced a highly concentrated condensate with 350-400 g/L butanol, whose solvent phase represented 61-66% volume and contained 477 g/L butanol. A butanol recovery higher than 80% was attained at 5 h, with an acetone recovery of 47% and an ethanol recovery of 61%. These results are in agreement with other works which observed that solutions containing butanol concentrations near or exceeding butanol solubility in water (~7.7 %, w/w) and subjected to gas stripping result in a significantly high concentrated butanol condensate, thus improving the energy-saving potential of gas stripping for energy-efficient dewatering technologies (Xue *et al.* 2013, 2014a,b; Chen et al., 2014).



a b

Figure 2: a) Dynamic evolution of ABE solvent concentration in the condensate during second-stage gas stripping; b) Dynamic evolution of ABE solvents recovery during second-stage gas stripping.

3.3. Fed-batch fermentation system coupled with gas stripping

The optimal working conditions established with synthetic medium were tested with cheese whey fermentation broth. According to the previous experiments, the selected time for gas stripping was 4 h (Table 1). It must be noted that it was necessary to add 0.3 mL of an antifoaming agent to the solution before connecting the gas stripping. The results of this experiment are given in Table 2 (First stripping cycle). A good selectivity (α_B = 11.08) and a high butanol concentration (119 g/L) were found in the condensate. Once more, this butanol concentration was above its solubility limit in water and two phases could be separated. The upper solvent phase represented 7% of the total condensate volume with concentrations of 661.5 g/L butanol, 13.4 g/L

acetone and 7.6 g/L ethanol, whereas the bottom aqueous phase represented 93% of the total condensate volume and its concentrations were 77.1 g/L butanol, 14.1 g/L acetone and 2.2 g/L ethanol.

Table 2: Gas stripping performance indicators for the fermentation broth (cheese whey with C. beijerinckii *CECT 508). Stripping conditions T feed = 60 °C, Gas flow = 1.34 L/min, T refrigeration = 5 °C, t = 4 h. Initial concentrations (1st stripping): 3.02 g/L A; 12.04 g/L B; 0.23 g/L E. Initial concentrations (2nd stripping): 4.71 g/L A; 9.63 g/L B; 0.29 g/L E. A: Acetone, B: Butanol, E: Ethanol.*

	Selectivity, α			Recovery efficiency, η (%)			Concentration in condensate (g/L)		
	A	B	E	A	B	E	A	B	E
1st stripping cycle	4.65	11.08	9.32	27.60	59.29	55.83	13.89	118.9	2.14
2nd stripping cycle	4.79	13.95	17.11	25.41	66.97	91.99	22.16	119.43	4.94

The effect of gas stripping conditions (particularly T feed = 60 °C) on bacterial development was assessed by performing two consecutive stripping processes on the same fermentation broth. Therefore, after the first 4-h stripping process, the circuit was closed and the feed was supplemented as explained in section 2.4. Despite the extreme temperature, bacteria could recover and produce butanol again (9.6 g/L were attained). Afterwards, a second 4-h stripping was run. Table 2 and Figure 3 show the results of these stripping/resting cycles. It was corroborated that bacteria were negatively affected by this high temperature (mortality ~85%) and the time to reach again the stationary phase after nutrient addition was 6-7 days. Nevertheless, bacteria could recover and produce butanol titers of 9.6 g/L. Under the tested conditions, stripping offered good selectivity and recovery efficiency values (α_B: 10-14; η_B: 59-80 %), for both synthetic media and fermentation broths. Butanol selectivity values of 5.6-23 have been reported in model solutions (Ezeji et al., 2005; Yang and Lu, 2013; Qureshi et al., 2014) and 4-31 in fermentation broths (Qureshi and Blaschek, 2001; Ezeji et al., 2005). This work agrees with publications reporting condensates with 151 g/L butanol, which could be phase-separated to get an organic phase with 445-610 g/L butanol (Xue et al., 2012; Rochón et al., 2017). Others, on the contrary, reported lower butanol concentrations between 66-76 g/L in the condensate (Cai et al., 2016).

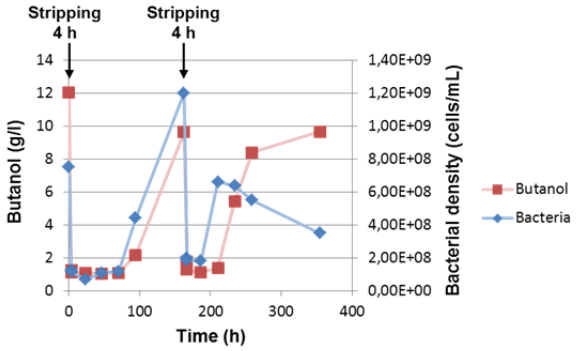


Figure 3: Evolution of ABE fermentation by C. beijerinckii *in a fed-batch system with cheese whey during two gas stripping/resting cycles. Stripping conditions T feed = 60 °C, Gas flow = 1.34 L/min, T refr = 5 °C, t = 4 h.*

On the other hand, the optimal feed temperature (60 °C) was not very appropriate for bacterial growth, and cells needed 6-7 days to recover after applying stripping. However, Xue et al. (2012) reported that the asporogenic mutant *C. acetobutylicum* JB200 was successfully subjected to eight gas stripping periods with six feeding cycles over 326 h, keeping butanol concentrations of 6-13 g/L in the broth. These data differ significantly from our results with *C. beijerinckii* CECT 508, which needs longer recovery times.

4. Conclusions

Process parameters of gas stripping, including feed temperature, gas flow rate and refrigeration temperature, were crucial for butanol recovery. A two-stage gas stripping process was proven as an efficient technique to obtain a highly concentrated butanol stream, attaining 350-400 g/L butanol in the condensate, with a concentration of 477 g/L of butanol in the organic phase. This contributes to reducing energy consumption for dewatering in a final butanol purification process. Besides, the resistance of *Clostridium beijerinckii* CECT 508

to gas stripping was assessed and, although bacteria were negatively affected by the stripping process (T feed = 60 ºC), they could recover and produce butanol again.

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