MODELLING OF ACETONE-BUTANOL-ETHANOL BATCH BIOSYNTHESIS

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The influence of fermentation medium composition and process temperature on the performances of ABE batch biosynthesis was assessed by mathematical modelling. A kinetic model based on the structured growth approach was applied to simulate the fermentation dynamics. Final concentrations (for a process duration of 100 h) of each solvent under various operation conditions were determined based on these simulations. The effects of process factors, i.e., initial concentrations of substrate (c_{S0}=17.58-102.4 g/L) and butyric acid (c_{BA0}=0.758-9.242 g/L) in the fermentation medium, initial C/N mass ratio ($R_{C/N0}$=3.45-116.5), and fermentation temperature (t=26.35-37.65 ºC), on concentration of butanol, total concentration of ABE solvents, and ratio between the concentrations of butanol and acetone were determined using a second order response surface statistical model. The highest values of concentrations of butanol and ABE solvents (12.41 g/L and 17.38 g/L) were obtained under the following optimal conditions: $c_{S0,opt}$=102.4 g/L, $c_{BA0,opt}$=0.758 g/L, $R_{C/N0,opt}$=60, and t_{opt}=32 ºC. The values of solvent ratio increased linearly with an increase in $R_{C/N0}$.

Keywords: ABE fermentation, *Clostridium acetobutylicum*, statistical model, kinetic model

1. Introduction

At the present time, worldwide, the main reason for concern regarding energy is that the current vegetation on Earth cannot treat by photosynthesis the carbon dioxide emitted due to the widespread use of the fossil fuels formed and stored underground for millions of years. One of the obvious consequences is the greenhouse effect accompanied by climate changes. These climate changes, attributed to the increase in emissions of CO_2 and other greenhouse gases, lead researchers to look for alternative energy sources [1-3]. An abundant and cheap resource is the biomass, either fresh or residual, which must be processed in such a way as to become effectively usable as an energy vector [4,5]. Another reason to

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develop renewable energy resources is related to the demographic explosion, which amplifies the concern about the depletion of general oil reserves [6-8].

Of the four universally recognized biofuels, *i.e.*, biodisel, bioethanol, biobutanol, and biogas, the last three have in common that they are a result of the anaerobic fermentation of organic substrates. Bioethanol (B) is produced along with small amounts of acetone (A) and ethanol (E) by ABE anaerobic fermentation in the presence of *Clostridium acetobutylicum* bacteria. ABE technology has become competitive for industrial application due to the need for high-performing biofuels and strong advances in processes related to ABE synthesis, including genetic modification of *Clostridium* bacteria, hydrolysis of sugars from different types of biomass, fermentation, and separation of fermentation products.

Solvent-generating *Clostridium* species can use a wide variety of substrates, from simple monosaccharides, *e.g.*, pentoses (fructose), hexoses (glucose), to more or less complex polysaccharides. Glucose derived from molasses, starch, cellulose or hemicelluloses is the basic substrate in ABE synthesis [9]. Yeast extract, which is rich in proteins, is used as a source of nitrogen to grow bacterial cells and achieve a high production of ABE solvents.

ABE synthesis takes place in two stages. In the first stage (acidogenesis), the enzyme systems of clostridial bacteria activate the formation of acetate (acetic acid) and butyrate (butyric acid), resulting in hydrogen and carbon dioxide as main products. In relation to the cell growth curve, the acidogenic stage usually occurs during the logarithmic growth phase of the bacterial system [10,11]. In the second stage (solventogenesis), acids are re-assimilated to be used in the production of acetone, butanol and ethanol (or isopropanol instead of acetone in some cases as is used the *Clostridium beijerinckii* culture).

Mathematical modelling is an effective tool for design, scale-up, and optimization of ABE fermentation process [2,12,13]. A statistical model and a kinetic model were used in this paper to assess the influence of fermentation medium composition and process temperature on the performances of ABE batch biosynthesis.

2. Case study

Some data reported in the related literature [14-18] as well as predictions obtained based on the mathematical models proposed in this paper were used to assess the influence of culture medium composition and fermentation temperature on the transformation of carbon substrate into ABE solvents. Standard composition of the culture medium used in experimental research, used as case study [14-18] is given in Table 1. Glucose was the main C source, butyric acid the secondary C source, whereas ammonium acetate, tryptone, and yeast extract were

N sources for the bacterial system. Batch fermentation tests were conducted under anaerobic conditions in the presence of *Clostridium acetobutylicum* bacteria. Preparation of inoculum was almost similar, the pH was kept between 4.8 and 5.8, and the duration of each fermentation test was 100 h.

Table 1

Standard composition of ABE fermentation medium with *Clostridium acetobutylicum* inoculum

No.	Compound	Concentration	
		Value	Units
1	Inoculum	75	mL/L
2	Glucose	50	g/L
3	Butyric acid	5	g/L
4	Ammonium acetate	2	g/L
5	Tryptone	5	g/L
6	Yeast extract	2	g/L
7	Magnesium sulphate	0.3	g/L
8	Iron sulfate heptahydrate	0.001	g/L

3. Modelling of ABE fermentation in a fed batch reactor

3.1. Statistical model

A second order response surface model with 4 factors and 5 factor levels was applied to asses the influence of process factors on its performances [19]. Table 2 contains values of final concentrations (for a fermentation duration of 100 h) of butanol (c_B), acetone (c_A), and ethanol (c_E) at different levels of process factors, *i.e.*, initial concentrations of substrate (c_{S0}) and butyric acid (c_{BA0}) in the fermentation medium, initial C/N mass ratio ($R_{C/N0}$), and fermentation temperature (t).

Table 2

Values of concentrations of butanol (c_B), acetone (c_A), and ethanol (c_E) at different levels of process factors (initial concentration of substrate (c_{S0}), initial concentration of butyric acid (c_{BA0}), initial C/N mass ratio ($R_{C/N0}$), and fermentation temperature (t))

No.	c_{S0} (g/L)	c_{BA0} (g/L)	$R_{C/N0}$	t (ºC)	c_B (g/L)	c_A (g/L)	c_E (g/L)
1	90	8	100	36	12.2	2.48	1.24
2	30	2	100	36	3.60	0.75	0.32
3	90	2	20	36	11.9	4.05	2.16
4	30	8	20	36	8.50	2.93	1.61
5	90	2	100	28	11.3	2.40	1.12
6	30	8	100	28	3.70	0.79	0.34
7	90	8	20	28	8.70	3.12	1.61
8	30	2	20	28	5.10	1.76	1.01
9	90	2	100	36	12.6	2.66	1.21
10	30	8	100	36	4.10	0.85	0.39
11	90	8	20	36	9.50	3.40	2.02
12	30	2	20	36	5.70	2.06	1.23

13	90	8	100	28	11.1	2.31	1.09
14	30	2	100	28	3.10	0.66	0.29
15	90	2	20	28	10.4	3.58	1.89
16	30	8	20	28	7.10	2.53	1.35
17	60	5	60	32	10.9	2.86	1.45
18	102.4	5	60	32	13.9	3.61	1.81
19	17.58	5	60	32	1.90	0.51	0.25
20	60	9.242	60	32	10.9	2.80	1.45
21	60	0.758	60	32	9.10	2.35	1.22
22	60	5	116.5	32	9.10	1.82	1.22
23	60	5	3.45	32	9.60	3.84	1.28
24	60	5	60	37.65	11.5	2.98	1.05
25	60	5	60	26.35	7.80	2.00	1.67

Tabulated data were obtained based on results reported in the literature [14-18] and the kinetic model presented in the section 3.2.

Dimensionless process factors, x_j (j=1…4), having values of -√2=-1.414, -1, 0, 1, and √2=1.414, were determined using Eqs. (1)-(4). The concentration of butanol (c_B), the ratio between the concentrations of butanol and acetone ($R_{B/A}=c_B/c_A$), and the total concentration of solvents ($c_{TS}=c_B+c_A+c_E$) were selected as process performances. Values of process performances at different levels of natural and dimensionless process factors are summarized in Table 3. Second-order polynomial relationships, expressed by Eq. (5), were used to predict the process performances, *i.e.*, $y_1=c_{B,p}$, $y_2=R_{B/A,p}$, and $y_3=c_{TS,p}$, depending on dimensionless factors. Characteristic regression coefficients of statistical models described by Eq. (5), β_{0i}, $\beta_{1i}\ldots\beta_{44i}$ (i=1…3), were calculated according to equations presented in a previous paper [16] and $\overline{x_j^2}$ is given by Eq. (6).

$$x_1 = \frac{c_{S0} - 60}{30} \tag{1}$$

$$x_2 = \frac{c_{BA0} - 5}{3} \tag{2}$$

$$x_3 = \frac{R_{C/N} - 60}{40} \tag{3}$$

$$x_4 = \frac{t - 32}{4} \tag{4}$$

$$\begin{aligned} y_i = {} & \beta_{0i} + \beta_{1i}x_1 + \beta_{2i}x_2 + \beta_{3i}x_3 + \beta_{4i}x_4 + \\ & + \beta_{12i}x_1x_2 + \beta_{13i}x_1x_3 + \beta_{14i}x_1x_4 + \beta_{23i}x_2x_3 + \beta_{24i}x_2x_4 + \beta_{34i}x_3x_4 + \\ & + \beta_{123i}x_1x_2x_3 + \beta_{124i}x_1x_2x_4 + \beta_{134i}x_1x_3x_4 + \beta_{234i}x_2x_3x_4 + \beta_{1234i}x_1x_2x_3x_4 + \\ & + \beta_{11i}\left(x_1^2 - \overline{x_1^2}\right) + \beta_{22i}\left(x_2^2 - \overline{x_2^2}\right) + \beta_{33i}\left(x_3^2 - \overline{x_3^2}\right) + \beta_{44i}\left(x_4^2 - \overline{x_4^2}\right) \end{aligned}, \quad i=1\ldots3 \tag{5}$$

$$\overline{x_j^2} = \frac{\sum_{k=1}^{25} x_{jk}^2}{\sum_{k=1}^{25} k} = \frac{20}{25} = 0.8\,,\ j=1\ldots4 \tag{6}$$

Table 3

Values of process performances (concentrations of butanol (c_B), ratio between concentrations of butanol and acetone ($R_{B/A}=c_B/c_A$), and total concentration of solvents ($c_{TS}=c_B+c_A+c_E$) at different levels of natural and dimensionless process factors (initial concentration of substrate (c_{S0} and x_1), initial concentration of butyric acid (c_{BA0} and x_2), initial C/N mass ratio ($R_{C/N0}$ and x_3), and fermentation temperature (t and x_4))

No.	c_{S0} (g/L)	x_1	c_{BA0} (g/L)	x_2	$R_{C/N0}$	x_3	t (ºC)	x_4	c_B (g/L)	$R_{B/A}$	c_{TS} (g/L)
1	90	1	8	1	100	1	36	1	12.2	4.92	15.92
2	30	-1	2	-1	100	1	36	1	3.60	4.80	4.67
3	90	1	2	-1	20	-1	36	1	11.9	2.94	18.11
4	30	-1	8	1	20	-1	36	1	8.50	2.90	13.04
5	90	1	2	-1	100	1	28	-1	11.3	4.71	14.82
6	30	-1	8	1	100	1	28	-1	3.70	4.68	4.83
7	90	1	8	1	20	-1	28	-1	8.70	2.79	13.43
8	30	-1	2	-1	20	-1	28	-1	5.10	2.90	7.87
9	90	1	2	-1	100	1	36	1	12.6	4.74	16.47
10	30	-1	8	1	100	1	36	1	4.10	4.82	5.34
11	90	1	8	1	20	-1	36	1	9.50	2.79	14.92
12	30	-1	2	-1	20	-1	36	1	5.70	2.77	8.99
13	90	1	8	1	100	1	28	-1	11.1	4.81	14.5
14	30	-1	2	-1	100	1	28	-1	3.10	4.70	4.05
15	90	1	2	-1	20	-1	28	-1	10.4	2.91	15.87
16	30	-1	8	1	20	-1	28	-1	7.10	2.81	10.98
17	60	0	5	0	60	0	32	0	10.9	3.81	15.21
18	102.4	√2	5	0	60	0	32	0	13.9	3.85	19.32
19	17.58	-√2	5	0	60	0	32	0	1.90	3.73	2.66
20	60	0	9.242	√2	60	0	32	0	10.9	3.89	15.15
21	60	0	0.758	-√2	60	0	32	0	9.10	3.87	12.67
22	60	0	5	0	116.5	√2	32	0	9.10	5.00	12.14
23	60	0	5	0	3.45	-√2	32	0	9.60	2.50	14.72
24	60	0	5	0	60	0	37.65	√2	11.5	3.86	15.53
25	60	0	5	0	60	0	26.35	-√2	7.80	3.90	11.47

3.2. Kinetic model

Values of concentrations of ABE solvents in Table 2, *i.e.*, c_B=1.9-13.9 g/L, c_A=0.51-4.05 g/L, and c_E=0.25-2.16 g/L, were obtained depending on process factors (c_{S0}=17.58-102.4 g/L, c_{BA0}=0.758-9.242 g/L, $R_{C/N0}$=3.45-116.5, and t=26.35-37.65 ºC) by applying a kinetic model based on the structured growth approach [13]. Specific growth rate of biomass (X) is given by Eq. (7), where y is

the dimensionless cellular ribonucleic acid (RNA) concentration and $c_{RNA_{\min}}$ the RNA concentration at μ=0 [9,13,19].

$$\mu = 0.56(y-1) = 0.56\left(\frac{c_{RNA}}{c_{RNA_{\min}}} - 1\right) \tag{7}$$

Fermentation process in a batch reactor with perfect mixing is described by Eqs. (8)-(17), where τ is the time, c_n (n=1…9) are the concentrations of biomass (X), glucose substrate (S), butyric acid (BA), butanol (B), acetic acid (AA), acetone (A), ethanol (E), carbon dioxide (CO_2), and hydrogen (H_2), k_1… k_{14} the kinetic constants, K_I, K_S, K_{AA}, and K_{BA}, the Monod constants. The values of kinetic and Monod constants are given in Table 4 [9,19].

$$\frac{dy}{d\tau} = \left[k_1 \frac{K_I}{K_I + c_B} c_S - 0.56(y-1)\right] y \tag{8}$$

$$\frac{dc_X}{d\tau} = 0.56(y-1)c_X - k_2 c_B c_X \tag{9}$$

$$\frac{dc_S}{d\tau} = -k_3 c_S c_X - k_4 \frac{c_S}{K_S + c_S} c_X \tag{10}$$

$$\frac{dc_{BA}}{d\tau} = k_5 \frac{K_I}{K_I + c_B} c_S c_X - k_6 \frac{c_{BA}}{K_{BA} + c_{BA}} c_X \tag{11}$$

$$\frac{dc_B}{d\tau} = k_7 c_S c_X - 0.8 \frac{dc_{BA}}{d\tau} \tag{12}$$

$$\frac{dc_{AA}}{d\tau} = \left(k_8 \frac{K_I}{K_I + c_B} - k_9 \frac{c_{AA}}{K_{AA} + c_{AA}}\right) \frac{c_S}{K_S + c_S} c_X \tag{13}$$

$$\frac{dc_A}{d\tau} = k_{10} \frac{c_S}{c_S + K_S} c_X - 0.5 \frac{dc_{AA}}{d\tau} \tag{14}$$

$$\frac{dc_E}{d\tau} = k_{11} \frac{c_S}{K_S + c_S} c_X \tag{15}$$

$$\frac{dc_{CO_2}}{d\tau} = k_{12} \frac{c_S}{K_S + c_S} c_X \tag{16}$$

$$\frac{dc_{H_2}}{d\tau} = k_{13}\frac{c_S}{K_S + c_S}c_X + k_{14}c_S c_X \tag{17}$$

Table 4

Values of kinetic and Monod constants in Eqs. (8)-(17)

Parameter	Unit	Value	Parameter	Unit	Value
k_1	L/(g·h)	0.0090	k_{10}	h^{-1}	0.1558
k_2	L/(g·h)	0.0008	k_{11}	h^{-1}	0.0258
k_3	L/(g·h)	0.0255	k_{12}	h^{-1}	0.6139
k_4	h^{-1}	0.6764	k_{13}	h^{-1}	0.0185
k_5	L/(g·h)	0.0136	k_{14}	L/(g·h)	0.00013
k_6	h^{-1}	0.1170	K_I	g/L	0.8330
k_7	L/(g·h)	0.0113	K_S	g/L	2.0
k_8	h^{-1}	0.7150	K_{AA}	g/L	0.5
k_9	h^{-1}	0.1350	K_{BA}	g/L	0.5

4. Results and discussions

After removing the non significant factors in Eq. (5), the statistical models described by Eqs. (18)-(20) were obtained. An acceptable agreement between the values of process performances specified in Table 3 and those predicted by Eqs. (18)-(20) was obtained, the values of coefficients of variation being in the range of 5-17%. It is observed that predicted concentrations of butanol ($y_1=c_{B,p}$) and ABE solvents ($y_3=c_{TS,p}$) depend on all process factors, whereas predicted solvent ratio ($y_2=c_{B/A,p}$) depends only on initial C/N mass ratio in the fermentation medium ($R_{C/N0}$).

$$\begin{aligned} y_1 = c_{B,p} &= 8.532 + 2.343x_1 + 0.380x_4 - 0.663x_1x_2 + 1.162x_1x_3 + \\ &+ 0.450x_1x_2x_3 - 1.305\left(x_1^2 - 0.8\right) - 0.580\left(x_3^2 - 0.8\right) - 0.430\left(x_4^2 - 0.8\right) \end{aligned} \tag{18}$$

$$y_2 = c_{B/A,p} = \frac{c_{B,p}}{c_{A,p}} = 3.815 + 0.770x_3 \tag{19}$$

$$\begin{aligned} y_3 = c_{TS,p} &= 12.107 + 3.217x_1 - 1.131x_3 + 0.556x_4 - 0.994x_1x_2 + 1.336x_1x_3 + \\ &+ 0.654x_1x_2x_3 - 1.797\left(x_1^2 - 0.8\right) - 0.577\left(x_3^2 - 0.8\right) - 0.542\left(x_4^2 - 0.8\right) \end{aligned} \tag{20}$$

The statistical model described by Eqs. (18)-(20) were used to determine the optimum factor levels to maximize the process performances. The influence of dimensionless factors on concentration of butanol, $y_1=y_1(x_1, x_2, x_3, x_4)$ (Eq. (18)) is shown in 2D contour plots represented in Fig. 1.

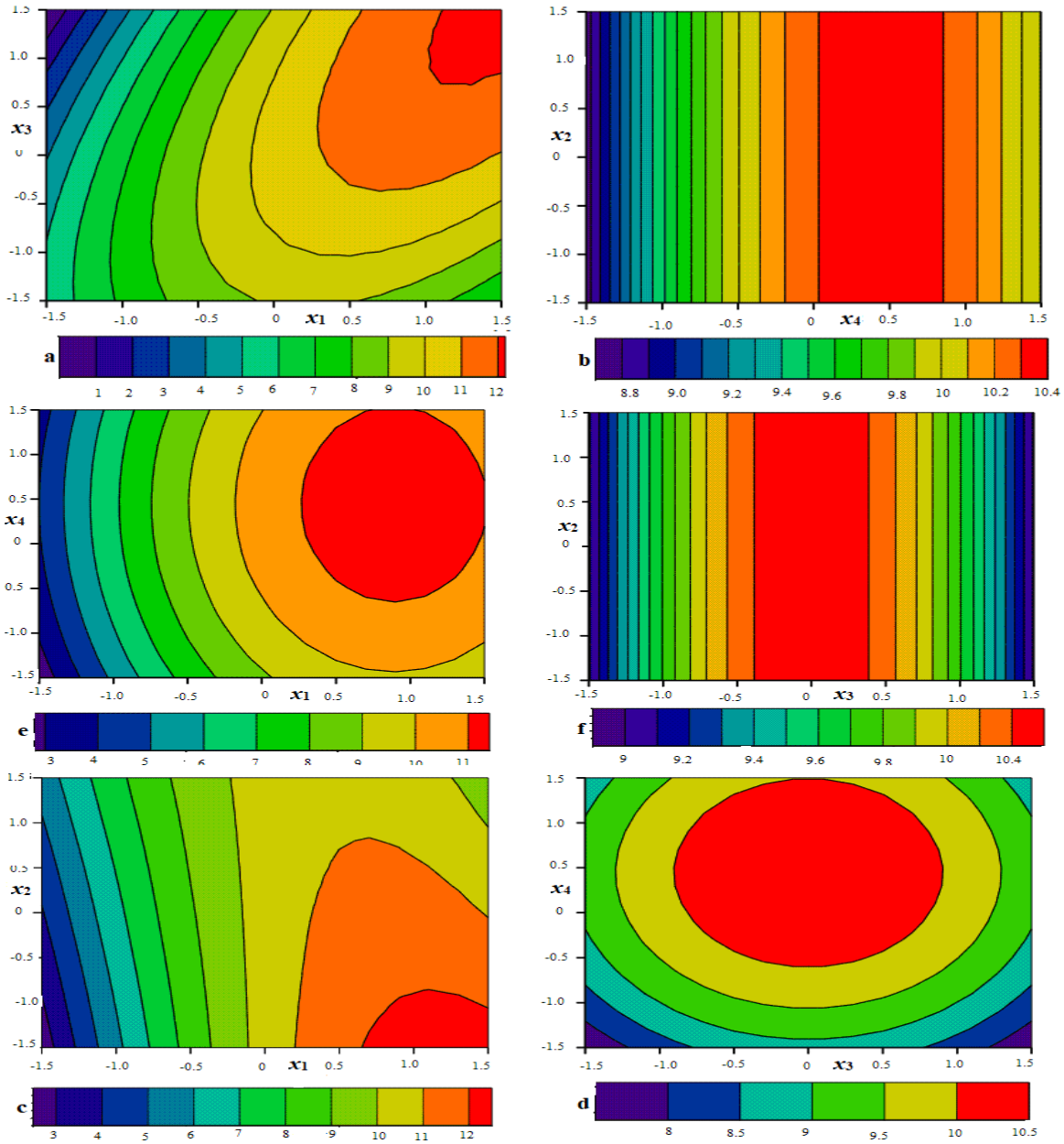

Fig. 1. Influence of dimensionless factors on concentration of butanol (c_B) in ABE fermentation: (a) x_2=x_4=0, (b) x_1=x_3=0, (c) x_3=x_4=0, (d) x_1=x_2=0, (e) x_2=x_3=0, (f) x_1=x_4=0

Maximum values of y_1 for each case presented in Fig. 1 (a-f) are summarized in Table 5.

Table 5

Maximum values of concentration of butanol ($y_{1,max}$) and total concentration of ABE solvents ($y_{3,max}$) depending on process factors

Fig. 1	x_1	c_{S0} (g/L)	x_2	c_{BA0} (g/L)	x_3	$R_{C/N0}$	x_4	t (ºC)	$y_{1,max}$ (g/L)	$y_{3,max}$ (g/L)
a	√2	102.4	0	5	√2	116.5	0	32	12.25	15.31
b	0	60	-√2÷√2	0.758÷9.242	0	60	0.4÷0.5	33.6÷34	10.47	14.58
c	√2	102.4	-√2	0.758	0	60	0	32	12.41	17.38
d	0	60	0	5	0	60	0.4÷0.5	33.6÷34	10.47	14.58
e	0.9	87	0	5	0	60	0.4÷0.5	33.6÷34	11.52	16.02
f	0	60	-√2÷√2	0.758÷9.242	0	60	0.4÷0.5	33.6÷34	10.47	14.58

Tabulated data highlight that the highest value of $y_{1,max}$ is 12.41 g/L (Fig. 1c) and corresponds to c_{S0}=102.4 g/L (x_1=√2), c_{BA0}=0.758 g/L (x_2=-√2), $R_{C/N0}$=60 (x_3=0), and t=32 ºC (x_4=0). A similar value, *i.e.*, $y_{1,max}$=12.25 g/L (Fig. 1a) was obtained for c_{S0}=102.4 g/L (x_1=√2), c_{BA0}=5 g/L (x_2=0), $R_{C/N0}$=116.5 (x_3=√2), and t=32 ºC (x_4=0). Moreover, in Fig. 1b and Fig. 2c it is observed that c_{BA0} does not affect the value of $y_{1,max}$.

According to Eq. (19), the ratio between the concentrations of butanol and acetone (y_2=2.7÷4.9) increases linearly with an increase in the initial C/N mass ratio (x_3=-√2÷√2, $R_{C/N0}$=3.45÷116.5). This dependence is shown in Fig. 2.

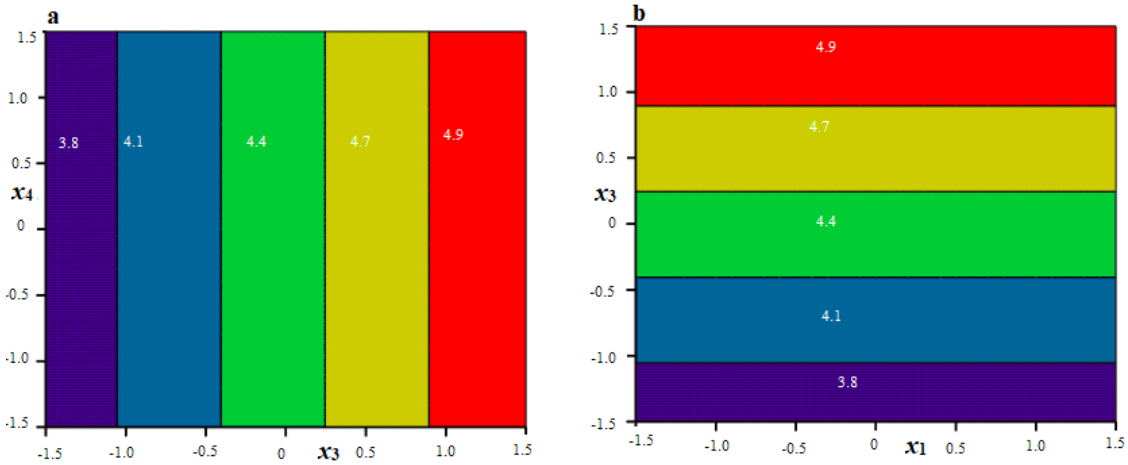

Fig. 2. Influence of dimensionless factors on the ratio between the concentrations of butanol and acetone (y_2) in ABE fermentation: (a) x_1=x_2=0, (b) x_2=x_4=0

Maximum values of y_3 for each case presented in Fig. 1 (a-f), which were determined using Eq. (20), are summarized in Table 5. Tabulated data highlight that the values of $y_{3,max}$ are 25÷40% higher than those of $y_{1,max}$. The highest value of $y_{3,max}$ is 17.38 g/L, corresponding to $y_{1,max}$=12.41 g/L (c_{S0}=102.4 g/L (x_1=√2), c_{BA0}=0.758 g/L (x_2=-√2), $R_{C/N0}$=60 (x_3=0), t=32 ºC (x_4=0)).

A low level of final total concentration of ABE solvents in the fermentation medium (<20 g/L) is an effect of butanol inhibition on the bacterial system [6]. Fed-batch operation or fermentation coupled with gas stripping or solvent pervaporation can be used for more efficient production and separation of ABE solvents [9,19].

5. Conclusions

A second order response surface model with 4 factors and 5 factor levels was used to determine the effects of fermentation medium composition and process temperature on the performances of ABE batch biosynthesis, *i.e.*, concentration of butanol (c_B), ratio between the concentrations of butanol and acetone ($R_{B/A}$=c_B/c_A), and total concentration of ABE solvents (c_{TS}=c_B+c_A+c_E) for a process duration of 100 h. Initial concentrations of substrate (c_{S0}=17.58-102.4 g/L) and butyric acid in the fermentation medium (c_{BA0}=0.758-9.242 g/L), initial C/N mass ratio ($R_{C/N0}$=3.45-116.5), and fermentation temperature (t=26.35-37.65 ºC) were selected as process factors. Values of concentrations of ABE solvents (c_B=1.9-13.9 g/L, c_A=0.51-4.05 g/L, and c_E=0.25-2.16 g/L) were obtained depending on process factors using a kinetic model based on the structured growth approach and data reported in the related literature.

Process performances, *i.e.*, y_1=$c_{B,p}$, y_2=$R_{B/A,p}$, and y_3=$c_{TS,p}$, were predicted depending on dimensionless factors by second-order polynomial relationships, which were used to determine the optimum factor levels to maximize the process performances. Concentrations of butanol (y_1=$c_{B,p}$) and ABE solvents (y_3=$c_{TS,p}$) depended on all process factors, whereas the ratio between the concentrations of butanol and acetone (y_2=$R_{B/A,p}$) depended only on initial C/N mass ratio in the fermentation medium ($R_{C/N0}$). The highest values of concentrations of butanol and ABE solvents, $y_{1,max}$=12.41 g/L and $y_{3,max}$=17.38 g/L, were obtained under the following optimal conditions: $c_{S0,opt}$=102.4 g/L, $c_{BA0,opt}$=0.758 g/L, $R_{C/N0,opt}$=60, and t_{opt}=32 ºC. The values of y_2 (2.7÷4.9) increased linearly with an increase in $R_{C/N0}$. Fed-batch operation or fermentation coupled with a suitable technique of solvent recovery could improve significantly the solvent production.

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