Evaluation of biobutanol production from non-pretreated rice straw hydrolysate under non-sterile environmental conditions

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HIGHLIGHTS

▶ Producing biobutanol under non-sterile environmental condition is feasible.
▶ High cell concentration ensures a reproducible butanol production under non-sterile condition.
▶ The order of sugar preference during fermentation is as glucose > galactose > arabinose.

ARTICLE INFO

ABSTRACT

The study aims to investigate a cost-effective approach to convert non-pretreated rice straw hydrolysate into biobutanol. The influences of the initial cell concentration and incubation temperature on biobutanol production were evaluated under both sterile and non-sterile conditions. Results indicate that 100% glucose utilization could be achieved for initial cell concentrations greater than 2100 mg/L under both sterile and non-sterile conditions. Regression analyses resolve that under the sterile condition, the maximum butanol productivity of 1.45 g/L/d was projected at 1.96 g/L of cells and 32.3 °C, while the maximum butanol yield of 0.22 g/g was predicted at 2.01 g/L of cells and 26.3 °C. These two maximum values could not be projected by the regression analyses for the non-sterile condition. However, this study confirms that a high initial cell concentration of *Clostridium saccharoperbutylacetonicum* N1-4 can minimize interference from other microbes so that non-sterile biobutanol production is comparable to sterile biobutanol production.

1. Introduction

Rice is one of the major crops grown in Taiwan. According to data published by the Council of Agriculture, Executive Yuan in the Agricultural Statistics Yearbook 2009, the total area of rice fields in Taiwan is 254,590 ha, and 6.2 tons of rice can be harvested per hectare. The harvest of rice leads to significant production of agricultural residues, mainly rice straw. These residues can be recycled as an economical and environmentally friendly renewable resource by converting them to biofuels using available biological processes (Demain, 2009). At present, ethanol fermentation and ABE (Acetone–Butanol–Ethanol) fermentation are the mature biotechnologies converting fermentable sugars into bioethanol and biobutanol, respectively. Comparing these two biofuels via a

life-cycle assessment (LCA), Swana et al. (2011) found that the advantage of biobutanol over bioethanol is its net energy return. Their results showed that corn grain-to-butanol conversion yielded a net energy return of 6.53 MJ/L if the butanol was purified only during distillation. The net energy return associated with corn grain-to-ethanol conversion was only 0.40 MJ/L.

Butanol is the major chemical end product in ABE fermentation, and the fermentation reaction requires a carbon source to act as an electron acceptor and donor to proceed. A high carbohydrate concentration (e.g., glucose), which provides both the necessary carbon source and energy for the microbial species, is critical to ensure successful ABE fermentation (Dabrock et al., 1992; Karakashev et al., 2007). ABE fermentation is an anaerobic process that produces three major classes of products: solvents (acetone, butanol, and ethanol), organic acids (acetic acid, butyric acid, and lactic acid), and gases (CO_2 and H_2) (Mariano et al., 2012). The total solvent concentration obtained in traditional batch fermentation is approximately 20–30 g/L (Karakashev et al., 2007; Qureshi and Blaschek, 2001). However, adding supplements of electron shuttle compounds, which cycle between oxidized and reduced states in

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redox reactions, can modify the fermentation pattern and increase biobutanol production (Tashiro et al., 2007). Most recently, Yarlagadda et al. (2012) reported that the addition of methyl viologen during fermentation increased butanol production 12-fold but significantly decreased hydrogen and organic acid production.

In ABE fermentation, the high substrates cost due to the use of carbohydrate substrates decreases the economic viability of ABE fermentation compared with fossil fuels. The cost of converting raw material into carbohydrate makes up approximately 60% of the overall cost of ABE fermentation (Qureshi and Blaschek, 2000). Additionally, traditional ABE fermentation must be carried out under sterile conditions to avoid contamination, limiting its cost effectiveness. This limitation can be solved by a recently developed membrane separation technology that removes contaminant microbes out of the feedstock (Lipnizki, 2010). This technology implements a microfiltration membrane with pore sizes ranging from 0.1 to 0.4 μm to separate cells and other solids from the mixed liquor (Metcalf and Eddy, 2004). Furthermore, the enzymatic hydrolysis of lignocellulosic biomass is typically performed at 45 °C (Abedinifar et al., 2009) or 50 °C (Wang and Chen, 2011). Carrying out this process at this temperature inhibits mesophilic anaerobic microbes so that they will not interfere with ABE fermentation. Based on this information, the technical viability of biobutanol production should be re-evaluated given the possibility of effective non-sterile ABE fermentation. The purpose of this study is to investigate a cost-effective approach to convert non-pretreated rice straw (NPRS) hydrolysate into biobutanol. The feasibility of performing ABE fermentation under non-sterile conditions was evaluated by comparing production results to those obtained under sterile conditions. The optimal initial cell concentration and incubation temperature were determined using a central composite design (CCD) in conjunction with response surface methodology (RSM) for projecting the maximum potential of biobutanol production.

2. Methods

2.1. Culture development

Clostridium saccharoperbutylacetonicum N1-4 (ATCC 27021) was obtained from American Type Culture Collection (ATCC 27021), USA. Freeze-dried powder cells were activated in 250 mL peptone-yeast extract-glucose (PYG) medium anaerobically at 35 ± 1 °C and 1.7 Hz for 30 h. The PYG medium was composed of glucose (10 g/L), yeast extract (10 g/L), peptone (5 g/L), tryptone (5 g/L), cysteine–HCl (0.5 g/L), glutathione (0.25 g/L), K_2HPO_4 (2.04 g/L), KH_2PO_4 (0.04 g/L), $FeSO_4 \cdot 7H_2O$ (1.1 mg/L), $CaCl_2$ (0.008 g/L), $MgSO_4 \cdot 7H_2O$ (0.0192 g/L), NaCl (0.08 g/L), $NaHCO_3$ (0.4 g/L), and resazurin (1 mg/L). The activated cells were mixed with glycerol at a ratio of 7–3 (volume to volume basis) in a microtube and preserved in a −80 °C freezer as laboratory stock to be used at a later time. Each microtube contained 1 mL of a mixed solution of activated cells and glycerol.

Two microtubes (2 mL each) of the mixed active cell and glycerol solution were subcultured anaerobically in 500 mL of PYG medium under sterile conditions. After incubation in a shaker chamber at 35 ± 1 °C and 1.7 Hz (100 rpm) for 36 h, the cells were centrifuged, and the resulting pellets were collected and inoculated in ABE fermentation media. The subculturing process eliminates any residual glucose contained in the PYG medium.

2.2. Experimental design

A central composite design (CCD) in conjunction with response surface methodology (RSM) has been employed in many biological experiments. A CCD involves factorial points, axial points, and center points to describe a second-order response (Myers and Montgomery, 1995). Each point represents a single experimental condition or experimental run. In this study, ABE fermentation experiments were designed statistically according to a two-factor CCD. MINITAB® software (Version 15; LEAD Technologies, Inc.) was used to create this CCD and to analyze responses using response surface methodology. The total number of experimental runs can be determined using Eq. (1) (Cho and Zoh, 2007; Lu et al., 2008):

$$N = 2^K + 2K + n_c \tag{1}$$

where N is the total number of experimental runs, K is the number of independent variables (initial cell concentration and incubation temperature being the two variables in this study), and n_c is the number of center points. Three center points were evaluated to provide an internal estimate of the CCD error. Initial cell concentration, X_1 (640–2331 mg/L), and incubation temperature, X_2 (25–45 °C), were chosen as the two independent variables (factors) in the experimental design. An initial cell concentration of 1429 ± 214 mg/L and an incubation temperature of 35 °C were determined to be the center point. A total of 11 batch experiments, as listed in Table 1, were performed in this study.

2.3. Batch experiments

The ABE fermentation experiments were performed under sterile conditions (A group) and non-sterile conditions (B group). Corresponding runs in Group A and Group B, e.g., run 1 in Group A and run 1 in Group B, were performed under similar conditions except for the sterile/non-sterile condition. The experiments were conducted in 500-mL batch reactors, each containing centrifuged cells and synthetic NPRS hydrolysate. The NPRS is dried rice straw that has not undergone any dilute acid/base pretreatments before enzymatic hydrolysis. Our earlier study (data not published yet) showed that NPRS hydrolysate contains no inhibitory chemical compounds, only reducing sugars. Therefore, the NPRS hydrolysate was synthesized with 2.73 g/L arabinose, 28.10 g/L glucose, and 10.00 g/L galactose. A volume of 3.33 mL of nutrient solution, composed of NH_4HCO_3 (160 g/L), KH_2PO_4 (80 g/L), NaCl (0.4 g/L), $FeCl_2$ (0.28 g/L), $MgSO_4 \cdot 7H_2O$ (4.0 g/L), $Na_2MoO_4 \cdot 2H_2O$ (0.4 g/L), $CaCl_2$ (0.28 g/L), and $MnSO_4 \cdot H_2O$ (0.37 g/L), was added to the batch reactors. Then, the batch reactors were filled with acetate buffer solution (5000 mg/L) to achieve a final working volume of 500 mL. The acetate buffer was added to mimic the NPRS hydrolysate solution. To ensure anaerobic conditions, the batch reactors were purged with nitrogen gas for 20 min. Then, 1.5 mL of 0.25 M Na_2S was injected into the medium to consume the remaining O_2 prior to beginning the ABE fermentation batch experiments. The batch reactors were incubated in a shaker chamber at a frequency of 1.7 Hz (100 rpm). The initial pH in the batch reactor was fixed at 5.42 ± 0.03. Samples were taken periodically to determine the cell concentration, pH, fermentation products, and residual sugar concentration.

2.4. Data analysis

The modified Gompertz equation, Eq. (2), is a sigmoid function; it has been implemented to statistically describe cumulative hydrogen production (Chen et al., 2006) and cumulative methane production (Chen et al., 2003). In this study, the cumulative butanol production curves with respect to time were first obtained from the butanol production experiments. Then, the modified Gompertz equation was employed to determine the butanol production potential (P), butanol production rate (R), and lag phase (I).

Table 1
2^2 Factorial central composite design matrix.

Sequence of run	Coded variables		Natural variables	
	X_1	X_2	Cell concentration, X_1 (mg/L)	Temperature, X_2 (°C)
1	0	−1.414	1429 ± 214	25
2	0	+1.414	1429 ± 214	45
3	−1.414	0	640 ± 57	35
4	−1	−1	808 ± 74	28
5	0	0	1429 ± 214	35
6	−1	+1	808 ± 74	42
7	+1	−1	2170 ± 157	28
8	+1	+1	2170 ± 157	42
9	0	0	1429 ± 214	35
10	0	0	1429 ± 214	35
11	+1.414	0	2331 + 28	35

$$P(t) = P \cdot \exp\left\{-\exp\left[\frac{R \cdot e}{P}(I - t) + 1\right]\right\} \qquad (2)$$

In Eq. (2), $P(t)$ is the cumulative butanol production (g/L) at time t; I is the time of lag-phase (day); P is butanol production potential (g/L); R is butanol production rate (g/L-day); and e is exp(1), i.e., 2.71828.

Butanol productivity is the rate of butanol production in batch experiments from the time of inoculation to the time of the concentration plateau. Butanol yield is the ratio of the butanol concentration to the total utilized sugar concentration. The predicted butanol production rate calculated using the Gompertz equation, butanol productivity, and butanol yield for the 11 batch experiments were fitted into a quadratic response model (Eq. (3)):

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \qquad (3)$$

where Y_i is the predicted response, X_1 represents the cell concentration (mg/L), X_2 represents the temperature (°C), β_0 is the intercept of the model, and β_1, β_2, β_{11}, β_{22}, and β_{12} are the coefficients. The maximum response can be obtained by computing ($\partial Y_i/\partial X_1$, $\partial Y_i/\partial X_2$) and setting the equation equal to zero. In this study, the surface response analysis was performed using MINITAB® software (Version 15; LEAD Technologies, Inc.).

2.5. Analytical methods

The concentration of carbohydrate in the fermentation broth was analyzed using high pressure liquid chromatography (HPLC) equipped with a refractive index detector (Waters 410), carbohydrate analysis column (3.9 × 300 mm, Waters), and pump (Hitachi L-2130). Fermentation products including acetone, butanol, ethanol, acetic acid, and butyric acid were analyzed by a gas chromatograph (Aglient 7890A) equipped with a flame ionized detector (FID). The capillary column used was a 30 m by 0.53 mm packed nitroterephthalic acid modified polyethylene glycol column. The solution pH was measured following the procedures described by Standard Methods (1998). The cell concentration was determined using procedures described by Piarpuzán et al. (2011).

3. Results and discussion

3.1. Performance of ABE fermentation

Fig. 1 shows the sugar consumption during the course of ABE fermentation for run 11. As illustrated in the figure, the glucose concentration dramatically decreased to 0 within 4 days under both sterile (Fig. 1a) and non-sterile conditions (Fig. 1b). Galactose (initial concentration of approximately 7 g/L) was gradually used by *C. saccharoperbutylacetonicum* N1-4 after the consumption of glucose during the remaining fermentation period. Consumption
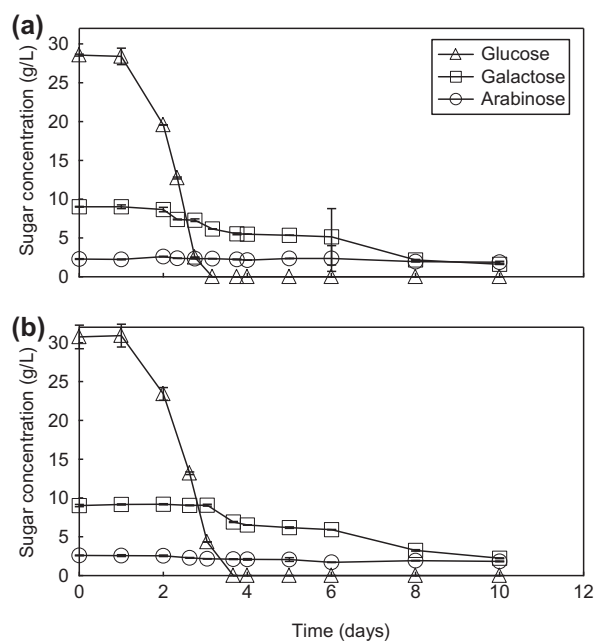

Fig. 1. Courses of glucose, galactose, and arabinose concentrations under (a) sterile conditions and (b) non-sterile conditions for run 11. Data are presented as means ± standard deviations from triplicate technical repeats of measurements.

of arabinose by *C. saccharoperbutylacetonicum* N1-4 did not occur. This result reveals that microbes in ABE fermentation exhibit a preference for specific sugars, agreeing with observations made by Ezeji et al. (2007). The metabolite production during ABE fermentation for run 11 is shown in Fig. 2. As revealed in the figure, acetone, butanol, ethanol, and the total solvent concentrations (data not shown) under both sterile (Fig. 2a) and non-sterile (Fig. 2b) conditions reached a maximum after day 4 and then plateaued. These results indicate that these metabolites were mainly produced by the conversion of glucose, not galactose or arabinose. In addition, the acetate concentration decreased from approximately 5 to 2 g/L. Apparently, acetate was assimilated by *C. saccharoperbutylacetonicum* N1-4 during the ABE fermentation. This uptake of acetate, which is used as a buffer for the hydrolysis of various lignocellulosic biomasses (Guo et al., 2009; Várnai et al., 2010), for acetone production using ABE fermentation has been reported elsewhere (Lee et al., 2008). Acetate can prevent strain degeneration (Chen and Blaschek, 1999), a phenomenon where solvent-producing clostridia lose the ability to produce solvents. At the end of fermentation, butyrate concentration increased to approximately 1.5 g/L for both sterile and non-sterile conditions. Because glucose was exhausted by this time, it can be concluded
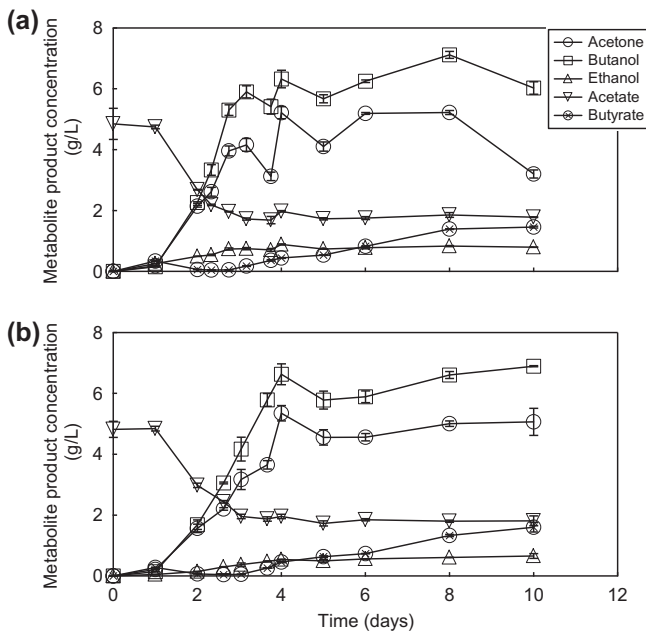
Fig. 2. Courses of metabolite product concentrations under (a) sterile conditions and (b) non-sterile conditions for run 11. Data are presented as means ± standard deviations from triplicate technical repeats of measurements.

that butyrate was mainly produced by the conversion of galactose. It seems that the dominant metabolic mechanism at this stage was acidogenesis. This finding is consistent with our earlier report (Chen et al., 2011) but contrary to the well-known biphasic process of ABE fermentation. Typically, it is believed that acids are produced in the first stage (acidogenesis phase). Then, the acids are re-assimilated, and solvents are produced (solventogenic phase). A possible explanation for this process is that some cells remain in the acidogenesis phase throughout ABE fermentation, and these cells begin to produce butyrate under low carbohydrate concentrations. Figs. 1 and 2 indicate that all runs show similar tendencies of substrate utilization and solvent production if ABE fermentation occurred.

Table 2 summarizes the results of ABE fermentation of the 11 runs at the harvest time under both sterile and non-sterile conditions. The harvest time is defined as the time when the total

solvent concentration plateaued. As is evident from the table, glucose is the main carbon source during ABE fermentation, whereas arabinose is barely utilized by *C. saccharoperbutylacetonicum* N1-4. Approximately 100% of the glucose and more than 70% of the total sugar were consumed in runs 1, 7, and 11 as well as three center runs (runs 5, 9, and 10) under the sterile condition. However, the average values of glucose utilization and total sugar utilization from the three center runs under the non-sterile condition were significantly less, 62% and 45%, respectively. Additionally, the statistical variances, or standard deviations (data not shown), of glucose utilization and total sugar utilization from the three center runs under the sterile condition were both less than those under the non-sterile condition. The variances represent the internal estimate of errors (experimental errors) of the CCD in the system. Thus, maintaining sterile conditions will ensure consistent system performance. The results of runs 7 and 11 indicate that high cell concentrations in ABE fermentation significantly reduce the metabolite production discrepancy between the sterile and non-sterile conditions. These results indicate that microbial interference under the non-sterile condition can be profoundly reduced if the solvent-producing clostridia are maintained at high levels. Runs 3 and 4 had low initial cell concentrations, and thus, low sugar utilization was observed under both the sterile and non-sterile conditions. Fermentation was not observed in runs 2, 6, and 8 owing to high incubation temperatures, 42–45 °C, which inhibit all microbial activities.

The triplicate results from the center runs reveal that the butanol concentration, butanol productivity, butanol yield, and ABE concentration for the sterile condition were 5.1 ± 0.3 g/L, 1.26 ± 0.06 g/L/d, 0.16 ± 0.01 g/g sugar$_{consumed}$, and 8.1 ± 0.8 g/L, respectively. Moreover, a butanol concentration of 2.8 ± 2.1 g/L, butanol productivity of 0.46 ± 0.26 g/L/d, butanol yield of 0.13 ± 0.04 g/g sugar$_{consumed}$, and ABE concentration of 4.6 ± 3.3 g/L were observed for the non-sterile condition. Solvent production apparently decreases under the non-sterile condition. In addition, the variances, or standard deviations, of these parameters under the non-sterile condition were dramatically increased in comparison with those under the sterile condition. The variance of solvent production from the three center runs positively correlates with the inconsistent sugar utilization. Throughout the entire CCD, the results of runs 7 and 11 reveal that the difference of solvent production between the sterile and non-sterile conditions can be minimized if high initial cell concentrations are used. In non-sterile conditions, fermentation may be contaminated by other anaerobic

Table 2
Performance of ABE fermentation from NPRS hydrolysate.

Run	Harvest time[a] (day)		Sugar utilization (%)								Butanol concentration (g/L)		Butanol productivity (g/L/d)		Butanol yield (g/g sugar $_{consumed}$)		B/A[b] (g/g)		ABE concentration (g/L)	
			Glucose		Galactose		Arabinose		Total sugar											
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
11	4	4	100	100	39	28	6	19	84	80	6.3	6.6	1.58	1.66	0.20	0.20	1.4	1.28	12.4	12.5
5[c]	4	5	100	70	31	5	20	25	78	49	5.3	2.8	1.32	0.55	0.16	0.13	2.0	2.3	8.9	4.3
9[c]	4	7	100	88	25	29	3.6	2	76	66	5.1	4.8	1.26	0.67	0.16	0.17	2.0	2.5	8.1	8.0
10[c]	4	4.25	94	27	12	8	14	0.3	69	20	4.8	0.7	1.21	0.17	0.17	0.09	2.0	1.2	7.4	1.4
7	7	11	100	100	56	68	0	12	84	87	7.3	7.9	1.04	0.71	0.21	0.22	1.6	1.38	13.1	14.3
1	11	11	100	97	35	26	0	0	77	73	6.3	6.4	0.57	0.58	0.20	0.21	2.5	2.3	9.3	9.5
4	11	ND[d]	67	0	21	0	0	0	52	0	4.3	0	0.39	0	0.20	0	1.3	ND	8.1	0
3	6	13.4	22	25	11	3.6	0	2.6	17	18	0.6	0.23	0.10	0.02	0.09	0.03	0.9	0.6	1.5	0.54
2	ND[d]	ND	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ND	ND	0	0
8	ND	ND	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ND	ND	0	0
6	ND	ND	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ND	ND	0	0

[a] The harvest time is defined as the period when a maximum concentration of ABE was achieved.
[b] Butanol to acetone ratio.
[c] Three replicated center points.
[d] Not determined.

bacteria. However, when *C. saccharoperbutylacetonicum* N1-4 is dominant in the fermentation system, ABE production is not influenced by microbial contamination. Only when an insufficient initial concentration of *C. saccharoperbutylacetonicum* N1-4 has been inoculated will ABE fermentation have poor reproducibility or low ABE production. This low ABE production results because a lower population of *C. saccharoperbutylacetonicum* N1-4 can be easily wiped out or inhibited by other anaerobic bacteria. The results confirm that ABE fermentation by *C. saccharoperbutylacetonicum* N1-4 conducted under non-sterile conditions is feasible when the initial cell concentration is greater than 2200 mg/L. In addition, the results of runs 7 and 11 and the three center runs indicate that increasing the initial cell concentration enhances solvent production in terms of butanol concentration, butanol productivity, butanol yield, and ABE concentration in both the sterile and non-sterile condition. Notice that the butanol to acetone (B:A) ratio is not significantly influenced by either the cell concentration or temperature in this investigation. Runs 2, 6, and 8 had no metabolite production, from neither solvents nor organic acids. This result illustrates that *C. saccharoperbutylacetonicum* N1-4 is either inactive or dead at an incubation temperature between 42 and 45 °C.

3.2. Kinetics of butanol production

The modified Gompertz equation was employed to describe the kinetics of butanol production. Fig. 3 shows the predicted values for cumulative butanol production as best-fit smooth curves for experimental data obtained from run 5 in Group A. Similar plots were generated for all active corresponding batch runs in both the sterile and non-sterile conditions. The kinetic parameters estimated using Eq. (2) are listed in Table 3. The butanol production data are fitted well by the modified Gompertz equation ($R^2 > 0.97$). As shown in Table 3, the butanol production potential (P) and butanol production rate (R) for runs under the sterile condition were 0–7.27 g/L and 0–4.82 g/L/d, respectively. The P and R values for the corresponding runs under the non-sterile condition were 0–7.70 g/L and 0–3.21 g/L/d, respectively. In general, the butanol production potential and butanol production rate under the sterile condition were greater than those under the non-sterile condition. However, for runs 7 and 11 with cell concentrations higher than the other runs, the butanol production potential and butanol production rate were not significantly different between
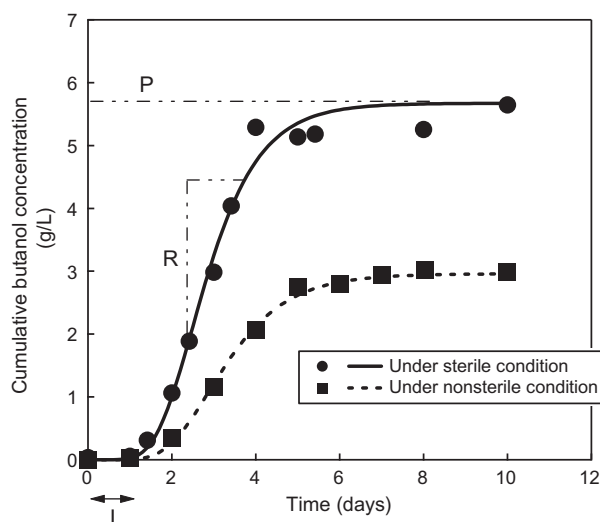
Table 3
Kinetic parameters of the modified Gompertz equation for butanol production.

Run	Sterile condition (A)				Non-sterile condition (B)			
	P (g/L)	R (g/L/d)	I (d)	R^2	P (g/L)	R (g/L/d)	I (d)	R^2
11	6.23	4.82	1.56	0.97	6.51	3.21	1.57	0.98
5	5.64	2.39	1.61	0.98	2.96	1.08	1.88	0.99
9	5.43	2.92	1.68	0.97	4.78	1.16	1.62	0.99
10	5.55	2.1	1.69	0.98	0.69	0.42	1.5	0.97
7	7.27	2.73	2.64	0.99	7.70	2.06	2.41	0.99
1	6.27	1.47	5.76	0.99	6.72	1.43	6.24	0.99
4	4.35	1.07	3.43	0.99	0	0	ND	–
3	0.55	0.27	1.54	0.98	0.22	0.11	3.08	0.99
2	0	0	ND	–	0	0	ND	–
8	0	0	ND	–	0	0	ND	–
6	0	0	ND	–	0	0	ND	–

ND: not determined.

the sterile and non-sterile conditions. In addition, the values were positively correlated with the cell concentration in both conditions. The inoculated cell concentration seems to be more influential than the incubation temperature on the efficiency of ABE fermentation. It is interesting to note that both the butanol production potential and the butanol production rate for run 1 exhibit little difference between the sterile and non-sterile conditions. Run 1 was performed with a cell concentration of approximately 1400 mg/L at 25 °C. A possible explanation for this result is that *C. saccharoperbutylacetonicum* N1-4 under these conditions is prominent to suppress microbial interference.

All ABE fermentation runs in this study experienced a lag period, and only the temperatures and initial cell concentrations were varied for all runs. Incubation temperature was the main factor affecting the duration of the lag phase. Runs 3, 5, 9, 10, and 11 under the sterile condition were all performed at 35 °C; they had identical lag time despite their different initial cell concentrations. Run 1 had a relatively longer lag time because of its lesser incubation temperature of 25 °C. Decreasing temperature from 35 to 25 °C increased the duration of the lag time. At higher temperatures, e.g., 42 °C or 45 °C, as in runs 2, 6, and 8, *C. saccharoperbutylacetonicum* N1-4 was either inhibited or killed; therefore, these runs had indefinite lag periods. Under the non-sterile condition, the incubation temperature and initial cell concentration were noted to affect the kinetics of ABE fermentation. Lag time decreased with increasing incubation temperatures, the same pattern as that under the sterile condition. However, it is possible that contamination led to longer lag times in runs 3 and 4 owing to the low initial cell concentration in the batch reactor. As described earlier, a high initial cell concentration of *C. saccharoperbutylacetonicum* N1-4 can overcome the effect of contamination, but inhibition of the functional microorganisms can occur for runs with low initial cell concentration under the non-sterile condition.

3.3. Response surface analysis

The butanol productivity and butanol yield, as shown in Table 2, and the Gompertz predicted butanol production rate, as shown in Table 3, were subjected to polynomial regression analyses to yield second-order polynomial equations. The second-order polynomial equations were used to describe predicted responses of butanol productivity (Y_1), butanol yield (Y_2), and the Gompertz predicted butanol production rate (Y_3) with initial cell concentration (X_1) and incubation temperature (X_2) as parameters. The regression equations for Y_1, Y_2, and Y_3 with respect to their coefficient of determination (R^2) and the level of significance (p value) are given in Table 4. The goodness of fit of the regression equation to the data is indicated by the magnitude of R^2, and the acceptable level of

Fig. 3. Cumulative butanol production curves for ABE fermentation of run 5. (Markers—experimental data; nonlinear lines—data estimated by Eq. (2)).

Table 4
A summary of regression analyses.

Experimental group	Regression equations	R^2	p-Value
Sterile condition (A)	$Y_1 = -14.6073 + 0.0041X_1 + 0.7545X_2 - 0.0000X_1^2 - 0.0106X_2^2 - 0.0000X_1X_2$	0.89	0.019
	$Y_2 = -0.479560 + 0.000193X_1 + 0.039767X_2 - 0.000000X_1^2 - 0.000727X_2^2 - 0.000001X_1X_2$	0.93	0.008
	$Y_3 = -28.0070 + 0.0068X_1 + 1.5062X_2 - 0.0000X_1^2 - 0.0211X_2^2 - 0.0001X_1X_2$	0.79	0.089
Non-sterile condition (B)	$Y_1 = -5.98816 + 0.00163X_1 + 0.30756X_2 + 0.00000X_1^2 - 0.00401X_2^2 - 0.00004X_1X_2$	0.66	0.244
	$Y_2 = -1.06068 + 0.00071X_1 + 0.04399X_2 - 0.00000X_1^2 - 0.00052X_2^2 - 0.00001X_1X_2$	0.89	0.020
	$Y_3 = -10.2550 + 0.0042X_1 + 0.4894X_2 + 0.0000X_1^2 - 0.0058X_2^2 - 0.0001X_1X_2$	0.78	0.092

Y_1 is butanol productivity; Y_2 is butanol yield; Y_3 Gompertz predicted butanol production rate; X_1 is initial cell concentration; X_2 is incubation temperature.
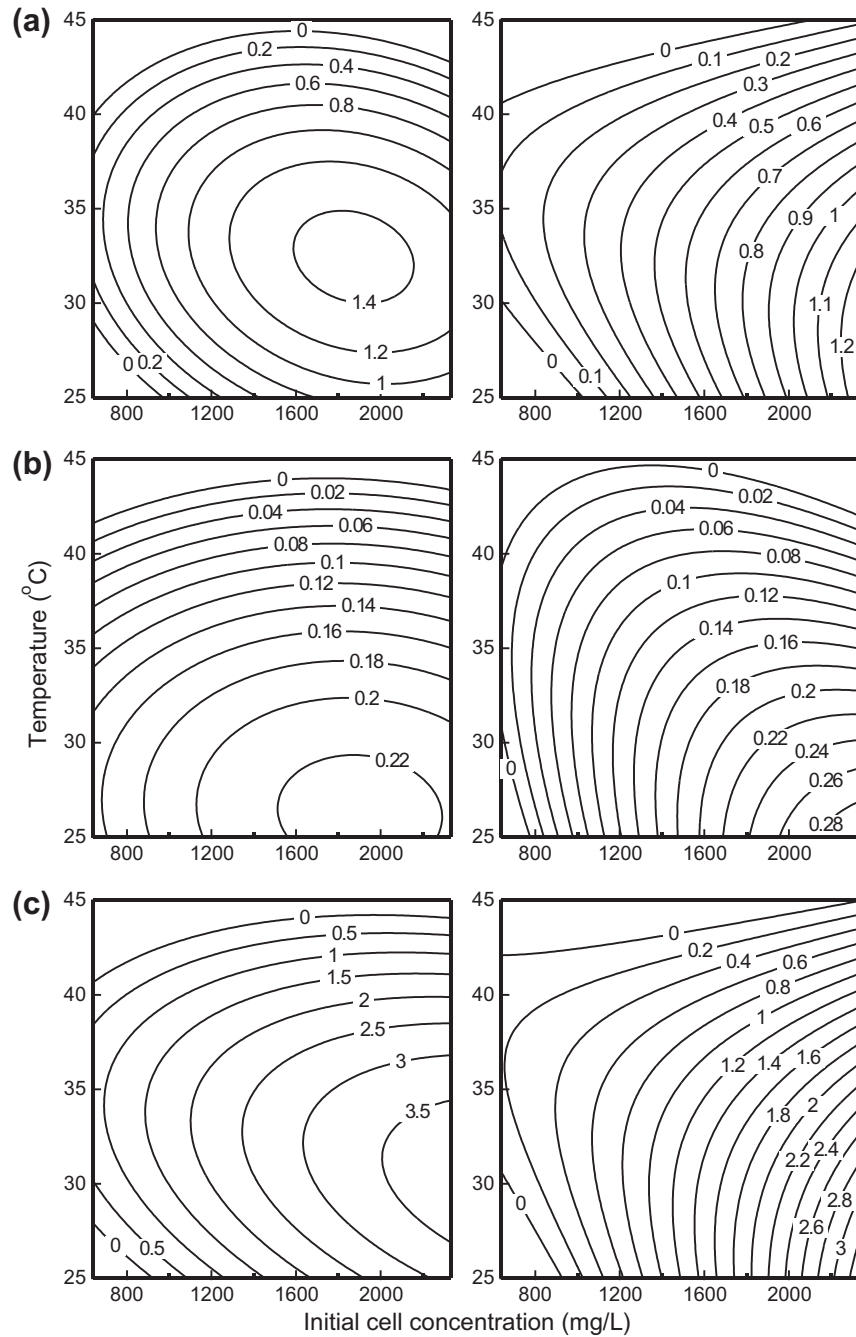

Fig. 4. Contour plots of (a) butanol productivity (Y_1, g/L/d), (b) butanol yield (Y_2, g/g), and (c) butanol production rate (Y_3, g/L/d) for Group A (left column) and Group B (right column).

significance is given by p values less than 0.05. Based on the p values, the second-order polynomial equations gave significant results for butanol productivity (Y_1) and butanol yield (Y_2) for Group A (sterile condition) and butanol yield (Y_2) for Group B (non-sterile condition). The p values of the Gompertz-predicted butanol production rate (Y_3) for Group A and B were 0.089 and 0.092, respectively. Although these values are slightly higher than 0.05, the R^2 values are considered to be acceptable for biochemical reactions. The quadratic model did not fit butanol productivity (Y_1) for Group B.

Using the regression equations, two-dimensional contour plots were obtained for Groups A and B, as shown in Fig. 4. Under the sterile condition, the maximum Y_1 (1.45 g/L/d) and Y_2 (0.22 g/g) could be obtained at (X_1, X_2) of (1.96 g/L, 32.3 °C), and (2.01 g/L, 26.3 °C), respectively. However, the maximum Y_3 could not be resolved by response surface methodology (RSM) because of its incapability of determining the optimum X_1 and X_2. Similarly, the maximums of Y_1, Y_2, and Y_3 under the non-sterile condition could not be calculated without the optimum X_1 and X_2. The predicted maximum butanol productivity of NPRS hydrolysate is 86% lower than glucose (Hipolito et al., 2008) and 65% lower than mixed sugars (Ezeji et al., 2007). This lower maximum butanol productivity resulted because lag time was included when solving for the maximum butanol productivity. However, the Gompertz predicted butanol production rate can show the efficiency of butanol production even though the actual maximum value could not be determined in this study. Notice that the effect of inhibitory compounds such as furfural and hydroxymethyl furfural (HMF) (by-products from diluted acid pretreatment) on biobutanol production can be neglected. The presence of furfural and HMF can hamper ABE fermentation from barley straw hydrolysate (BSH) (Qureshi et al., 2010). However, these inhibitory chemicals do not exist in the NPRS hydrolysate because the NPRS was not subjected to any chemical pretreatments. The potential of converting NPRS hydrolysate into biobutanol is confirmed by the Gompertz predicted butanol production rate, butanol productivity, and butanol yield in this study.

4. Conclusions

ABE fermentation has long been considered to be effective only if it is performed under sterile conditions in order to avoid microbial interference. However, the cost of maintaining sterile conditions during ABE fermentation is one of the issues that limits cost-effective biobutanol production. This study demonstrates that using high inoculated cell concentration ensures reproducible performance of ABE fermentation under the non-sterile condition. The pre-sterilization step of producing biofuels from agricultural residues can be eliminated. The use of C. saccharoperbutylacetonicum Nl-4 to ferment NPRS hydrolysate into biofuels can be made more cost effective by using non-sterile conditions.

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