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Intensification of biobutanol production in batch oscillatory baffled bioreactor

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Abstract

Biobutanol is a high value biofuel and potentially a better fuel extender than ethanol. The market demand is expected to increase dramatically, if biobutanol can be produced economically via the ABE (acetone, butanol ethanol) fermentation. One way to improve the ABE yield and productivity is through intensification of the fermentation process. In this work, a novel intensified bioreactor called the oscillatory baffled bioreactor (OBB) was evaluated for this process. Batch ABE fermentations were conducted in OBBs at various “oscillatory Reynolds numbers” in the range 0 to 1870 and in stirred tank reactors (STRs) at various stirrer speeds in the range 0 to 200 rpm. The fermentations were performed anaerobically using clostridia strain *Clostridium* GBL1082 at constant temperature. In the OBB, the highest ABE yield achieved was 0.35 g/g (of g ABE produced per g glucose used) which was comparable to yield achieved in the STR. The highest productivity achieved in the OBB was 0.22 g/L/h, 38% higher than the maximum achieved in the STR. It was also observed that use of the highest mixing intensities (for both OBBs and STRs) within the studied range resulted in higher acid concentration, suggesting that acids were not reassimilated efficiently. It is likely that there is a balance between achieving uniform suspension of the bio-fluid and the adverse effects of shear in both bioreactors.

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Keywords: Acetone-butanol-ethanol fermentation; process intensification; oscillatory baffled bioreactor

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1. Introduction

Butanol has significant potential as a ‘next generation’ biofuel due to its superior technical properties such as hydrophobicity, high energy content, and ease of storage and transportation, compared to ethanol. Recent interest in butanol fermentation via the “acetone-butanol-ethanol (ABE)” fermentation has led to re-assessment of ABE fermentation with the aim to improve solvent yield, volumetric productivity and final solvent concentration. Various strategies have been implemented, includes the use of inexpensive carbon source, i.e. sago starch [1], corn steep liquor and corn molasses, state-of-the-art fermentation techniques, i.e. cell recycling and bleeding [2], immobilized cell [3], co-culture fermentation [4] and also economical solvent recovery methods [5].

This ABE fermentation performed by *Clostridium* species can be divided into two distinct phases, acidogenic and solventogenic phases. Acetic acid and butyric acid were produced during the acidogenic phase, and after that acetone, butanol and ethanol were produced during solventogenic phase. In general, the biphasic metabolism of *Clostridium* species is directly associated with cell growth status. Exponentially growing cells mainly produce acids, while solvent is usually produced when cells enter the late exponential phase [6]. If solvent production could be initiated in early exponential phase, the solvent productivity might be increased as the fermentation time would be shortened. In industry, established fermentation technologies are using several parallel static vessels in staggered sequence to provide a steady flow of fermentation broth and to overcome inhibition [7]. This requires a huge floor space to accommodate multiple process equipment which leads to increase in capital cost. In addition, several intrinsic problems (such as low solvent yield, expensive raw material and costly recovery process) also contribute to the incompetence of this fermentation compared to the chemical route.

Oscillatory baffled bioreactor (OBB) might offer an intensification approach to the ABE fermentation. Ni *et al.* [8] suggested that this bioreactor can be applied to bio-processing due to its efficient yet gentle mixing and improved mass transfer, in their case they using the reactor for ethanol fermentation from *Saccharomyces cerevisiae*. The OBB niche application is converting long reactions (such as fermentation) from batch to continuous reactor. Its greatly reduced length-to-diameter ratio in comparison to conventional tubular reactors makes it more practical for performing long reactions i.e. ABE fermentation in continuous plug flow mode. The objective of this study was to examine the application of OBB with respect to its ability to perform ABE fermentation in comparison with STR in batch fermentation using 5% glucose as the carbohydrate source.

Nomenclature

ABE	acetone-butanol-ethanol
CDW	cell dry weight (g/L)
BOBB	batch oscillatory baffled bioreactor
STR	stirred tank reactor
Re_o	oscillatory Reynolds number
St_r	Strouhal number
D	column diameter (m)
D_o	orifice diameter (m)

H	BOBB height (m)
L	space between each baffle plate
ρ	fluid density (kg/m ³)
μ	fluid viscosity (kg/m. s)
x_o	oscillation amplitude (m)
f	oscillation frequency (1/s)

1.1. Oscillatory Baffle Bioreactor

Oscillatory Baffled Bioreactor (OBB) is a unique design of plug flow bioreactor consisting of a column fitted with series of orifice baffle plates mounted transverse to the flow and equally spaced. The key feature of OBB is the interaction of baffles and a periodically reversing flow in a column. The fluid accelerates and decelerates as a result of the oscillation continually forming short-lived vortices due to the interaction with the baffles. Each inter-baffle region act as a continuous stirred tank in which the radial velocity components are comparable to the axial ones [9]. The dynamic nature of batch oscillatory flow in a baffled column was affected by frequency and amplitude of the oscillation. These were characterized by dimensionless oscillatory Reynolds number (Re_o) and Strouhal number (St_r), defined below:

$$Re_o = \frac{2\pi f x_o D}{\mu} \quad (1)$$

$$St_r = \frac{D}{4\pi x_o} \quad (2)$$

where D is the column diameter (m), ρ the fluid density (kg/m³), μ is the fluid viscosity (kg/m/s), x_o is the oscillation amplitude (m) and f is the oscillation frequency (1/s). The Re_o describes the mixing rate applied to the column while the St_r is the ratio of column diameter to stroke length, indicates the effectiveness of eddy propagation [9].

2. Materials and methods

2.1. Fermentation set-up in oscillatory baffled bioreactor

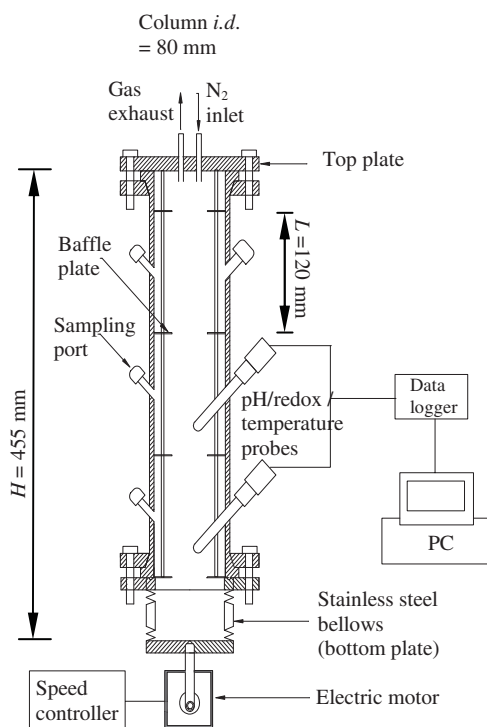


Fig. 1. Schematic diagram of batch oscillatory baffled bioreactor (BOBB) setup

A schematic diagram of the batch oscillatory baffled bioreactor (BOBB) setup is shown in Fig. 1. The bioreactor used for the experiments was installed vertically and consisting of an 80 mm *i.d.* glass column with a wall thickness of 10 mm and total height, $H = 455$ mm. Baffle train consist of three orifice-type plates which arranged at equal space between each plates, $L = 120$ mm with orifice diameter, $D_o = 40$ mm. Two pieces of stainless steel rods with 3 mm *o.d.* were used to support baffle plates inside the glass column. A stainless steel bellows at the bottom end of the column was important as not only act as a bottom plate but also provide liquid oscillation inside the column. This was initiated by an oscillation system which driven by electrical motor. The rotating speed of electrical motor was controlled by a speed controller, able to provide an oscillation frequency up to 1 Hz and oscillation amplitude of 2 and 4 mm (measure from center-to-peak). Liquid temperature inside the BOBB was maintained at 32°C by a silicone tube coiled around the glass column and connected to circulate water bath. pH, redox and temperature readings were measured using Mettler Toledo InPro3253i combine pH electrode with temperature

compensation. Data collected (pH, redox and temperature) over the course of the fermentation were recorded by PICO data logger from Pico Technology.

2.2. Strain and culture condition

Clostridium GBL1082, obtained from Green Biologics Ltd. (Oxford, UK), was used in this study. A glycerol stock of *Clostridium* GBL1082 was cultivated in deoxygenated reinforced clostridial medium (RCM: yeast extract 3.0 g/L, peptone 10.0 g/L, meat extract 10.0 g/L, glucose 5.0 g/L, starch 1.0 g/L, agar 0.5 g/L, sodium chloride 5.0 g/L, sodium acetate 3.0 g/L and cystein hydrochloride 0.5 g/L) (Oxoid, UK). Ten percent (v/v) of activated cell (16-18 h culture) were then inoculated into fermentation media contain yeast extract 5.0 g/L, $(\text{NH}_4)_2\text{SO}_4$ 3.0 g/L, CaCO_3 5.0 g/L and glucose 50 g/L. Fermentations were conducted at 32°C in 2 L batch oscillatory baffled bioreactor (BOBB) at various oscillatory Reynolds numbers and 2 L stirred tank reactor (STR) at various stirrer speeds with 1.5 L working volume, respectively. The pH was initially adjusted to 6.5 with 3 M H_2SO_4 prior to inoculation. Oxygen-free N_2 gas was swept over the headspace of the reactor until the culture initiated production of its own gases (CO_2 and H_2). Samples were withdrawn at intervals for analysis.

2.3. Analytical procedures

Cell density was determined by measuring the optical density at 600 nm using UV-visible spectrophotometer (Jenway 6705). The cell dry weight (CDW) could be calculated using correlation between CDW and OD_{600} which was developed earlier, that is $\text{CDW (g/L)} = (0.407 \times \text{OD}_{600}) + 0.0014$. The concentration of acetone, butanol, ethanol, acetic acid and butyric acid were determined by gas chromatography (GC) analysis (5890; Hewlett Packard, USA). Two microliter samples were injected into a stainless steel column (2 by 2000 mm) packed with Porapak Q, 50/80 mesh. The column temperature was held at 150°C for 20 min, programmed at 15°C/min to 180°C with a 15 min final hold. The injector and detector temperature both were set at 250°C. A flame ionization detector (FID) was used for signal detection with helium as a carrier gas. Glucose concentration was determined using dinitrosalicylic acid (DNS) reagent [10]. The fermentation broth was centrifuge at 13,000 rpm for 10 min. One mL of the supernatant was mixed with 3 mL of 1% DNS reagent and incubated at 90°C for 10 min. After the mixture cooled to room temperature, the absorbance was measured at 540nm, using a Jenway UV-visible spectrophotometer. Standard solutions of anhydrous D-glucose containing 0.5–3 g glucose/L deionized water were prepared and 1 mL of each standard solution were mixed with 3 mL 1% DNS reagent and incubated 90°C for 10 min. A blank (deionized water) was incubated with the reagent and was used for zero adjustment of the spectrophotometer. The glucose concentration in the sample was compute by least squares linear regression, using a standard curve.

Productivity was calculated as total ABE produced (grams per liter culture volume) divided by fermentation time (h). Yield was defined as total grams ABE produced per total grams glucose utilized.

3. Results and discussion

Anaerobic fermentations of *Clostridium* GBL1082 were carried out in BOBB at an oscillatory Reynolds numbers (Re_o) 470. Solvent, acid production and the corresponding biomass and pH values during this batch fermentation can be seen in Fig. 2Fig.. Under this condition, biphasic fermentation was observed: acidogenic phase where acetic acid and butyric acid were accumulated mainly in the first 6 h; both acids were then reutilized, while solvents were produced during solventogenic phase. An absent of lag phase can be observed here as cells rapidly went into the exponential growth phase before reaching the stationary phase after ~ 24 h fermentation (Fig. 2, a1). The maximum biomass of 1.54 g/L was observed at 54 h. It is interesting that an earlier initiation of solventogenic phase happened here, as solvents were produced at the same time as acetic acid and butyric acid (Fig. 2, a2). In the middle of the solventogenic phase, the biomass concentration starts to decrease (at 54 h), suggesting cell death occurred owing to the lack of nutrients and/or unfavourable environment due to accumulation of metabolite in the media. At the end of the fermentation, glucose was nearly fully utilized with only 7 g/L left in the medium. The highest titre of butanol was 6.7 g/L followed by acetone at 3.5 g/L and ethanol at 0.3 g/L, respectively. At the end of fermentation the acid concentration was 2.78 g/L.

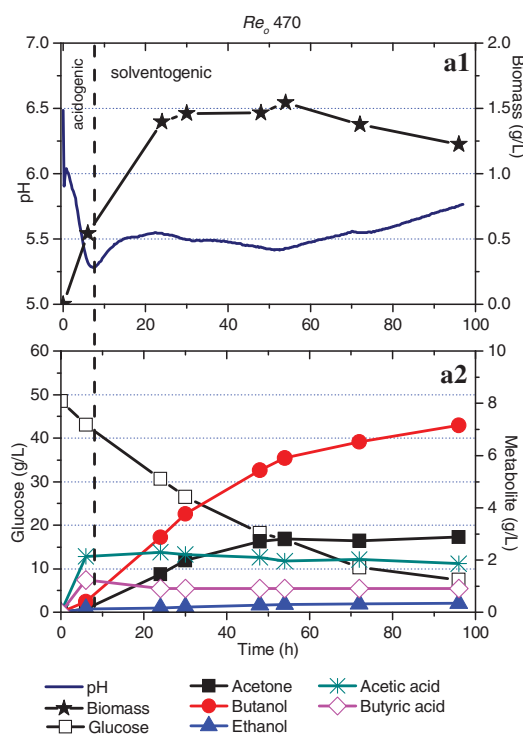


Fig. 2. Fermentation profiles of *Clostridium* GBL1082 in BOBB at Re_o 470. *Clostridium* GBL1082 was cultivated in growth media containing 5% glucose at 32°C with 1.5 L working volume

Table 1. Batch fermentation of *Clostridium* GBL1082 in BOBB at different oscillatory Reynolds number (Re_o)

BOBB mixing rate (Re_o)	Biomass (g/L)	Solvent concentration (g/L)	Acid concentration (g/L)	Butanol % from total solvent
0	1.85	12.82	2.13	67%
470	1.54	10.40	2.78	69%
938	1.52	10.04	2.66	67%
1870	1.33	4.48	4.50	77%

In order to examine the effect of mixing rate in BOBB, a series of batch fermentation experiment were conducted at different Re_o (0-1870) which indicates the mixing rate inside BOBB. Table 1 shows the batch fermentation data in BOBB at different Re_o . The data represent herein the average of duplication determination. As the mixing rate increased (depicted by an increase in Re_o), it negatively affect the biomass and solvent concentration. At Re_o 1870, the biomass concentration decreased about 28% from the highest obtained here (at Re_o 0). In addition, total solvent concentration was also decreased to 4.48 g/L that is 65% reduction from the highest concentration obtained at Re_o 0 (12.8 g/L). As a consequence of low solvent production at Re_o 1870, high acid concentration (4.50 g/L) was accumulated in the broth as most acid did not converted to solvent. It can also be observed here that over the course of the fermentation, BOBB at Re_o 0 produced the highest level of butanol yield, ranging between 0.20 - 0.26 g butanol/g glucose (g/g) as shown in Fig. 3. This was followed by Re_o 470 and 938, with butanol yield between 0.15 – 0.17 g/g. In the mean time, the butanol productivity for Re_o 0, 470 and 938 were at similar level, ranging between 0.10 -0.13 g/L/h during the first 48 h of fermentation before dropping to the lowest level of 0.07 g/L/h when reaching the end of fermentation time (Fig.). Interestingly, at the higher Re_o , there was a substantial increase in butanol percentage with the highest percentage of 77% of butanol produced at Re_o 1870 (Table 1). It is likely that the highest mixing rate caused changes in the microorganism metabolism pathways and resulted in a favourable carbon flow to butanol production instead of acetone and ethanol. Unfortunately, these mixing conditions do not favour cell growth nor the metabolic shift from acidogenic to solventogenic phase, so at high Re_o the final butanol concentration is low with only 3.46 g/L. It has been shown that increased in shear stress induces morphological variations and causes cell lysis [11]. This may explain the low solvent yield and productivity at high Re_o .

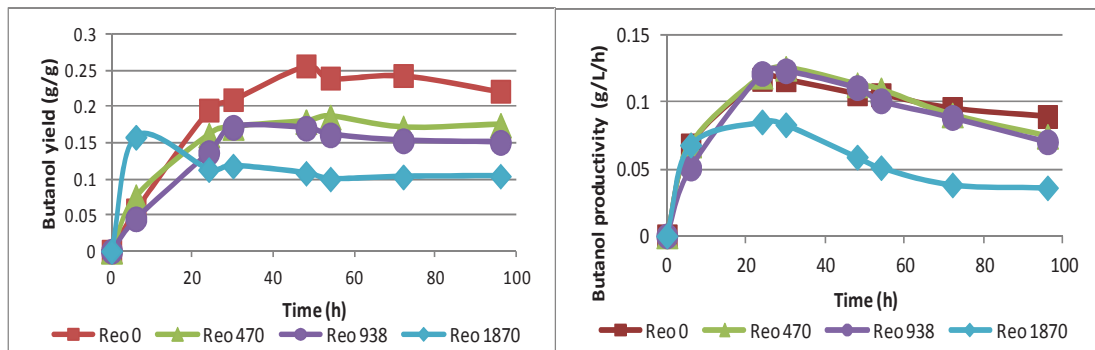


Fig. 3. Butanol yield and productivity of batch fermentation in oscillatory baffled bioreactor (BOBB) at different Re_o .

Table 2. Batch fermentation data of *Clostridium* GBL1082 carried out in BOBB and STR at different mixing rate

Bioreactor/ Oscillatory Reynolds numbers/ Stirrer speeds	Total solvent (g/L)	ABE Productivity (g/ L/h)	ABE yield (g/g) ^c	Power density (W/m ³)
BOBB				
Re_o 0	7.81	0.16	0.35	0
Re_o 470	9.02	0.18	0.28	0.0161
Re_o 938	6.49	0.22	0.30	0.1043
Re_o 1870	3.00	0.12	0.17	0.8343
STR				
0 rpm	11.20	0.16	0.24	0
50 rpm	7.35	0.14	0.33	0.8075
100 rpm	5.79	0.08	0.35	6.4602
200 rpm	8.10	0.15	0.27	51.6815

Table 2 shows the data summary for batch fermentation of *Clostridium* GBL1082 in two different bioreactor systems, BOBB and STR. It is been observed here in the BOBB, that the solvent concentration decreased as the mixing rate increased (represent by increased in Re_o value) and there was no particular trend following the solvent productivity and its yield. While in STR, there was no exact trend can be observed for all fermentation data represent here. The highest solvent productivity was achieved in BOBB at Re_o 938 with 0.22 g/L/h producing about 6.49 g/L of total solvent. This solvent productivity was considered as an average achieved in general batch ABE fermentation as reported elsewhere, ranging between 0.2-0.3 g/L/h. Nevertheless it is 38% higher than the maximum achieved in the STR. Higher solvent productivity in BOBB is likely to be due to early initiation of solventogenic phase, eventually accumulating more solvent earlier than the STR. It was also observed here that the highest total solvent yield was achieved in the BOBB at Re_o 0 with 0.35 g ABE/ g glucose (g/g) which also be achieved in STR at 100 rpm.

In order to compare the fermentation between these two bioreactor systems, it is essential to compare the metabolites produced on the basis of power consumed per unit volume (power density). Herewith, power density (W/m³) was calculated for these two bioreactors: BOBB power density was determined using a quasi steady equation suggested by Hewgil *et. al.* [12] and STR as defined by Holland and Chapman [13]. For the fermentation data represent in Table 2 here, BOBB mostly had lower power density than STR, with the only comparable power density at 0.8 W/m³, calculated at the highest mixing rate in BOBB but it was the lowest mixing rate in STR.

4. Conclusion

Butanol was produced in a novel batch oscillatory baffled bioreactor (BOBB) in comparison with a conventional reactor, stirred tank reactor (STR). The maximum productivity of the BOBB was 0.22 g/L/h (38% higher) as compared to 0.16 g/L/h in the STR. In addition, a comparable solvent yield with the STR was achieved in BOBB with 0.35 g/g. Interestingly, at similar power density the percentage of butanol produced in BOBB was always higher than in STR, suggesting more carbon flows along the butanol production pathway rather than ethanol or acetone. Butanol production in BOBBs proved to be feasible as

it is not only able to compete with conventional reactor (STR) but also offers advantages of straight-forward scale-up, whereas it is complicated and unpredictable in STRs. In addition BOBB may offer potential for “whole process intensification” via integration of separation process, as the reactor must not be treated in isolation.

Acknowledgements

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