Butanol production in acetone-butanol-ethanol fermentation with *in situ* product recovery by adsorption

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Abstract

Activated carbon Norit ROW 0.8, zeolite CBV901, and polymeric resins Dowex Optipore L-493 and SD-2 with high specific loadings and partition coefficients were studied for *n*-butanol adsorption. Adsorption isotherms were found to follow Langmuir model, which can be used to estimate the amount of butanol adsorbed in acetone-butanol-ethanol (ABE) fermentation. In serum-bottle fermentation with *in situ* adsorption, activated carbon showed the best performance with 21.9 g/L of butanol production. When operated in a fermentor, free- and immobilized-cell fermentations with adsorption produced 31.6 g/L and 54.6 g/L butanol with productivities of 0.30 g/L·h and 0.45 g/L·h, respectively. Thermal desorption produced a condensate containing ~167 g/L butanol, which resulted in a highly concentrated butanol solution of ~640 g/L after spontaneous phase separation. This *in situ* product recovery process with activated carbon is energy efficient and can be easily integrated with ABE fermentation for *n*-butanol production.

Keywords: Acetone-butanol-ethanol fermentation; Activated carbon; Adsorption; Butanol; *in situ* product recovery

1. Introduction

Due to concerns about depletion of crude oils and escalating prices of petroleum-derived products, biobutanol has attracted attention as a liquid transportation fuel (Dürre, P., 1998; Wang et al., 2014; Xue et al., 2013a). However, due to severe product inhibition caused by butanol, conventional acetone-butanol-ethanol (ABE) fermentation is limited by low product titer, yield, and productivity (Kumar and Gayen, 2011), resulting in intensive energy consumption for product recovery by distillation (Abdehagh et al., 2014; Vane 2008; Xue et al., 2014a). Although intensive efforts have been made on improving butanol tolerance and production by solventogenic clostridia through adaptation, mutagenesis and metabolic engineering (Lee et al., 2008; Xu et al., 2015; Yang and Zhao, 2013), 2% (w/v) of butanol production is still a bottleneck in the development of ABE fermentation (Zhao et al., 2013). Fermentation with *in situ* product recovery (ISPR) can alleviate product inhibition and thus improve conversion and productivity (Staggs and Nielsen, 2015; Xue et al., 2014a; Yang and Lu, 2013). Several online integrated butanol recovery methods, including adsorption (Nielsen and Prather, 2009; Qureshi et al., 2005; Wiehn et al., 2014; Yang et al., 1994), liquid-liquid extraction (Roffler et al. 1987), pervaporation (Matsumura et al., 1988; Xue et al., 2014b) and gas stripping (Lu et al., 2013; Xue et al., 2012; 2013b; 2014c) have been investigated. Among them, adsorption requires less energy, is easier to operate (Agueda et al., 2013; Oudshoorn et al., 2009b), and has been demonstrated as an effective process for separating butanol and reducing its inhibition in ABE fermentation (Liu et al., 2014; Yang and Tsao, 1995).

Butanol recovery from dilute solution by adsorption is usually carried out in two steps: adsorption followed by desorption to obtain a concentrated butanol solution with adsorbent regeneration (Agueda et al., 2013; Vane, 2008). Three types of adsorbents have been widely

1 studied for butanol adsorption: activated carbon (AC), zeolite ($\text{SiO}_2/\text{Al}_2\text{O}_3$), and polymeric
2 (typically ion-exchange) resins (Abdehagh et al., 2015; Liu et al., 2014; Nielsen and Prather,
3 2009; Oudshoorn et al., 2009a; Qureshi et al., 2005). In general, adsorbents should have a high
4 butanol adsorption capacity, affinity, and selectivity, and be inexpensive and easy to be
5 regenerated for reuse. Although some zeolites and silicalite could adsorb almost all butanol from
6 a dilute solution of less than 10 g/L and be thermally regenerated to produce a butanol solution
7 of >80% (w/v) (Milestone and Bibby, 1981; Saravanan et al., 2010), their biocompatibility for *in*
8 *situ* butanol recovery during fermentation has not been studied. Meanwhile, a recent study
9 showed that activated carbon F-400 was the best butanol adsorbent with the highest adsorption
10 capacity and fastest adsorption rate among ACs and zeolites tested (Abdehagh et al., 2013).
11 However, its application in ISPR for butanol production has not been demonstrated. To date,
12 most of prior studies of ISPR by adsorption focused on polymeric resins, which are relatively
13 expensive and have only moderate butanol adsorption capacity and selectivity (Liu et al., 2014;
14 Nielsen and Prather, 2009; Nielsen et al., 2010; Wiehn et al., 2014; Yang et al., 1994), which
15 have limited their commercial applications.

16 The goal of this study was to investigate and demonstrate the feasibility of *in situ* adsorption
17 for high-titer butanol production in fed-batch fermentation with *Clostridium acetobutylicum*
18 JB200 (Yang and Zhao, 2013). We first screened a variety of commercial adsorbents, including
19 AC, ion exchange resins and zeolites, for their ability to adsorb n-butanol from model solutions.
20 Promising adsorbents were then evaluated for their adsorption isotherms and uses in ISPR for
21 butanol production in ABE fermentation with cells immobilized in a fibrous bed bioreactor (Du
22 et al., 2015). For the first time, our results demonstrate that activated carbon Norit ROW 0.8, a
23 relatively inexpensive adsorbent, is biocompatible and effective for *in situ* butanol recovery for

high-titer butanol production in ABE fermentation.

2. Materials and Methods

2.1 Screening adsorbents for butanol adsorption

Nine different adsorbents (activated carbon Norit ROW 0.8, ion exchange resins Amberlite IRA-900, Amberlite XAD-4, Diaion HP-2MG, Diaion HP-20, Dowex Optipore L-493, Dowex Optipore SD-2, and zeolites CBV901 and CBV28014) representing different types were selected based on literature data (Abdehagh et al., 2013; Nielsen & Prather, 2009; Nielsen et al., 2010; Oudshoorn et al., 2009a; Qureshi et al., 2005; Saravanan et al., 2010). The zeolites were obtained from Zeolyst International (Conshohocken, PA) while all other adsorbents were purchased from Sigma-Aldrich (St. Louis, MO). They were tested for their ability to adsorb *n*-butanol in capped tubes, each containing 25 mL of the model solution (~10 g/L butanol) and 1 g of a tested adsorbent, at 37 °C. The amount of butanol adsorbed per unit weight of the adsorbent or specific loading (*q*) was determined from the difference between the initial and final (equilibrium) butanol concentrations, as follows:

$$q = (C_{eq} - C_o)V/W \quad [1]$$

where *V* is the solution volume, *W* is the mass of adsorbent, and *C_o* and *C_{eq}* are the butanol concentrations in the solution at initial and equilibrium, respectively. The partition coefficient (*K_r*), which indicates the affinity or potential of an adsorbent to take up butanol from the aqueous solution, can be determined from the following equation:

$$K_r = q/C_{eq} \quad [2]$$

2.2 Adsorption isotherm

Norit ROW 0.8, Dowex Optipore L-493 and SD-2, and CBV901 with high butanol adsorption

capacity found in the screening tests were further studied for their adsorption isotherms at 37 °C and 60 °C. Various amounts of adsorbents, ranging from 0.25 g to 6 g, were added to 15 mL of the model solution (butanol concentration: 39.6 ± 1.8 g/L) and allowed to equilibrate for 24 h with agitation at 150 rpm. The adsorption isotherm for activated carbon, which was the best among the four adsorbents studied, was further studied with a model solution containing ~20 g/L glucose, ~20 g/L acetone, ~40 g/L butanol, ~4 g/L ethanol, ~10 g/L acetic acid, and ~4 g/L butyric acid to evaluate butanol and acetone adsorption in the presence of glucose and acids.

2.3 Adsorption dynamics in a packed column of activated carbon

Continuous adsorption with 25 g Norit ROW 0.8 packed in a glass column (15 cm in length; 2.1 cm in inner diameter) by feeding 10 g/L butanol solution at 20 ml/min was studied for ~90 min. Samples from the effluent were collected periodically to monitor butanol concentration until complete breakthrough was confirmed. The fully loaded activated carbon was regenerated by heating at 200 °C overnight, and then washed with three volumes of distill water and reused for the next cycle of adsorption run. Continuous adsorption with a model solution containing 10 g/L butanol, 5 g/L acetone, 2 g/L ethanol, 4 g/L acetic acid and 2 g/L butyric acid was also studied.

2.4 Desorption dynamics

Desorption of butanol, water and ABE mixture from activated carbon was first studied by thermogravimetry. Briefly, butanol, water and ABE mixture with a ratio of 6:3:1 were first adsorbed onto activated carbon for 24 h to reach saturation. Then, free (un-adsorbed) water and solvents were removed from the ‘wet’ activated carbon by patting with filter paper. Butanol desorption from the “loaded” activated carbon (initial weight: 30.37 ± 0.18 g) was then carried out by heating at a gradually increased temperature (10 °C/min), from 25 °C to 250 °C, until reaching a constant weight. A two-step heating process for butanol desorption was then studied.

Briefly, 10 g of activated carbon was equilibrated in 300 ml model solution (~15 g/L butanol) at 37 °C with agitation at 25 rpm for 24 h. After removing free water and un-adsorbed butanol with filter paper and aspiration through a 50 ml syringe, the partially dried adsorbents were heated first at 40 °C to remove water, and then heated at 200 °C for butanol recovery.

2.5 Cultures and media

Clostridium beijerinckii CC101 (Lu et al., 2013) was used in serum bottle fermentation without pH control. *Clostridium acetobutylicum* JB200 (Yang and Zhao, 2013) derived from ATCC 55025 was used for ABE fermentation in bioreactor with pH control. The seed culture was prepared in Clostridial growth medium (CGM) containing 30 g/L glucose, 2 g/L yeast extract, 1 g/L Tryptone, minerals and vitamins in a phosphate buffer as described elsewhere (Xue et al., 2012), and incubated at 37 °C for ~16 h until active growth was observed. The medium was sterilized by autoclaving at 121 °C and 15 psig for 30 min. Batch fermentation was studied using P2 medium containing glucose (70 g/L), yeast extract (1 g/L), phosphate buffer (0.5 g/L KH₂PO₄ and 0.5 g/L K₂HPO₄), ammonium acetate (2.2 g/L), vitamins (1 mg/L para-amino-benzoic acid, 1 mg/L thiamin and 0.01 mg/L biotin), and mineral salts (0.2 g/L MgSO₄·7H₂O, 0.01 g/L MnSO₄·H₂O, 0.01 g/L FeSO₄·7H₂O, 0.01 g/L NaCl). All solutions were purged with nitrogen for 1 h through a sterile 0.2 µm filter, either before or after autoclaving.

2.6 Batch fermentation in serum bottles

Batch fermentation was first studied with *C. beijerinckii* CC101 in serum bottles containing 80 ml of P2 medium and 4 g of an adsorbent (Norit ROW 0.8, Dowex SD-2, Dowex L-493, or CBV901). Liquid samples were withdrawn with a syringe periodically for analysis of glucose, free cell density and fermentation products. To evaluate the reusability of AC and the reproducibility of fermentation performance with *in situ* adsorption, activated carbon in the

serum bottles was collected and regenerated by heating at 200 °C to a constant weight, and then washed with water for three times and used in the next batch fermentation. This was repeated for 5 cycles, with the initial and final glucose and butanol concentrations in the fermentation broth recorded for each cycle.

2.7 Fed-batch fermentation in bioreactor

The integrated ABE fermentation with adsorption system consisted of a stirred-tank reactor (1.5 L working volume) and an external glass column (i.d.: 50 mm, length: 400 mm, 250 ml working volume) packed with 75 g of activated carbon (**Fig. 1A**). The bioreactor with medium and the activated carbon column were autoclaved separately for 45 min, and aseptically connected after sterilization. Before inoculation with 100 ml of overnight culture of JB200 in serum bottles, the whole system was sparged with nitrogen for 30 min to ensure an oxygen-free environment. During fed-batch fermentation, a concentrated glucose solution (~360 g/L) was pulse-fed when glucose in the fermentation broth was nearly depleted. Adsorption was initiated by circulating the fermentation broth between the fermentor and the adsorption column at ~60 ml/min when the butanol concentration in the fermentation broth had reached ~10 g/L (at ~30 h). Butanol desorption and the regeneration of the adsorption column were done in an oven (200 °C) connected a cold trap with a closed-loop vapor recirculation via a peristaltic pump (**Fig. 1B**). The butanol collected in the cold trap was measured to determine the amount of butanol adsorbed in the column. Similar fed-batch fermentation was also carried out with cells immobilized in a fibrous matrix packed inside the stirred-tank bioreactor. The internal fibrous bed was made of a piece of cotton towel wound together with a stainless steel mesh affixed to the inner wall of the bioreactor. Batch fermentation without adsorption, as the control, was also carried out with an initial glucose concentration of ~85 g/L at 37 °C and pH 5.0.

2.8 Analytical methods

Cell biomass in the fermentation broth was estimated by measuring the optical density at 600 nm with a spectrophotometer (UV-16-1, Shimadzu, Columbia, MD). Glucose and fermentation products were analyzed after cell removal through centrifugation at 13,200 rpm for 5 min. The glucose concentration was determined with YSI 2700 Select Biochemistry Analyzer (Yellow Springs, Ohio). Butanol, acetone, ethanol, acetic acid and butyric acid were determined with a gas chromatograph (GC-2014 Shimadzu, Columbia, MD) equipped with a flame ionization detector (FID) and a fused silica column (Stabilwax-DA, 30 m long, 0.25 μ m film thickness and 0.25 mm ID, Restek, Bellefonte, PA) following the method previously described (Xue et al., 2012). All general lab supplies including pipette tips and centrifuge tubes were purchased from Guangzhou Jet Bio-Filtration Co., Ltd., unless otherwise noted.

3. Results and Discussion

3.1 Screening adsorbents for butanol adsorption

Activated carbon, 2 zeolites and 6 ion-exchange resins from a candidate pool of commercially available adsorbents were tested with a model solution containing ~10 g/L *n*-butanol at 37 °C. The specific loadings and partition coefficients of these adsorbents are compared and shown in **Figure 1**. Among them, activated carbon (Norit ROW 0.8) was the best adsorbent with the highest specific loading ($q = 0.21$ g butanol/g adsorbent) and partition coefficient ($K_r = 0.15$ g butanol/g adsorbent / g butanol/L). A high butanol adsorption capacity has also been reported for activated carbon F-400 ($q = 0.302$ g/g) (Abdehagh et al., 2015) and Norit ROW 0.8 ($q = 0.252$ g/g) (Groot and Luyben, 1986). Among the six ion-exchange resins tested, Dowex Optipore L-493 and SD-2 showed the best butanol adsorption capability ($q = 0.14$ g/g; $K_r = 0.034 \pm 0.01$ g butanol/g adsorbent / g butanol/L), which was somewhat lower than those previously reported (q

= 0.175 g/g for L-493 and 0.152 g/g for SD-2), probably because a higher butanol concentration of 20 g/L was used in the previous study (Nielsen and Prather, 2009). For the zeolite group, CBV901 ($q = 0.15$ g/g; $K_r = 0.037$ g butanol/g adsorbent / g butanol/L) was significantly better than CBV28014 ($q = 0.11$ g/g; $K_r = 0.032$ g butanol/g adsorbent / g butanol/L), which was consistent with the results from a previous study (Oudshoorn et al., 2009a). Norit ROW 0.8, Dowex L-493, Dowex SD-2, and CBV901 each representing a different type of adsorbents with a higher butanol adsorption capacity were thus further studied for their adsorption isotherms.

3.2 Adsorption isotherm

Figure 2 shows the adsorption isotherms at 37 °C and 60 °C for the four adsorbents studied. In general, the specific loading for these adsorbents increased with increasing the butanol concentration in solution and decreased with increasing the temperature from 37 °C to 60 °C. The adsorption isotherm was fitted, via least-squares regression using Minitab, to the Langmuir equation shown below:

$$q = \frac{q_m C_{eq}}{K + C_{eq}} \quad [3]$$

where q (g/g) is the specific loading, C_{eq} (g/L) is the equilibrium concentration of butanol in the solution, K (g/L) is the Langmuir coefficient, and q_m (g/g) is the maximum adsorption capacity.

In general, the adsorption data fitted well with the Langmuir isotherm, which is commonly used for modeling monolayer adsorption on homogeneous surfaces (Faisal et al., 2014; Lin et al., 2012; Saravanan et al., 2010). **Table 1** shows the model parameters for different adsorbents tested. As expected, q_m decreased with increasing the temperature from 37 °C to 60 °C for all adsorbents studied. The isotherm data confirmed that Norit ROW 0.8 was the best *n*-butanol adsorbent with the highest q_m value (0.48 g/g) and the lowest K value (1.72 g/L) at 37 °C, followed by Dowex SD-2 ($q_m = 0.38$ g/g, $K = 4.32$ g/L) and Dowex L-493 ($q_m = 0.36$ g/g, $K =$

4.02 g/L). CBV901 had the lowest adsorption capacity ($q_m = 0.24$ g/g), but a good affinity ($K = 1.80$ g/L) for *n*-butanol.

The adsorption isotherm of activated carbon was further studied with the model solution simulating ABE fermentation broth, and the results are shown in **Figure 2C**. Compared to the isotherm obtained with pure butanol solution (Fig. 2A), the amount of butanol adsorbed in the presence of glucose, acetone, ethanol, and acetic and butyric acids was significantly lower because of the competition for the active sites by mainly acetone and butyric acid (Abdehagh et al., 2013). Nevertheless, Norit ROW 0.8 had a high selectivity (4.7–10.3) for butanol over acetone and adsorbed much more butanol than acetone and acids (see supplemental **Table S1**).

3.3 Adsorption dynamics in a packed column of activated carbon

Figure 3 shows the breakthrough curves of continuous adsorption with butanol solution (Fig. 3A) and a model solution containing ABE and acetic and butyric acids (Fig. 3B). With the 10 g/L butanol solution, breakthrough started at ~30 min and complete breakthrough occurred at ~60 min in the first cycle, while breakthrough started a little earlier in the second cycle. Based on the breakthrough curve data, the amounts of butanol adsorbed were 8.88 g and 8.59 g in the first and second cycles, respectively, with the corresponding specific loadings of 0.355 g/g and 0.344 g/g, which were comparable to the value (~0.36 g/g) found in the adsorption isotherm (Fig. 2A). The data showed that AC could be regenerated and reused with a high efficiency (>97%) without losing much of its adsorption capacity. With the model solution containing ABE, butanol breakthrough occurred much earlier due to the occupation of activate sites by other chemical species. The order of breakthrough (acetic acid, acetone, ethanol, butyric acid, and butanol) clearly indicated that butanol had higher adsorption by AC than other chemical species. Overshoot observed with acetic acid, acetone, and ethanol suggested that their initial adsorption

was later replaced by the stronger adsorbate (butanol), which was also observed with other adsorbents (Abdehagh et al., 2015; Lin et al., 2012). Based on the breakthrough curves, the specific loadings were found to be: butanol 0.268 g/g, acetone 0.044 g/g, ethanol 0.011 g/g, acetic acid 0.014 g/g, butyric acid 0.038 g/g. The results were consistent with the values found in the adsorption isotherm (Fig. 2C) and confirmed that AC had higher adsorption capacity and selectivity for butanol over acetone, ethanol, acetic acid, and butyric acid.

3.4 Thermal desorption of butanol from activated carbon

Figure 4 shows thermogravimetric desorption of water, butanol and ABE mixture from Norit ROW 0.8. It should be noted that more water was adsorbed on AC than butanol and ABE mixtures. However, water adsorption was weaker than butanol adsorption, and water desorption was faster with complete desorption at ~125 °C, while complete butanol desorption occurred at a much higher temperature of ~200 °C. As can be seen in the thermogravimetric desorption curves, about 60% of the adsorbed water was desorbed when the heating temperature increased to 100 °C, which appeared to be an inflection point for decreased butanol desorption rate. The results suggested that about 40% of the adsorbed butanol was more strongly adsorbed. For ABE, about 80% of the adsorbed species were desorbed when the temperature had reached ~113 °C, where an inflection point for decreased desorption rate was observed. The condensate collected from desorption at 40 °C contained ~60 g/L butanol, while ~150 g/L butanol was obtained when the heating temperature increased to 200 °C. It is thus possible to obtain high-concentration butanol at higher temperatures after removing most of the adsorbed water at lower temperatures. Similar finding was reported for ACF-400 that thermal desorption generated a high butanol concentration of ~150 g/L in the desorbed phase with a consistent recovery rate of ~80% in 7 adsorption-desorption cycles using ABE model solutions (Abdehagh et al., 2015). A much higher

butanol concentration of ~80% (w/v) could be obtained from a two-step desorption process using silicalite and zeolites, which have a much higher selectivity for butanol over water (Milestone and Bibby, 1981; Saravanan et al., 2010). However, they are not compatible with ISPR due to their toxicity to cells.

Heat or energy required for thermal desorption includes evaporation heat or enthalpy for vaporizing butanol and water and sensible heat to heat up the temperature from 37 °C to 125 °C for water and 200 °C for butanol and AC, and is estimated to be ~14.1 kJ/g butanol desorbed (see Table 2), which is much smaller than the energy content of butanol (36 kJ/g). It is noted that the total energy consumption for butanol recovery and purification is mostly for product capture or concentrating the dilute butanol solution (~1%) to more than 50% (w/v) (Oudshoorn et al., 2009b). It is clear that adsorption with AC can provide an energy-efficient process for biobutanol production and is more favorable than conventional distillation, which requires more than 24–50 kJ/g butanol (Matsumura et al., 1988). Since the majority (~89%) of the heat for butanol desorption from AC was to vaporize water, which has a much higher heat capacity and evaporation enthalpy than butanol, it would be desirable to reduce the amount of water adsorbed in the process in order to further reduce energy consumption in desorption.

3.4 Batch fermentation with *in situ* adsorption in serum bottles

The effects of *in situ* butanol adsorption with Norit ROW 0.8, Dowex L-493, Dowex SD-2, and CBV901, respectively, on batch fermentation with *C. beijerinckii* CC101 were studied in serum bottles. The results are summarized and compared to the control without adsorption in Table 3. The initial glucose concentration in the medium was ~70 g/L, and the broth pH dropped from the initial value of ~6.5 to 4.2–4.6, depending on the adsorbent used, due to acids accumulation in the acidogenesis phase. With Norit ROW 0.8, almost all glucose was utilized in the fermentation,

1 producing a total of 21.9 g/L butanol at a yield of 0.31 g/g glucose consumed. The fermentations
2 with Dowex L-493 and SD-2 produced 14.5 g/L and 15.2 g/L butanol, respectively, at a yield of
3 0.31 g/g, with 24.5 g/L and 20.4 g/L glucose, respectively, remaining in the broth when the
4 fermentation ceased. In contrast, the control fermentation produced only 11.7 g/L butanol at a
5 yield of 0.27 g/g, with 28.5 g/L glucose remaining unused in the broth. The fermentation ceased
6 due to strong inhibition by butanol at >10 g/L (Xu et al., 2015). Clearly, *in situ* butanol
7 adsorption improved the fermentation by alleviating butanol toxicity. However, butanol
8 production was not improved with CBV901, which gave similar butanol production (11.5 g/L)
9 and glucose utilization to those of the control, even though a significant amount of produced
10 butanol was also adsorbed, suggesting that the hydrophobic CBV901 was toxic to cells and not
11 compatible with the fermentation for *in situ* butanol adsorption.

12 The reproducibility of the fermentation performance with the regenerated Norit ROW 0.8
13 was also evaluated in serum bottles, and the results showed that consistent fermentation results
14 were obtained in all five cycles (see Table 4), indicating that the heat-regenerated AC could be
15 reused with stable adsorption performance.

16 3.5 Fermentation with butanol adsorption in bioreactor

17 ABE fermentation was studied with free cells of *C. acetobutylicum* JB200 in a stirred-tank
18 bioreactor with pH controlled at ~5.0. With the medium initially containing ~85 g/L of glucose,
19 18.3 g/L butanol was produced in ~54 h (Fig. 5A), giving a butanol productivity of ~0.34 g/L·h
20 and yield of 0.22 g/g glucose consumed. Fed-batch fermentation was then studied with
21 adsorption through an external column of activated carbon. Butanol adsorption was initiated by
22 circulating the broth between the fermentor and adsorption column when butanol concentration
23 in the broth had reached ~10 g/L. Concentrated glucose solution was pulse-fed to the bioreactor

several times until the fermentation stopped at ~106 h when the butanol concentration in the fermentation broth reached 18.7 g/L (**Fig. 5B**). Thermal desorption by heating the activated carbon column at 200 °C resulted in a solution containing 167.1 g/L butanol, 20.5 g/L acetone, 2.8 g/L ethanol, 3.7 g/L acetic acid and 0.9 g/L butyric acid with a total volume of 112.5 ml. The total amount of butanol obtained from desorption was 18.8 g butanol, which was only ~80% of the butanol (~23.5 g) adsorbed in the column based on the estimation from the adsorption isotherm. The less than 100% recovery of butanol from thermal desorption was due to loss in collecting the condensate in the system. Thus, ~16.1 g/L butanol produced in the fermentation was adsorbed. With adsorption, the total butanol production increased ~90% due to alleviated butanol inhibition, but butanol yield and productivity were similar to those of the control without adsorption (see **Table 5**). The fermentation stopped due to the high level of butanol in the fermentation broth accumulated and not effectively removed by adsorption, due to the saturation of activated carbon with butanol by ~80 h as indicated by the rapid increase in butanol and acetone in the broth.

The fermentation was then studied with cells immobilized in the cotton cloth packed inside the bioreactor, initially operated in a repeated batch mode without adsorption (**Fig. 6A**), followed with adsorption operated in a fed-batch mode (**Fig. 6B**). In the repeated batch fermentation, the fermentation broth was drained and replaced with fresh medium at the end of each batch to start a new batch with cells already immobilized in the bioreactor. Consistent fermentation performance with 16–18 g/L butanol produced from ~77 g/L glucose consumed in ~48 h, with a butanol yield of ~0.21 g/g glucose and productivity of ~0.34 g/L·h, was obtained in all three batches, which were similar to those from the free-cell fermentation (see Table 5). In the subsequent fed-batch fermentation with activated carbon adsorption, the broth was circulated

between the fermentor and the adsorption column when the butanol concentration had reached ~10 g/L. The adsorption column was replaced with a new one containing fresh (regenerated) activated carbon when butanol concentration in the broth exceeded ~10 g/L. A total of three columns of activated carbon (connected at 170, 195.5 and 216.5 h, respectively) were used for butanol adsorption in the fed-batch fermentation, which kept the butanol concentration in the fermentation broth below ~10 g/L and allowed the fermentation to continue without butanol poisoning. However, the fermentation gradually slowed down to eventually halt at a high acetone concentration of ~18 g/L. The selective adsorption of butanol by activated carbon resulted in the accumulation of acetone, which became toxic and limited the ABE fermentation. Similar finding has also been reported ([Liu et al., 2014](#)).

Based on the adsorption isotherm (Fig. 3) and butanol concentration in the fermentation broth, the total amount of butanol adsorbed on the activated carbon was estimated to be ~73.0 g, suggesting that ~45.7 g/L butanol produced in the fed-batch fermentation were adsorbed. Thus, a total of ~54.6 g/L butanol was produced from 260 g/L glucose in 122 h in the fermentation, with an overall butanol yield of ~0.22 g/g and productivity of ~0.45 g/L·h. Compared to the control without adsorption, butanol adsorption with activated carbon increased total butanol production by ~230% and productivity by ~32% in the immobilized-cell fermentation (see Table 5). It is thus clear that activated carbon adsorption for *in situ* removal of butanol from the fermentation broth can greatly improve ABE fermentation. However, the process may be limited by acetone accumulation as activated carbon had a relatively low acetone adsorption capacity. One solution to this acetone inhibition problem is to use a *Clostridium* strain such as the engineered *C. tyrobutyricum* that does not produce acetone ([Du et al., 2015](#); [Yu et al., 2011](#)).

3.6 Comparison to other studies

Adsorption for *in situ* butanol recovery from butanol fermentation has been studied as an effective method to alleviate butanol toxicity and thus increase substrate utilization and butanol production in ABE fermentation (Groot et al., 1992; Qureshi et al., 2005; Staggs and Nielsen, 2015). Groot and Luyben (1986) first studied the effect of several adsorbents on fermentation by *C. beijerinckii* LMD 27.6 and found that only the polymeric resin XAD-8 increased butanol production to ~8.5 g/L (vs. 5.5 g/L for the control), while activated carbon Norit W52 and other polymeric resins had more acids but less butanol production (see **Table 6**), probably because adsorption of acids allowed cells to continue acidogenesis without shifting to solventogenesis. Yang et al. (1994) used polyvinylpyridine (PVP) ion exchange resin (Reillex 425) to adsorb butanol in batch fermentation with *C. acetobutylicum* and reported that total ABE production increased 54% (from 21 g/L to 29.8 g/L) and productivity increased 130% to 0.92 g/L·h when the fermentation broth contained ~30% (w/w) of the adsorbent, compared to the control without the adsorbent. In this study, the total butanol production increased to 14.1 g/L–17.5 g/L (vs. 11 g/L) with 5%–30% of the resin for *in situ* butanol adsorption. Later with the PVP resin in a packed column for butanol adsorption, a total of 27.7 g/L butanol at a productivity of 0.99 g/L·h was attained in repeated fed-batch fermentation with cell recycle (Yang and Tsao, 1995). Although these studies demonstrated the feasibility and benefits of adsorption in the ISPR for ABE fermentation, the adsorbents used had a low adsorption capacity ($q < 0.1$ g/g). More recently, Nielsen and Prather (2009) screened a variety of commercial resins and found that Dowex Optipore SD-2 had a high butanol adsorption capacity ($q = 0.266$ – 0.403 g/g resin) and the addition of 5% (w/v) SD-2 in the ABE fermentation by *C. acetobutylicum* ATCC 824 increased butanol production to 22.2 g/L, a 86% increase compared to the control without SD-2. However, adding more SD-2 in the fermentation was not beneficial and showed negative effect due to the

adsorption of glucose and other nutrients present in the medium. Similarly, butanol production by ATCC 824 increased ~124% to 27.1 g/L with butanol adsorption using an expanded bed of Dowex Optipore L-493 (Wiehn et al., 2014). Liu et al. (2014) used a macroporous cross-linked polystyrene resin KA-I for butanol adsorption in a fixed bed column connected to a biofilm reactor, and fed-batch fermentation with *C. acetobutylicum* B3 produced 38.2–58.3 g/L butanol with a butanol yield of 0.19–0.20 g/g glucose. The higher production was achieved when both butanol and acetone were adsorbed, while the lower butanol production was limited by acetone inhibition. Butanol production was further increased to 92.6 g/L when methyl viologen (MV) was added to modulate the redox. MV as an artificial electron carrier can inhibit hydrogen production and thus increase NADH availability for butanol production (Du et al., 2015).

In the present study, we used activated carbon Norit ROW 0.8 in a packed column for butanol adsorption in fed-batch fermentation, which gave a total butanol production of 54.6 g/L, comparable to those obtained with KA-I and higher than those with PVP, Dowex SD-2, and L-493 (see Table 6). It should be noted that Norit ROW 0.8 has a much higher butanol adsorption capacity than that of KA-I and PVP (see Table 6). Unlike KA-I and PVP, Norit ROW 0.8 also has a low affinity with acetic and butyric acids. Adsorption of these acids not only would reduce butanol adsorption capacity, but also could decrease butanol production (Groot and Luyben, 1986) and result in a butanol product containing undesirable acids after desorption. Furthermore, activated carbon is also the most economical adsorbent with a much lower price (less than \$5/kg) compared to polymeric resins (>\$100/kg) and zeolites (~\$250/kg). In addition, AC could detoxify plant biomass hydrolysates containing inhibitors, including furfural, organic acids, and phenolics, derived from degradation of sugars (glucose and xylose) and lignin in the pretreatment process (Zhang et al., 2016) and greatly increase butanol production in ABE fermentation (Liu et

al., 2015; Lu et al., 2013). These inhibitors are usually present at low concentrations (< 1 g/L) and should not interfere with butanol adsorption, which would require experimental verification in future studies.

Butanol can be desorbed from the adsorbent by heating, stripping, or displacement (Oudshoorn et al., 2012). Butanol adsorbed on PVP and KA-I resins was recovered by washing with methanol, resulting in a six-fold concentrated butanol solution (Lin et al., 2012). However, resins must be further regenerated by washing with water. Vacuum was used to recover 81% butanol from Dowex L-493, resulting in a condensate containing 85.8 g/L butanol (Wiehn et al., 2013). Butanol recovery from Dowex SD-2 via thermal treatment at 100 °C had a recovery rate of ~84% and the desorbed phase after condensation contained >107 g/L butanol (Nielsen and Prather, 2009). In the present study, butanol desorption from AC at 200 °C gave a high butanol concentration of ~167 g/L in the condensate, which was far exceeding *n*-butanol solubility, ~7.7% (w/w) in water at 20 °C, and spontaneously formed two phases with the upper organic phase having a high butanol concentration of ~640 g/L. Clearly, thermal desorption is effective for the regeneration of and butanol recovery from activated carbon.

In general, butanol adsorption with AC is competitive with other novel separation methods, including gas stripping, pervaporation and solvent extraction (Groot et al., 1992; Oudshoorn et al., 2009b; Qureshi et al., 2005; Xue et al., 2014a). Energy requirements (kJ/g butanol) for butanol recovery from dilute solution (~1.0% butanol) by various separation methods are compared in supplemental **Table S2**. The advantages and disadvantages of adsorption vs. other methods have been discussed in details in a recent review paper (Xue et al., 2014a). One advantage for adsorption is its relatively fast adsorption and desorption processes. The adsorbent could quickly remove ABE from the fermentation broth in a single pass. In contrast, gas

stripping, pervaporation, and extraction are slower processes, requiring a large volume of gas or extractant for stripping (extraction) in multiple passes and a large contacting surface area between the two phases for mass transfer. Gas stripping and pervaporation also require a sub-zero temperature for condensing ABE vapors. On the other hand, adsorption requires a desorption step for adsorbent regeneration, and direct contact of fermentation broth with the adsorbent could cause fouling and other operation difficulties. Both adsorption and gas stripping have a moderate selectivity (against water) of ~15 and energy requirement of ~20 kJ/g butanol. The selectivity of adsorption can be improved by screening and selecting from a large pool of adsorbents, while the selectivity of gas stripping is limited by gas-liquid equilibrium. The high cost of adsorbents and additional equipment (capital) cost have hindered the commercial application of adsorption in ABE fermentation, which, however, could be largely alleviated by choosing relatively inexpensive activated carbon such as the one used in this study. In summary, ABE fermentation with *in situ* butanol adsorption by activated carbon offers an energy-efficient and economically competitive process for biobutanol production.

4. Conclusions

Various adsorbents were screened and activated carbon was found to have the best specific loading and partitioning coefficient for butanol. Fed-batch fermentation with *in situ* adsorption by activated carbon produced 54.6 g/L butanol at a productivity of 0.45 g/L·h, increasing by 230% and 32%, respectively, compared to the control without adsorption. Thermal desorption produced a condensate containing ~167 g/L butanol, which was concentrated to ~640 g/L (80%, v/v) after phase separation. This ISPR process with a low energy consumption of ~14.1 kJ/g butanol exhibits great potential for economical biobutanol production.

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Figure Captions

Figure 1. Specific loadings and partition coefficients of various adsorbents for butanol in a screening test using a model solution containing 10 g/L butanol.

Figure 2. Equilibrium isotherms of *n*-butanol with Norit ROW0.8, CBV901, Dowex L-493 and Dowex SD-2 at 37 °C (A) and 60 °C (B). Data are fitted with the Langmuir model. C. Equilibrium isotherms of *n*-butanol and acetone with Norit ROW0.8 in a simulated ABE fermentation broth at 37 °C.

Figure 3. Dynamics of continuous adsorption in a packed column of activated carbon with butanol solution (A) and a model solution containing ABE and acetic and butyric acids (B).

Figure 4. Thermogravimetric desorption of *n*-butanol, water, and ABE mixture (3:6:1), respectively, from activated carbon. Curves show percentage weight changes in mass adsorbed (relative to the initial weight) as temperature increased from 25 to 250 °C.

Figure 5. Kinetics of ABE fermentation of *C. acetobutylicum* JB200 at 37 °C. A. Batch free-cell fermentation without adsorption (control); B. Fed-batch free-cell fermentation with adsorption. An activated carbon column was connected to the fermentation at 30 h when the butanol concentration in the fermentation broth was ~10 g/L

Figure 6. Kinetics of ABE fermentation of *C. acetobutylicum* JB200 at 37 °C. A. Repeated-batch immobilized-cell fermentation without adsorption (control); D. Fed-batch immobilized-cell fermentation with adsorption. Fresh or regenerated activated carbon column was connected to the fermentation at 170, 195.5 and 216.5 h, respectively.

Table 1. Langmuir isotherm parameters for butanol adsorption from least-squares regression

Adsorbents	37 °C		60 °C	
	K (g/L)	q_m (g/g)	K (g/L)	q_m (g/g)
Norit ROW 0.8	1.72	0.48	2.01	0.45
CBV901	1.80	0.24	1.11	0.21
Dowex L-493	4.02	0.36	4.02	0.33
Dowex SD-2	4.32	0.38	4.93	0.31

Table 2. Estimation of heat required for desorption of butanol from activated carbon

Species (amount)	C_p (J/g·K)	ΔH_{evap} (J/g)	Sensible heat (J/g) ¹	Total energy (J/g)	Total heat for desorption (J/g butanol) ²
Butanol (1 g)	2.39	585	389.57	974.57	975
Water (4.8 g)	4.18	2258	367.84	2625.84	12,604
AC (4 g)	0.84	-	136.92	136.92	548
Total heat per gram of butanol desorbed by thermal desorption:					14,127

1. Sensible heat for heating water from 37 to 125 °C and butanol and AC from 37 to 200 °C.
2. One gram of AC would adsorb ~0.25 g butanol (based on $q = 0.25$ g/g) and ~1.2 g water (based on 167 g/L butanol in the desorbed phase obtained after condensation). Therefore, for each gram of butanol desorbed, 4.8 g water would also be desorbed from 4 g AC.

Table 3. Comparison of *n*-butanol production in ABE fermentation with *in situ* butanol adsorption by various adsorbents in serum bottles

	Control	Norit ROW 0.8	L-493	SD-2	CBV901
Initial glucose titer (g/L)	71.5	72.5	70.5	69.0	71.0
Final glucose titer (g/L)	28.5	1.2	24.5	20.4	30.1
Butanol titer (g/L)	11.7	4.1	4.8	5.2	4.5
Final pH	4.85	4.60	4.25	4.39	4.56
Total butanol production (g/L) ^a	11.7	21.9	14.5	15.2	11.5
Butanol yield (g/g)	0.27	0.31	0.31	0.31	0.28

^aTotal butanol production including butanol remained in the fermentation broth and adsorbed, which was estimated from the final butanol concentration in the fermentation broth and the adsorption isotherm.

Table 4. Reusability of Norit ROW 0.8 in ABE fermentation with *in situ* butanol adsorption in serum bottles

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Initial glucose titer (g/L)	72.5	69.0	71.0	73.5	69.0
Final glucose titer (g/L)	1.2	2.6	0.6	5.2	1.0
Final butanol titer in broth (g/L)	4.1	3.5	4.8	3.3	4.9
Amount of butanol adsorbed (g)*	0.78	0.74	0.84	0.72	0.84
Sp. loading (g/g)*	0.196	0.185	0.209	0.181	0.211

*Specific loading was estimated from the final butanol concentration in the broth and the adsorption isotherm (Fig. 2C), and then used to estimate the amount of butanol adsorbed on AC in the fermentation.

Table 5. Comparison of *n*-butanol production in ABE fermentation in bioreactor with or without *in situ* butanol adsorption by activated carbon

	Free cell fermentation		Immobilized cell fermentation	
	w/o adsorption	w/ adsorption	w/o adsorption	w/ adsorption
Fermentation time (h)	54	106	47–48	122
Total glucose consumed (g/L)	82	158	76–77	260
Butanol in fermentation broth (g/L)	18.3	18.7	16.0–18.6	8.9
Total amount of butanol adsorbed (g)	-	18.8–23.5 ^a	-	53.8–92.2 ^b
Butanol removed from fermentation broth (g/L)	-	12.9–16.1	-	~45.7
Total butanol production (g/L)	18.3	31.6–34.8	16.0–16.6	~54.6
Butanol yield (g/g)	0.22	0.20–0.22	0.21–0.22	~0.22
Butanol productivity (g/L·h)	0.34	0.30–0.33	0.33–0.35	~0.45

^a The lower value is based on the total amount of butanol recovered from desorption, while the higher value is based on the assumption that only 80% of the adsorbed butanol was recovered in desorption.

^b The higher value is based on the adsorption isotherm obtained with the pure butanol solution (Fig. 3A) and the lower value is based on the isotherm in the presence of glucose and acetone (Fig. 3C). The actual value should be about the mean of these two values or 73.0 g/L, which is used in the calculation of total butanol production, yield, and productivity.

Table 6 Performance summary of different adsorbents during butanol fermentation

Adsorbent	Sp. loading (g/g)	BuOH in fermenter (g/L)	Total BuOH production (g/L)	BuOH Productivity (g/L·h)	BuOH Yield (g/g)	BuOH in desorbed phase (g/L)	Reference / Comments
Norit W52	0.005–0.012	0.7–1.2	0.7–1.4	–	–	5.3–10	Groot & Luyben, 1986
XAD-2	0.002–0.030	0.1–4.2	0.3–6.8	–	–	5.4–44	
XAD-4	0.007–0.015	0.3–1.0	1.1–1.7	–	–	15–30	
XAD-8	0.022–0.030	4.7–5.1	7.3–8.6	–	–	29–33	
PVP	~0.05	10.5	17.5	0.31–0.54	0.19	–	Yang et al., 1994
		16.2	27.7	0.99	0.19	–	Yang & Tsao, 1995
Dowex SD-2	0.26–0.40	3.4	22.2	0.31	0.16–0.24	107–169	Nielsen & Prather, 2009
Dowex L-493	~0.3	12.1	27.1	0.48	0.19	85.8	Wiehn et al., 2014
KA-I	0.084–0.093	>6.5	38.2–58.3	0.67–0.91	0.19–0.20	–	Liu et al., 2014 MV addition
			92.6	0.69	0.25	–	
Norit ROW0.8	0.25	8.9	54.6	0.45	0.22	167	This work

KA-I: Cross-linked polystyrene diethylbenzene; PVP: Polyvinylpyridine; –: Data not available

Figure 1

Figure 2

A

B

Figure 3

Figure 4

Figure 5

Figure 6

Graphical Abstract

An energy efficient process was developed for butanol production in acetone-butanol-ethanol fermentation with cells immobilized in a fibrous matrix attached to the inside wall of the stirred-tank bioreactor and butanol adsorption with activated carbon to alleviate butanol toxicity.