BIOPRODUCTS ENGINEERING

IN SITU RECOVERY OF HIGH-TITER BIOBUTANOL FROM ABE FERMENTATION BY CYCLIC VACUUM EVAPORATION

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ABSTRACT

Acetone-butanol-ethanol fermentation is product-limited because of butanol toxicity against solventogenic Clostridia species. In this study, extractive batch ABE fermentation using *C. saccharoperbutylacetonicum* DSMZ 14923 and vacuum cycles were applied for in situ butanol recovery. The condensation system and vacuum pump were coupled in series in a bioreactor. An ABE standard solution was used to characterize the vacuum recovery process at 56 and 25 mmHg absolute pressures at 30 °C. The lowest pressure was chosen to assess the impact of acetic acid, 5-hydroxymethylfurfural (HMF), and furfural on evaporation from the standard ABE solution during vacuum recovery. ABE co-fermentation of sugarcane bagasse hydrolysate (CHH) and molasses (SCM) with or without ~36 g/L sugars (control) with in situ product recovery by cycles of 2 h at an absolute pressure of 25 mmHg and 4-h of atmospheric pressure. In situ product recovery by vacuum evaporation decreased the fermentation time, almost completely depleted total sugars, improved cell growth, and increased ABE productivity and yield.

Keywords: in situ product recovery. Vacuum ABE fermentation. Sugarcane molasses. Hemicellulosic hydrolysate.

1 INTRODUCTION

Butanol is considered a valuable biofuel with the potential to substitute gasoline [1] as well as versatile chemicals, and as a feedstock for sustainable aviation fuel production.[2] Despite the interest in biobutanol production via acetone-butanol-ethanol (ABE) fermentation, a critical bottleneck in this process is product inhibition by butanol in *Clostridium* spp. Cells 1. Butanol toxicity results in low productivity, yield, and process energy inefficiency owing to product recovery from a diluted stream (lower than 13 g/L butanol in batch fermentation) and degeneration of the butanol-producing strains. [3]

Vacuum fermentation is a promising technique for in-situ product recovery. However, the application of vacuum recovery for ABE fermentation has been disregarded because the boiling point of water (100 °C) is lower than that of butanol (118 °C). Nevertheless, [4] demonstrated for the first time that this technique was viable for ABE fermentation once butanol and water formed a heteroazeotropic mixture that at concentrations below 70 g/L boiled at a lower temperature (or higher pressure in isothermal processes) than the boiling point of either butanol or water. The vapor phase is always more butanol-rich than the liquid phase.[5] studied the in situ removal of butanol by applying continuous and intermittent vacuums using synthetic media containing glucose as the carbon source. They found that both vacuum modes led to improved performance of ABE fermentation, with total glucose depletion, enhanced productivity, and cell growth, whereby a highly concentrated butanol stream was recovered. This study aimed to assess the performance of ABE co-fermentation integrated with cyclic product recovery by the hyper-butanol producer *C. saccharoperbutylacetonicum* DSMZ 14923, using sugarcane molasses (SCM) and concentrated hemicellulosic hydrolysate from sugarcane bagasse (CHH) as carbon sources.

2 MATERIAL & METHODS

The vacuum recovery process was conducted at 30 °C using 1 L of ABE standard solution containing (g/L) ethanol (1.1), acetone (4.8), and butanol (10.2) (concentrations usually reached in batch ABE fermentations) to evaluate the application of absolute pressures of 56 and 25 mmHg for ABE recovery from a dilute solution. A 3 L bioreactor (New Brunswick Bioflo®/Celligen®115) was connected in series to a condensation system at -10 °C and an oil-free vacuum pump (Edwards XDS 5 pump, AC motor, 250 W). A water bath was used to maintain the ABE solution temperature at 30 °C. A vacuum was applied to the reactor containing the ABE standard solution for one hour, where the vacuum was released to withdraw duplicate samples of 1 mL from the reactor to quantify the solvent concentrations. This procedure was repeated three more times, so 4h of vacuum was applied to the reactor. In the second evaporation experiment, 1 L of ABE standard solution was supplied with intermediate acetic acid, HMF, and furfural inhibitors. This solution contained (g/L) acetic acid, 4.9; ethanol, 0.9; butanol, 8.4; acetone, 2.3; HMF, 0.2; and furfural, 0.4. In this experiment, a vacuum was applied for a period of 0.5 h. After the vacuum was released, duplicate samples (1 mL) were withdrawn from the reactor to quantify the solvent concentrations. This procedure was repeated three more times, so 2h of vacuum was applied to the reactor. Mass balance was maintained during the experiments. A schematic of the reactor setup for the recovery experiments and ABE fermentation with in situ product recovery is shown in **Fig. 1**. The ABE fermentation study used *C. saccharoperbutylacetonicum* DSM 14923 (Collection of Cultures from the Leibniz Institute, German Collection of Microorganisms and Cell Cultures [DSMZ]). Culture maintenance, inoculum preparation, and fermentation were performed as described previously. [1] The initial concentration of total sugars was ~36 g/L [75 wt% and 25 wt% from sugarcane molasses (SCM) and concentrated hemicellulosic hydrolysate from sugarcane bagasse (CHH), respectively]. Anaerobic conditions were maintained by nitrogen sparging (1 L/min; i.e., 1 vvm). Nitrogen sparging was started 2h prior to inoculation. All experiments were conducted in

duplicate. Batch fermentation was performed for 20 h, after which vacuum evaporation was performed. During the vacuum application, the medium in the bioreactor was boiled at 30 °C to generate ABE and water vapor. The vacuum was cyclically applied to the fermenter for 2h (25 mmHg), followed by a period of 4h without vacuum (atmospheric pressure). After applying the vacuum, the pressure inside the bioreactor was restored to atmospheric pressure by stopping the vacuum and restarting N_2 flushing. During this period, the samples were withdrawn from the bioreactor. ABE and water vapor were condensed at -10 °C. Oxygen-free sterile water was added to the bioreactor at different intervals to maintain a constant liquid volume i. 1 L (to compensate for water loss due to evaporation). During the vacuum application, foam was formed in the bioreactor, which collapsed by applying 2-second pulses of N_2 gas for 10 min and adding antifoam when necessary. The samples were withdrawn at various intervals for further analysis.
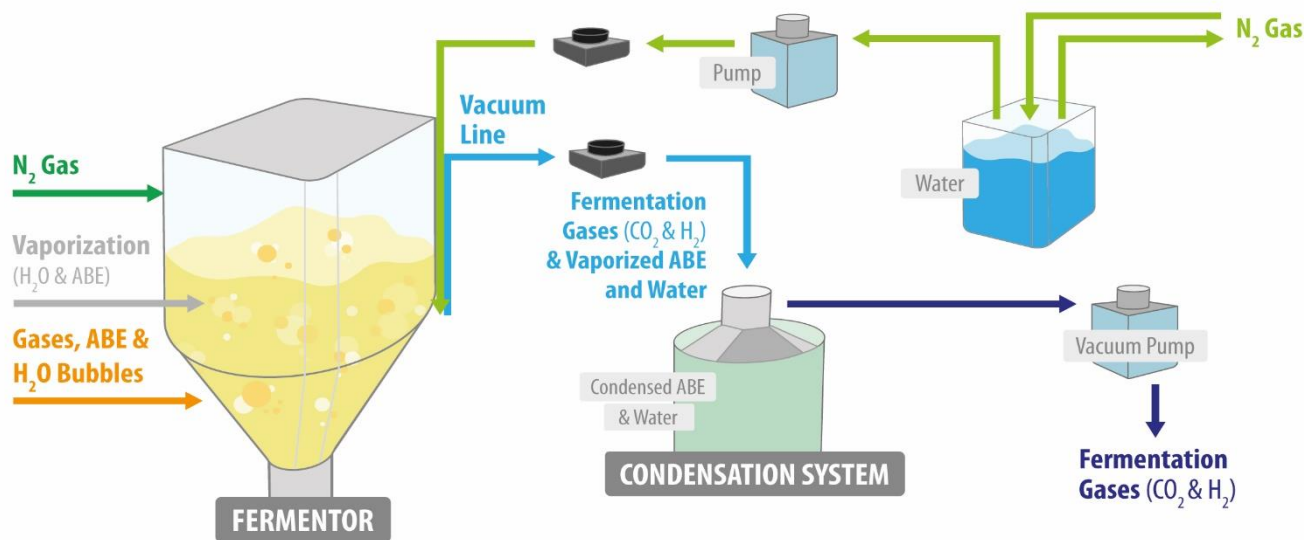

Figure 1. Schematic representation of batch ABE fermentation and recovery by cyclic vacuum application.

3 RESULTS & DISCUSSION

In order to assess the effectiveness of cyclic vacuum application during the ABE recovery process, experiments were performed using an ABE standard solution at absolute pressures of 56±2 and 25±2 mmHg and varying recovery times. As expected, the amount of all solvents in the reactor decreased over time owing to the vacuum evaporation (**Figure 2a**). After 1 h of vacuum application at absolute pressures of 56 and 25 mmHg, both ethanol and acetone were almost completely extracted, and butanol was largely removed. After the first hour of vacuum, the butanol removal rate slowed down for both pressures, whereby at the lowest pressure, butanol removal was negligible after 2-h, as the amount remained stable at approximately 0.5 g. Four hours of vacuum at an absolute pressure of 56 mmHg were required to remove 90% of the butanol that was initially present. In contrast, 95% and 98% of butanol was removed after 2h and 4h of vacuum at an absolute pressure of 25 mmHg, respectively, showing that butanol removal was more efficient at the lowest pressure. After 4h of vacuum, the recovered condensate contained 81% and 85% butanol, 64% and 66% acetone, and 70% and 75% ethanol in the initial solution fed into the reactor at 56 and 25 mmHg, respectively. At a constant temperature, the vapor pressure of the butanol-water mixture is lower at 25 mmHg than at 56 mmHg, and the butanol-water mixture evaporates faster in the former condition because it is in the region of superheated vapor, providing more suitable conditions for butanol extraction.

Based on the results of the batch vacuum extraction experiments, 25 mmHg of vacuum applied for 2-h was chosen as the operating condition to determine the effect of the three components present in the sugarcane bagasse hemicellulosic hydrolysate, namely 5-hydroxymethylfurfural (HMF), furfural, and acetic acid (which is also formed during the acidogenic phase) on butanol recovery. During these two hours of evaporation, the vacuum was released every half hour to obtain a sample to quantify the ABE solvents, acetic acid, and inhibitors. The ABE solvents and acetic acid were partly removed during vacuum extraction. Simultaneously, the removal of inhibitory compounds, namely furfural and 5-hydroxymethylfurfural, was insignificant. The butanol amount decreased from the initial amount of 8.4 g to 1.4 g after 2h of vacuum, which corresponded to a decrease of 83%, which was less than in the absence of the three additional components (almost 95% butanol removed after 2h). The recovered condensate stream is evaluated to assess the efficiency of the condensation system. After 2h of vacuum, the condensate contained 83% butanol, 56% acetone, and 72% ethanol as percentages of the solvents present in the initial solution. Remarkably, the amounts of butanol and ethanol in the condensate were almost unaffected by the presence of HMF, furfural, and acetic acid. While 85% butanol and 75% ethanol in the standard ABE solution (without additives) were condensed in 4 h, 83% and 72% butanol and ethanol were condensed in 2 h from the ABE solution in the presence of these additives (HMF, furfural, and acetic acid), respectively.

Nevertheless, although acetone effectively evaporated from the solution, it was less efficiently trapped in the condensation system. Only 56% and 66% of acetone was condensed from the ABE standard solution, with and without the abovementioned additives, respectively. The fermentation products were quantified only at the end of cultivation, that is, after 60 h. The primary solvents used were butanol, acetone, and a small amount of ethanol (**Figure 2b**). The ABE productivity and yield on the consumed sugar were 0.16 g/L/h and 0.31 g/g, respectively (**Table 1**). A low butanol concentration in the broth and low productivity are usually obtained in ABE fermentation because butanol is toxic to wild-type Clostridia spp. and is lethal in a concentration range of 10–12 g/L. [5] Although we reported that the strain used in this study, *C. saccharoperbutylacetonicum,* exhibits a higher tolerance to butanol, which becomes lethal at approximately 15 g/L [1], ABE fermentation is disturbed due to butanol toxicity. Consequently, sugar

depletion was not achieved. In *situ* product recovery by vacuum evaporation during ABE fermentation on a nonsynthetic medium was effective. After 20 h of fermentation, cyclic vacuum evaporation led to oscillatory behavior of the butanol, acetone, ethanol, and acetic acid concentrations, whereby the concentrations decreased during the vacuum and increased afterward. In contrast, butyric acid did not exhibit this behavior, likely because it had the highest boiling point of all the quantified components.
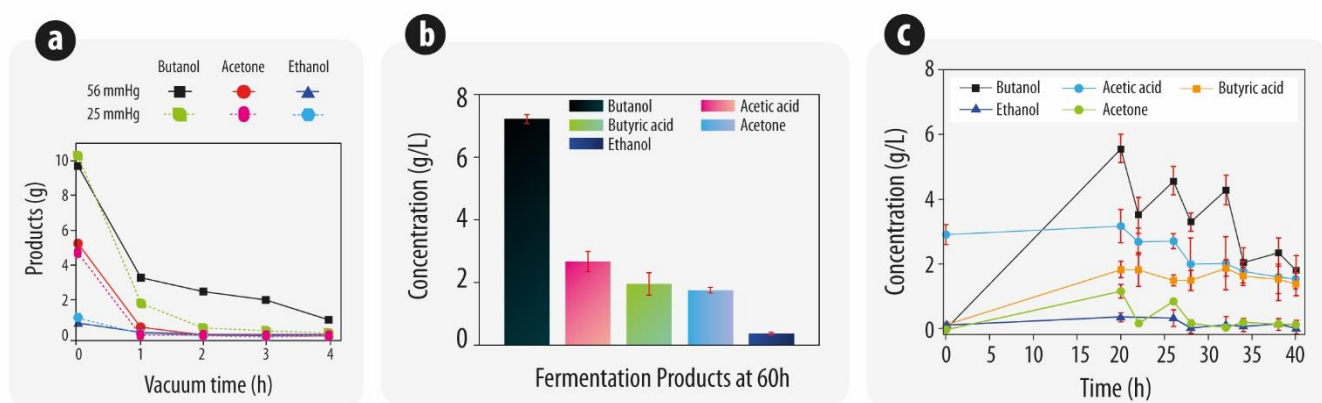

Figure 2 (a) ABE amounts at 56±2 and 25±2 mmHg at different vacuum recovery times; (b) ABE production in control co-fermentation at 60 h; (c) ABE Co-fermentation (using SCM and CHH) and in situ product recovery after 2h of cyclic vacuum separated by 4-h intervals of fermentation.

Table 1. ABE fermentation performance and product recovery under cyclic vacuum.

Parameters	Control experiment	Cyclic vacuum fermentation
Total ABE (g) / toal acetone (g) / total butanol (g) / total ethanol (g)	9.3 / 1.8 / 7.2 / 0.37	12.4 / 1.7 / 10.4 / 0.37
Cell concentration (OD_{600nm}) / Initial total sugars (g/L) / Residual total sugars (g/L)	6.46 / 34.4 / 4.1	8.17 / 39.9 / 1.1
Butanol productivity (g/L/h) / ABE productivity (g/L/h) / ABE yield (g/g)	0.12 / 0.16 / 0.31	0.26 / 0.31 / 0.32
Total sugars utilized (%) / Acetone – Butanol – Ethanol yields (g/g)	88 / 0.06 – 0.24 – 0.01	97 / 0.04 – 0.27 – 0.01
Fermentation time (h) / Vacuum time (h) / Condensate volume (L)	60 / - / -	40 / 8 / 0.112
Acetone – Butanol – Ethanol – Acetate - Butyrate concentrations in condensate (g/L)	-	13.8 – 78.9 – 3.3 – 0.40 – 0.10

The butanol concentration in the broth decreased to as low as 1.8 g/L and increased to 4.6 g/L before and after vacuum application, respectively (**Figure 2c**). Vacuum cycles were effective in maintaining the butanol concentration below the toxicity threshold for Clostridia spp., allowing *C. saccharoperbutylacetonicum* to attain a maximum optical density of 8.17, which was higher than that obtained in the control experiment (**Table 1**). This finding is consistent with a study by [6], who reported a 1.9 times increase in the cell density of *C. beijerinckii* 8052, measured as OD_{540nm}, when the fermentation was subjected to intermittent vacuum, compared to the control experiment. The amount of ABE produced during 1 L fermentation with vacuum extraction was 12.4 grams, which represents a two-fold improvement in productivity compared to the control experiment, amounting to 0.31 g ABE/L/h. Despite this increase, the ABE yield was similar to that of the control experiment, amounting to 0.32 g ABE/g. These results demonstrate that vacuum evaporation leads to significantly lower levels of butanol, which in turn reduces butanol stress on the bacteria. This may have enabled the bacteria to perform better even in the presence of inhibitors, as observed in the control experiment.

4 CONCLUSION

Cyclic and intermittent vacuum evaporation are promising solutions for enhancing the efficiency of solventogenic clostridia-based butanol fermentation processes. During in situ butanol recovery through ABE fermentation using *C. saccharoperbutylacetonicum*, the butanol concentration in the recovered condensate was more than ten times higher than that in the fermentation broth, which significantly simplifies downstream processing. Moreover, applying vacuum recovery during ABE fermentation reduces the fermentation time, resulting in a higher cell density, nearly complete depletion of total sugars in sugarcane molasses/bagasse hemicellulosic hydrolysate-based media, and increased ABE productivity and production. Notably, the strain was unaffected by vacuum evaporation.

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