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Full Length Research Paper

Evaluating the effect of acetic acid, furfural and catechol on the growth and lipid accumulation of *Trichosporon fermentans* by response surface methodology

Chao Huang[1, 3], Peng Wen[1], Hong Wu[1]*, Wen-yong Lou[2] and Min-hua Zong[2]*

[1]Laboratory of Applied Biocatalysis, College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China.
[2]State Key Laboratory of Pulp and Paper Engineering, College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China.
[3]Key Laboratory of Renewable Energy and Gas Hydrate, Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, Guangzhou 510640, China.

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The effect of combination of acetic acid, furfural and catechol on the growth and lipid accumulation of oleaginous yeast *Trichosporon fermentans* was systematically studied by response surface methodology (RSM). A 5-level 3-factor central composite design (CCD) was used to build the statistical model. The results measured by RSM showed that each inhibitor exhibited significant negative effect on the biomass, lipid content, lipid yield, and sugar consumption of *T. fermentans*. However, for the binary and ternary combinations of these compounds, only the binary combination of acetic acid and catechol showed significant effect, indicating there are no synergistic effects for these inhibitors in most cases. This work offers a simple way to evaluate the complex effect of various inhibitors on the growth and lipid accumulation of oleaginous microorganisms.

Key words: Central composite design, *Trichosporon fermentans*, acetic acid, furfural, catechol.

INTRODUCTION

For a long time, microbial oils, namely single cell oils (SCOs), were used as medically important poly-unsaturated fatty acids like *γ*-linolenic acid, or substitutes of lipid with rare fatty acid composition or structure such as cocoa-butter (Papanikolaou et al., 2003). Recently, they were also proved to be a promising feedstock for biodiesel production due to their similarity in fatty acid composition to that of vegetable oils (Li et al., 2008). Unfortunately, the high cost of fermentation substrate limits their practical application. Using inexpensive media, such as agro-industrial residues, especially lignocellulosic materials like rice straw, wheat straw, corncob, rice hull and etc., for lipid fermentation is one of the possible resolutions to this problem (Chen et al., 2012; Economou et al., 2011; Huang et al., 2009; Huang et al., 2012; Yu et al., 2011). However, during the dilute acid-treatment of lignocellulosic biomass, various inhibitory by-products such as organic acids, aldehydes and alcohols were generated (Palmqvist and Hahn-Hagerdal, 2000). All these compounds might cause negative effects on growth, metabolism, as well as product formation of microorganism cells during fermentation (Almeida et al., 2007).

In order to use lignocellulosic hydrolysates efficiently as substrate for lipid fermentation, it is critical to have an overall knowledge on the inhibitory effect of these compounds present in it on the growth and lipid accumulation of oleaginous microorganisms. The effects of individual inhibitor and binary combination of inhibitors on the growth and lipid accumulation of different

*Corresponding author. E-mail: bbhwu@scut.edu.cn. btmhzong@scut.edu.cn. Tel: +86-20-87111452. Fax: +86-20-22236669.

oleaginous microorganisms have been studied by many works (Chen et al., 2009; Hu et al., 2009; Huang et al., 2011; Huang et al., 2012; Zhang et al., 2011). However, the lignocellulosic hydrolysates generally contain more than one inhibitor and the synergistic effect of different inhibitors was complex (Duarte et al., 2005; Oliva et al., 2006; Sampaio et al., 2007). To date, little work has focused on the combined effect of several inhibitors on oleaginous microorganisms.

Response surface methodology (RSM) is a collection of certain statistical techniques for designing experiments, building models, evaluating the effect of the factors and searching for optimal conditions for desirable responses (Myers et al., 2009).

Acetic acid, furfural, and catechol are three typical inhibitors which represent organic acid, aldehyde and alcohol, the three kinds of inhibitors present in dilute acid-treated lignocellulosic hydrolysates, respectively (Oliva et al., 2006). Oleaginous yeast *Trichosporon fermentans* has been shown to be a potential strain for microbial oil production on lignocellulosic hydrolysates (Huang et al., 2009). In this work, the effect of combination of acetic acid, furfural, and catechol on the growth and lipid accumulation of *T. fermentans* were systematically investigated by RSM.

MATERIALS AND METHODS

Microorganism and chemicals

The oleaginous yeast *T. fermentans* CICC 1368 was obtained from the China Center of Industrial Culture Collection and kept on wort agar at 4°C. Furfural was purchased from Sigma (USA). Catechol was purchased from Alfa Aesar (UK). Acetic acid and other chemical compounds were from commercial source and were of the highest purity available.

Medium, precultivation and cultivation

The precultivation medium (pH 6.0) contained glucose and xylose (ratio 2:1) 20 g/l, peptone 10 g/l, yeast extract 10 g/l. And the fermentation medium (pH 6.5) contained glucose and xylose (ratio 2:1) 100 g/l, peptone 1.8 g/l, yeast extract 0.5 g/l, $MgSO_4 \cdot 7H_2O$ 0.4 g/l, KH_2PO_4 2.0 g/l, $MnSO_4 \cdot H_2O$ 0.003 g/l, $CuSO_4 \cdot 5H_2O$ 0.0001 g/l.

The preculture was performed in a 250 ml conical flask containing 50 ml precultivation medium at 28°C for 24 h in a rotary shaker (160 rpm). Seed culture (2.5 ml) was then inoculated to a 250 ml conical flask containing 47.5 ml fermentation medium and the cultivation was carried out at 25°C for 7 days in a rotary shaker (160 rpm).

Effects of inhibitors on growth and lipid accumulation

Seed culture (2.5 ml) prepared on the precultivation medium as described above, was inoculated into 47.5 ml of fermentation medium containing the selected inhibitors. Without adding the tested inhibitor, the biomass, lipid content, lipid yield, and sugar consumption of *T. fermentans* after 7 days' fermentation were 24.0 g/l, 61.7%, 14.8 g/l and 84.3 g/l, respectively. All reported data were averages of experiments performed at least in triplicate.

Experimental design and statistical analysis

A 5-level 3-factor central composite design (CCD) was adopted to evaluate the effects of acetic acid (X_1), furfural (X_2), and catechol (X_3) on the growth and lipid accumulation of *T. fermentans* on a fermentation medium mentioned above and then a model was developed. The highest concentration of these three compounds was about 2-fold greater than the highest concentration that they could be in the common lignocellulosic hydrolysates (Almeida et al., 2007). In this study, the experimental plan contained 20 trials and the independent variables were studied at five different levels, whose values were shown in Table 1.

The fermentation performance was evaluated by using the following fermentation parameters (response *Y*): biomass (g/l), lipid content (%), lipid yield (g/l), and sugar consumption (g/l). The experimental design used in this work was shown in Table 1. The response variable was fitted by a second-order model in order to correlate the response variables to the independent variables. The second order polynomial coefficients were calculated and analyzed using the "Design Expert" software (Version 7.0, Stat-Ease Inc., Minneapolis, USA). The general form of the second-degree polynomial equation is:

$$Y = b + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{123}X_1X_2X_3 + e \quad (1)$$

where Y is the predicted response (biomass, lipid content, lipid yield, and sugar consumption); b stands for offset term; X_1, X_2 and X_3 represent the concentrations (g/l) of factors 1, 2, and 3, respectively; b_1, b_2, and b_3 are the coefficients of linear effects; b_{11}, b_{22} and b_{33} refer to the coefficients for the quadratic effects; b_{12}, b_{13} and b_{23} are the coefficients for the interactions of factors 1 and 2, 1 and 3, and 2 and 3, respectively; and b_{123} is the coefficient for the interaction of factors 1, 2 and 3.

Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). This analysis included Fisher's *F*-test (overall model significance), it's associated probability *p(F)*, correlation coefficient *R*, determination coefficient R^2, which measures the goodness of fit of regression model. For each variable, the quadratic models were represented as contour plots (3D) and response surface curves were generated using the Design Expert software.

Analytical methods

Biomass was harvested by centrifugation and its weight was determined in its lyophilized form. Lipid was extracted with a mixture of chloroform: methanol (2:1, v/v) for 1 h. The extracted lipid was centrifuged to obtain a clear supernatant and the solvent was removed by evaporation under vacuum at 55°C and 100 rpm (NE-Series rotary evaporator EYELA, Japan). Lipid yield is expressed as the amount of lipid extracted from the cells in per liter fermentation broth (g/l) and lipid content is defined as the percentage of lipid to dry biomass (%, w/w).

Sugars were measured by High-performance liquid chromatography (HPLC) (Waters Corp., USA) with a RI detector (Waters 2410) and an Aminex HPX-87P column (300 × 7.8 mm, Bio Rad Corp., USA) at 85°C. Deionized water was used as the mobile phase at 0.5 mL/min.

RESULTS AND DISCUSSION

After lipid fermentation, the actual values and the predicted values of responses were summarized in Table 2. As shown in Table 2, the values for biomass (g/l), lipid

Table 1. Central composite design arrangement.

Design point	Code independent variable level			Factors' concentration		
	A	B	C	Acetic acid (g/l)	Furfural (g/l)	Catechol (g/l)
1	-1	-1	-1	2	0.2	0.16
2	1	-1	-1	8	0.2	0.16
3	-1	1	-1	2	0.8	0.16
4	1	1	-1	8	0.8	0.16
5	-1	-1	1	2	0.2	0.64
6	1	-1	1	8	0.2	0.64
7	-1	1	1	2	0.8	0.64
8	1	1	1	8	0.8	0.64
9	-1.682	0	0	0	0.5	0.4
10	1.682	0	0	10	0.5	0.4
11	0	-1.682	0	5	0	0.4
12	0	1.682	0	5	1	0.4
13	0	0	-1.682	5	0.5	0
14	0	0	1.682	5	0.5	0.8
15	0	0	0	5	0.5	0.4
16	0	0	0	5	0.5	0.4
17	0	0	0	5	0.5	0.4
18	0	0	0	5	0.5	0.4
19	0	0	0	5	0.5	0.4
20	0	0	0	5	0.5	0.4
21 (Control)	-1.682	-1.682	-1.682	0	0	0

content (%, w/w), lipid yield (g/l), and sugar consumption (g/l), obtained in the fermentation experiments, varied with different concentrations of inhibitors. The coefficients of Equation 1 were calculated using regression analysis from the experimental results shown in Table 2. The values of R^2 for biomass, lipid content, lipid yield, and sugar consumption were 0.9904, 0.9633, 0.9826 and 0.9843, respectively, showing a good model fit.

Effect of combination of acetic acid, furfural and catechol on the biomass of *T. fermentans*

The effect of combination of acetic acid, furfural and catechol on the biomass of *T. fermentans* was shown in Table 3. Base on these data, the resulting equation, which predicts the biomass in the linear regression model (1), is expressed as follows:

$$\text{Biomass}=11.40-3.72*A-3.90*B-2.72*C-0.34*A*B+2.28*A*C-0.45*B*C-0.52*A^2-1.75*B^2-0.45*C^2+0.37*A*B*C \quad (2)$$

As can be seen in Table 3, each inhibitor showed great negative effect on the biomass of *T. fermentans*. The inhibitory effect of acetic acid, furfural, and catechol on the growth of different microorganisms has been well known in many works (Palmqvist and Hahn-Hagerdal, 2000) and the results in Table 3 further supported this. Interestingly, the effect of individual inhibitor on the growth of *T. fermentans* was more significant than the effect of binary or ternary combination of these compounds, indicating that no obvious synergistic inhibitory effect existed for these inhibitors. This was in contrast with the phenomenon observed in ethanologenic yeasts that the compounds mentioned above usually have strong synergetic effect on their growth (Palmqvist and Hahn-Hagerdal, 2000).

It is worth noting that the binary interaction of furfural and other two compounds (AB and BC in Table 3) did not show statistically significant effects despite that the coefficients of AB and BC were negative. This means that the simultaneous presence of furfural and acetic acid or catechol in the lignocellulosic hydrolysates might affect little on the growth of *T. fermentans*. Surprisingly, the binary combination of acetic acid and catechol exhibited certain stimulated effect on the growth of *T. femrentans* and the *P* value showed this effect was significant, suggesting the inhibition was relieved in the case of binary combination of these two compounds. Similarly, the effect of ternary combination of these three compounds on the biomass of *T. fermentans* was positive, indicating no synergistic inhibitory effect was occurred. The three-dimensional response surface plots are shown in Figure 1. Despite that the *P*-value of AC was less than 0.0001, the relative flat surface and parallel contour lines reflected that the binary interaction among

Table 2. Actual and predicted value of different responses.

Run	Biomass (g/l)		Lipid content (%)		Lipid yield (g/l)		Sugar consumption (g/l)	
	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	19.1	20.2	51.9	65.2	9.9	11.2	65.6	68.2
2	9.4	9.5	42.0	40.1	3.9	3.9	30.3	32.7
3	14.6	14.7	44.6	45.9	6.5	7.0	46.3	48.8
4	0.4	1.2	2.7	5.7	0.01	0.5	9.1	11.4
5	12.7	11.8	39.9	38.9	5.1	4.8	41.8	43.0
6	9.1	8.8	37.4	38.2	3.4	3.1	26.0	27.0
7	3.3	3.0	9.0	13.0	0.3	0.5	21.1	22.3
8	0.2	0.2	1.5	3.0	0.003	-0.2	6.5	7.5
9	15.7	16.2	52.1	48.5	8.2	7.7	63.2	60.4
10	3.9	3.7	16.3	16.8	0.6	0.9	19.4	17.1
11	12.5	13.0	46.7	47.1	5.8	6.0	42.7	40.1
12	0.2	-0.1	2.2	-1.1	0.004	-0.4	8.5	6.1
13	15.4	14.7	57.0	54.7	8.6	7.8	54.2	50.1
14	4.6	5.6	28.6	28.0	1.3	1.8	25.3	24.4
15	11.8	11.4	49.8	48.8	5.9	5.7	41.3	39.6
16	11.4	11.4	49.5	48.8	5.7	5.7	38.3	39.6
17	10.6	11.4	48.8	48.8	5.2	5.7	35.7	39.6
18	11.6	11.4	48.9	48.8	5.7	5.7	37.0	39.6
19	12.7	11.4	51.8	48.8	6.6	5.7	44.1	39.6
20	11.1	11.4	51.8	48.8	5.8	5.7	40.3	39.6
21	24.0	23.6	61.7	57.2	14.8	14.5	84.3	84.3

Table 3. Analysis of variance (ANOVA) for the quadratic model of biomass.

Source	Sum of Squares	DF	Source Source	F-Value	P-value	Coefficient estimate
Model	772.88	10	77.29	110.02	< 0.0001	11.40
A-Acetic acid	192.98	1	192.98	274.70	< 0.0001	-3.69
B-Furfural	209.25	1	209.25	297.86	< 0.0001	-3.87
C-Catechol	105.37	1	105.37	149.99	< 0.0001	-2.74
AB	0.82	1	0.82	1.16	0.3063	-0.30
AC	45.53	1	45.53	64.81	< 0.0001	2.22
BC	2.30	1	2.30	3.28	0.1003	-0.50
A2	4.04	1	4.04	5.75	0.0375	-0.51
B2	46.00	1	46.00	65.48	< 0.0001	-1.74
C2	2.98	1	2.98	4.24	0.0666	-0.44
ABC	1.28	1	1.28	1.82	0.2067	0.33
Residual	7.03	10	0.70			
Lack of Fit	4.51	5	0.90	1.80	0.2682	
Pure Error	2.51	5	0.50			
Total	779.90	20				

R^2=0.9904; Adj. R^2=0.9808.

the inhibitory compounds showed little synergetic effect on the growth of *T. fermentans* and thus might be beneficial for its lipid production on lignocellulosic hydrolysates.

Effect of combination of acetic acid, furfural and catechol on the lipid accumulation of *T. fermentans*

Besides the effect on the growth, inhibitors could also

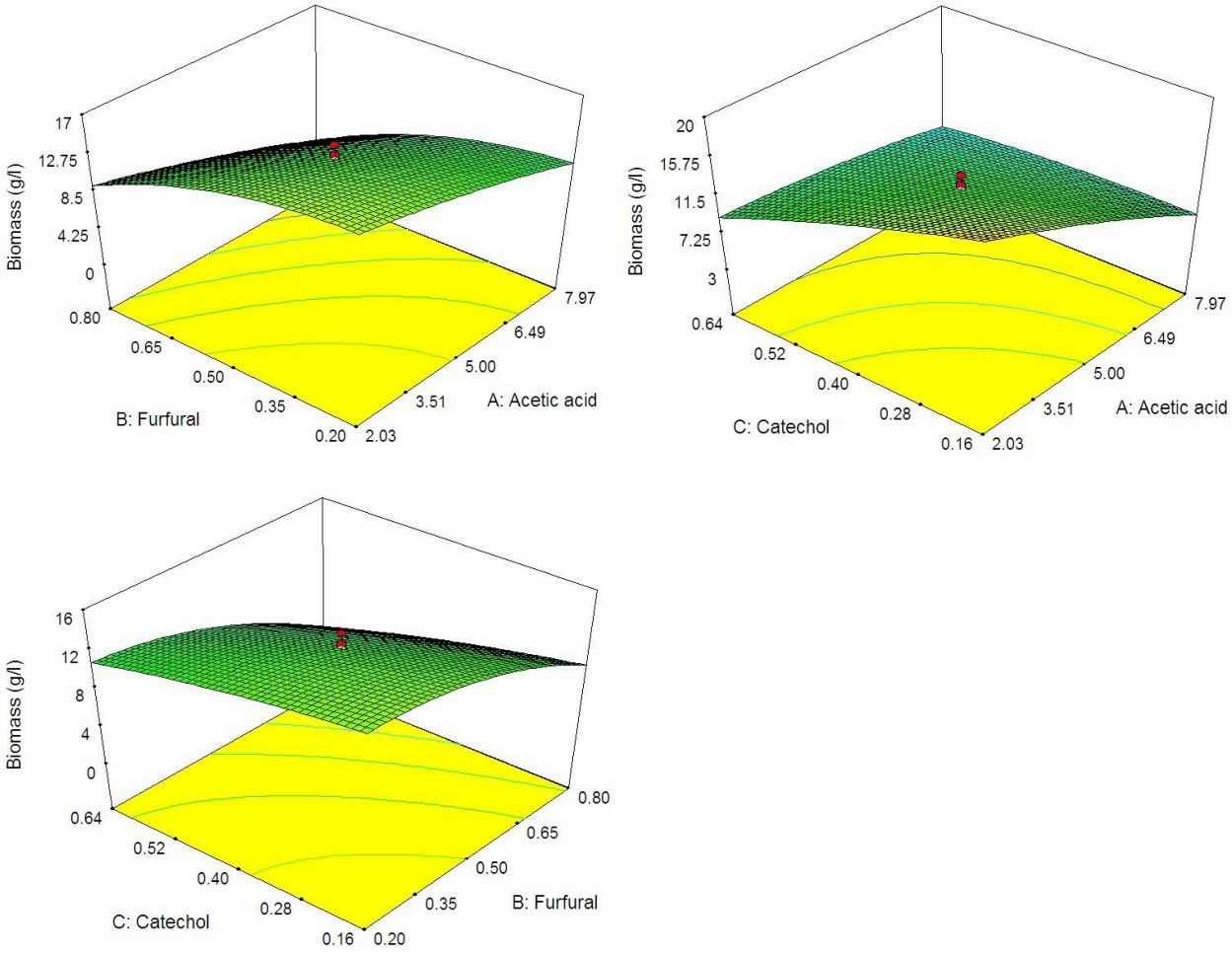

Figure 1. Response surface plots showing binary interaction of different variables on the biomass of *T. fermentans.* Huang et al. (2012).

affect the lipid accumulation of oleaginous yeasts (Hu et al., 2009; Huang et al., 2011). Similar to the effect of combination of acetic acid, furfural and catechol on the biomass, the effect of individual inhibitor on the lipid content of *T. fermentans* was also more significant than the binary or ternary combination of them (as depicted by their *P*-value in Table 4). Among these three inhibitors, furfural (with the highest *F*-value) showed the most significant effect on the lipid content of *T. fermentans* and the effect of catechol was the least (as indicated by its lowest *F*-value). Also, in the binary combination of the inhibitors, the interaction effect between acetic acid and catechol was significant (*P*-value <0.01) while the other two were not. That means most of these inhibitors had little interaction effect, namely, simultaneous existence of these three typical inhibitors in the lignocellulosic hydrolysates would not cause serious synergistic inhibition on the lipid accumulation of *T. fermentans*. It is worth noting that the effect of binary combination of acetic acid and catechol on the lipid accumulation was positive (Table 4), which was similar to their effect on the growth of *T. fermentans*. Also, the ternary combination of these three compounds showed no synergistic effect on the lipid accumulation of *T. fermentans*. The equation which predicts the lipid content is expressed as:

$$\text{Lipid content} = 48.83 - 9.43*A - 14.34*B - 7.96*C - 3.02*A*B + 6.74*A*C - 0.93*B*C - 5.71*A^2 - 9.13*B^2 - 2.65*C^2 + 0.72*A*B*C \quad (3)$$

The three-dimensional response surface plots were shown in Figure 2. Compared with the contours of binary combination of catechol and furfural or acetic acid in Figure 1, the corresponding contours in Figure 2 were obviously more parallel. This suggests that the effect of combination of catechol and furfural or acetic acid were more significant on the growth of *T. fermentans* than that on its lipid accumulation. In contrast, the binary combination of furfural and acetic acid affect less on the growth of *T. fermentans*. The effect of combination of acetic acid, furfural, and catechol on the lipid yield of *T. fermentans* was also analyzed (Table 5). Similar to the effect on the

Table 4. Analysis of variance (ANOVA) for the quadratic model of lipid content.

Source	Sum of Squares	DF	Mean square	F-Value	P-value	Coefficient estimate
Model	7322.35	10	732.24	26.25	< 0.0001	
A-Acetic acid	1257.13	1	1257.13	45.07	< 0.0001	-9.43
B-Furfural	2877.17	1	2877.17	103.15	< 0.0001	-14.34
C-Catechol	887.19	1	887.19	31.81	0.0002	-7.96
AB	83.98	1	83.98	3.01	0.1134	-3.02
AC	418.16	1	418.16	14.99	0.0031	6.74
BC	7.90	1	7.90	0.28	0.6062	-0.93
A2	499.69	1	499.69	17.91	0.0017	-5.71
B2	1270.63	1	1270.63	45.55	< 0.0001	-9.13
C2	106.61	1	106.61	3.82	0.0791	-2.65
ABC	6.20	1	6.20	0.22	0.6476	0.72
Residual	278.94	10	27.89			
Lack of Fit	269.58	5	53.92	28.80	0.0011	
Pure Error	9.36	5	1.87			
Total	7601.29	20				

R^2=0.9633; Adj. R^2=0.9266.

biomass and lipid content, the effect of individual inhibitor was negative to the lipid yield of *T. fermentans*, and the effect was of high significant (*P*-value<0.0001). Different from the effect on the growth and lipid content of *T. fermentans*, the effect of acetic acid on the lipid yield of *T. fermentans* was more significant than that of furfural and catechol (shown by its higher *F*-value). Similarly, only the binary combination of acetic acid and catechol show significant effect on the lipid yield of *T. fermentans* while the other two binary combinations were not. Also the ternary combination of these three compounds had no significant effect on the lipid yield of *T. fermentans*. The equation which predicts the lipid yield is expressed as:

$$\text{Lipid yield} = 5.72 - 2.03 * A - 1.89 * B - 1.78 * C + 0.23 * A * B + 1.41 * A * C + 0.003612 * B * C - 0.51 * A^2 - 1.04 * B^2 - 0.32 * C^2 + 0.041 * A * B * C \quad (4)$$

Figure 3 depicted the three-dimensional response surface plots of response referring to lipid yield. Intestinally, the response surfaces and contours in Figure 3 were similar to that of Figure 1. However, the contours were more parallel and the response surfaces were more flatter than that in Figure 1, indicating that the combination effect among acetic acid, furfural and catechol were more significant on the biomass of *T. fermentans* than on its lipid yield.

Effect of combination of acetic acid, furfural and catechol on the sugar consumption of *T. Fermentans*

Generally, oleaginous microorganisms would continue assimilate the carbon source after their fast exhaustion of limited nitrogen sources at lipid-producing conditions, and then the carbon flux would turn into lipid synthesis (Ratledge, 2004). Hence, the inhibition on sugar utilization might influence both the growth and lipid accumulation of oleaginous microorganisms. In our previous work, the sugar consumption capacity could reflect the effect of the inhibitor on the growth and lipid accumulation of *T. fermentans* (Huang et al., 2011). Thus, the effect of acetic acid, furfural, and catechol on the sugar consumption of *T. fermentans* was systematically measured by RSM.

As shown in Table 6, all these three compounds individually showed significant negative effect on the sugar consumption of *T. fermentans* (*P* value<0.0001). This could help to explain their great inhibitions on the growth and lipid accumulation of *T. fermentans.* Interestingly, only the binary combination of acetic acid and furfural showed negative influence (shown by its negative coefficient), and the binary combination of acetic acid and catechol, furfural and catechol exhibited positive effect on the sugar consumption of *T. fermentans* instead, indicating the interaction of these compounds would not influence the sugar utilization of *T. fermentans* seriously. Similarly, the effect of ternary combination of acetic acid, furfural, and catechol on the sugar consumption of *T. fermentans* was positive, suggesting there is no synergistic effect among these three typical inhibitors present in lignocellulosic hydrolysates. Like the situation of the effect on the biomass, lipid content, and lipid yield, merely the binary combination of acetic acid and catechol showed significant effect on the sugarconsumption of *T. fermentans*, suggesting that the synergetic effect of these compounds was little. The equation which predicts the sugar consumption is

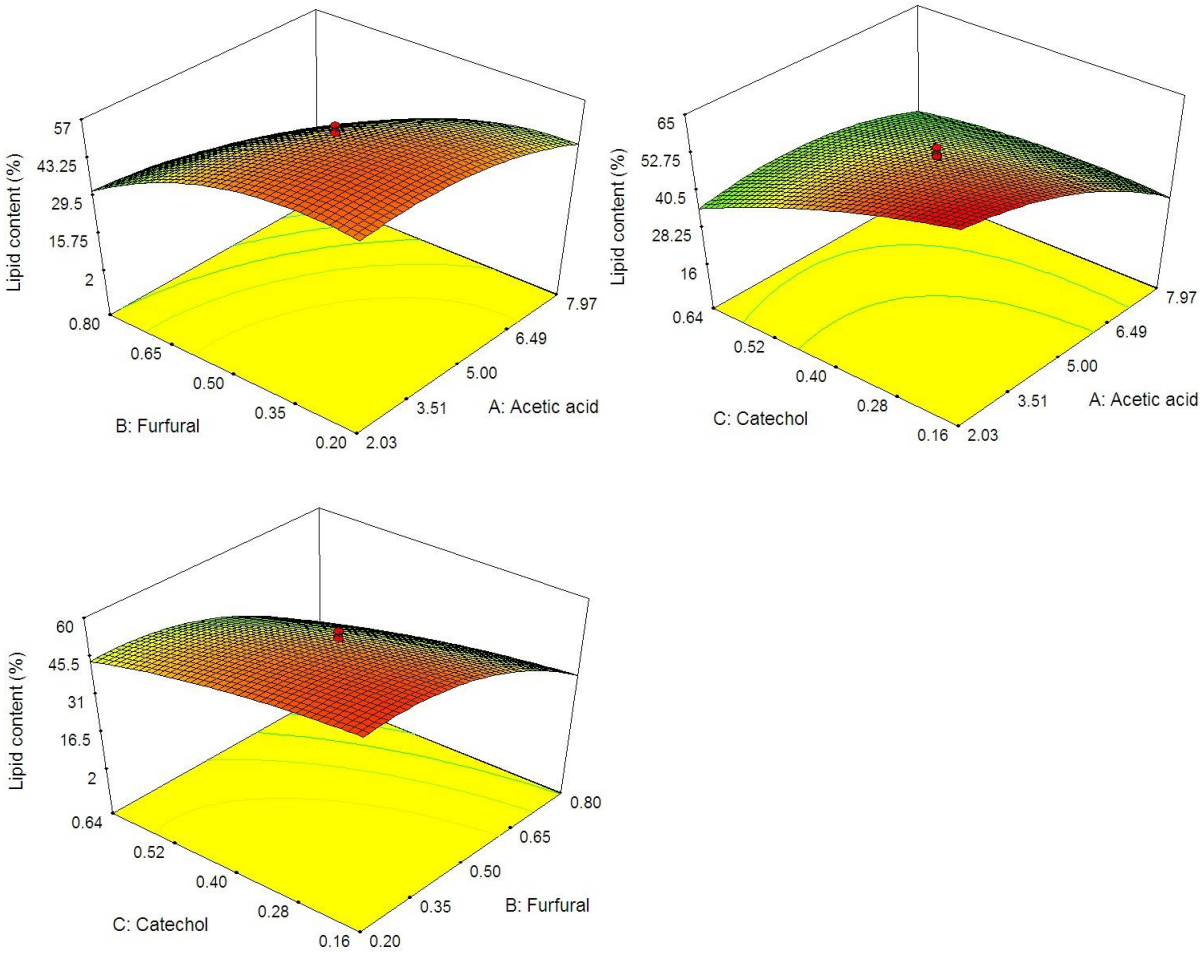

Figure 2. Response surface plots showing binary interaction of different variables on the lipid content of *T. fermentans*. Huang et al. (2012).

Table 5. Analysis of variance (ANOVA) for the quadratic model of lipid yield.

Source	Sum of Squares	DF	Mean square	F-Value	P-value	Coefficient estimate
Model	279.83	10	27.98	56.45	< 0.0001	5.72
A-Acetic acid	58.02	1	58.02	117.03	< 0.0001	-2.03
B-Furfural	49.92	1	49.92	100.69	< 0.0001	-1.89
C-Catechol	44.46	1	44.46	89.68	< 0.0001	-1.78
AB	0.48	1	0.48	0.96	0.3491	0.23
AC	18.42	1	18.42	37.16	0.0001	1.41
BC	0.00	1	0.00	0.00	0.9880	0.00
A2	4.04	1	4.04	8.16	0.0171	-0.51
B2	16.52	1	16.52	33.32	0.0002	-1.04
C2	1.53	1	1.53	3.09	0.1091	-0.32
ABC	0.02	1	0.02	0.04	0.8427	0.04
Residual	4.96	10	0.50			
Lack of Fit	3.93	5	0.79	3.82	0.0838	
Pure Error	1.03	5	0.21			
Total	284.79	20				

R^2= 0.9826; Adj. R^2=0.9652.

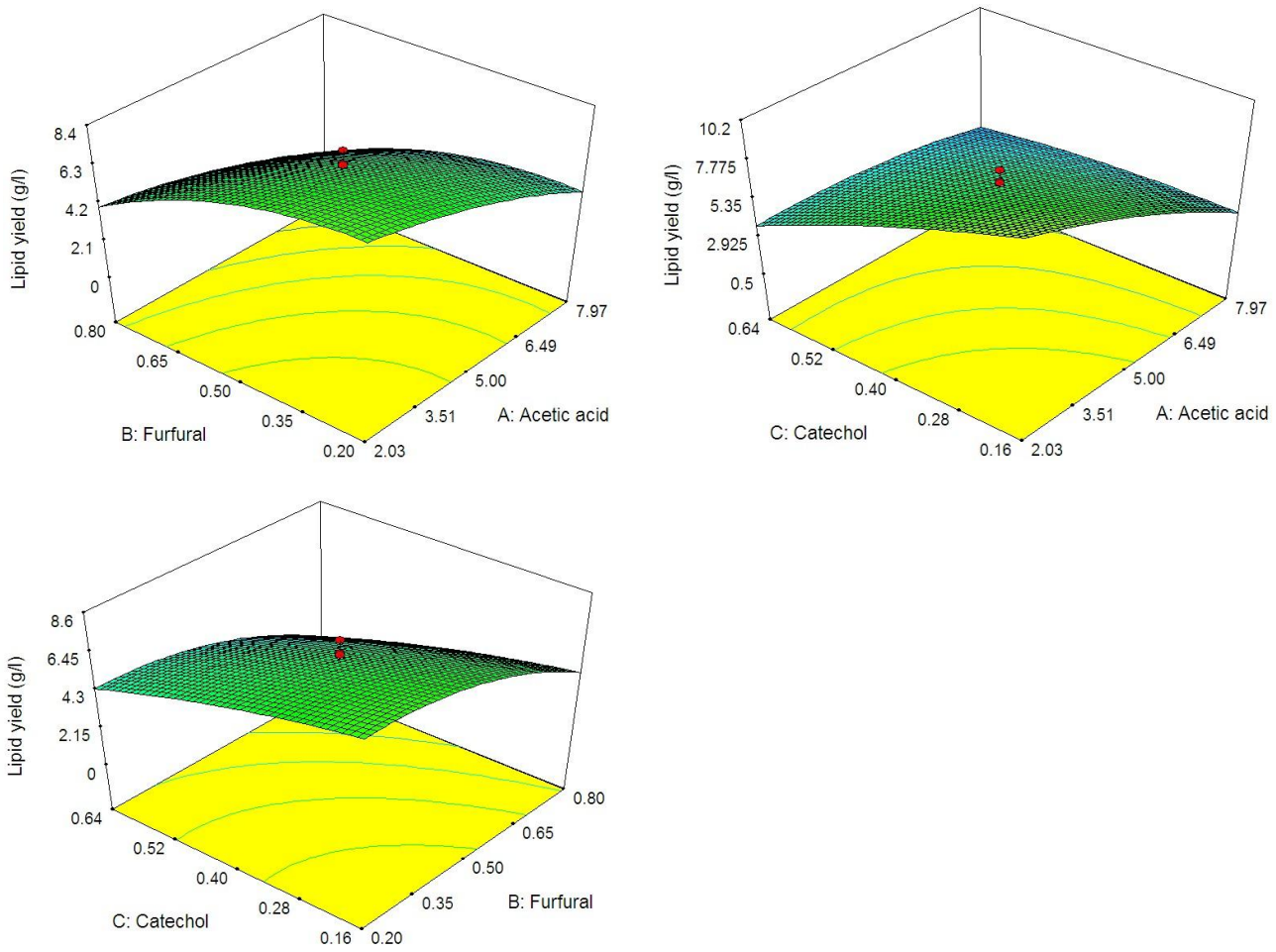

Figure 3. Response surface plots showing binary interaction of different variables on the lipid yield of *T. fermentans.* Huang et al. (2012).

Table 6. Analysis of variance (ANOVA) for the quadratic model of sugar consumption.

Source	Sum of Squares	DF	Mean square	F-Value	P-value	Coefficient estimate
Model	7528.43	10	752.84	62.85	< 0.0001	
A-Acetic acid	2339.38	1	2339.38	195.29	< 0.0001	-12.86
B-Furfural	1434.22	1	1434.22	119.73	< 0.0001	-10.12
C-Catechol	820.01	1	820.01	68.45	< 0.0001	-7.65
AB	0.06	1	0.06	0.00	0.9452	-0.08
AC	250.91	1	250.91	20.95	0.0010	5.22
BC	0.02	1	0.02	0.00	0.9703	0.04
A2	1.42	1	1.42	0.12	0.7380	-0.30
B2	519.26	1	519.26	43.35	< 0.0001	-5.84
C2	10.64	1	10.64	0.89	0.3681	-0.84
ABC	1.68	1	1.68	0.14	0.7156	0.37
Residual	119.79	10	11.98			
Lack of Fit	72.64	5	14.53	1.54	0.3235	
Pure Error	47.16	5	9.43			
Total	7648.22	20				

R^2= 0.9843; Adj. R^2=0.9687.

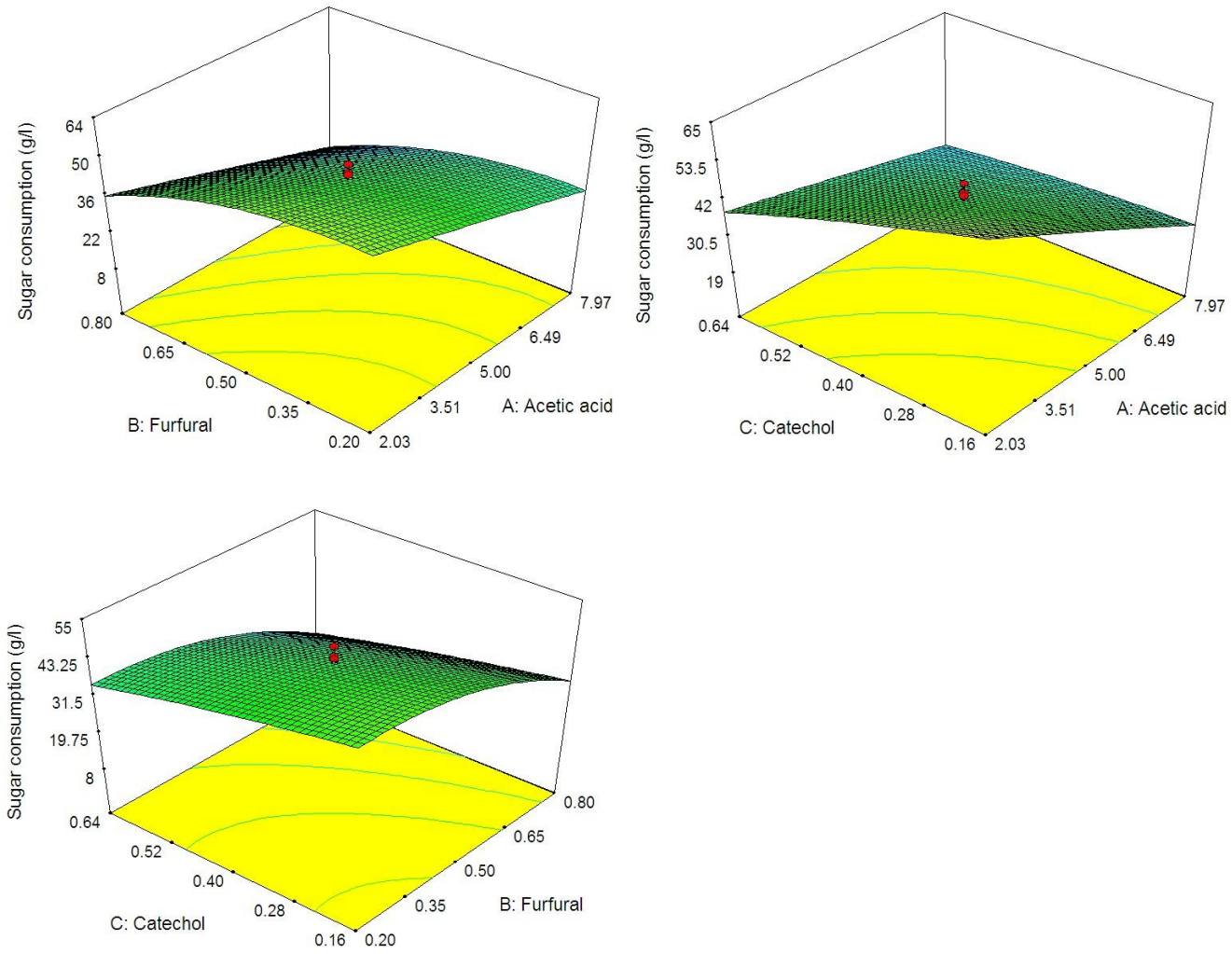

Figure 4. Response surface plots showing binary interaction of different variables on the sugar consumption of *T. fermentans*. Huang et al. (2012).

expressed as:

$$\text{Sugar consumption} = 39.60 - 12.86 * A - 10.12 * B - 7.65 * C - 0.080 * A * B + 5.22 * A * C + 0.044 * B * C - 0.30 * A^2 - 5.84 * B^2 - 0.84 * C^2 + 0.37 * A * B * C \quad (5)$$

The three-dimensional response surface plots of responses were depicted in Figure 4. All these curves were similar to that in Figure 1. The flat response surfaces indicated the interaction effect among different inhibitors on the sugar consumption of *T. fermentans* was less significant than their individual effect.

Conclusions

The inhibitory laws of these inhibitors (acetic acid, furfural, and catechol) including their individual, binary, and ternary combinations on the biomass, lipid content, lipid yield and sugar consumption of *T. fermentans* are similar. There was little synergistic inhibition on the growth, lipid accumulation, and sugar metabolism of *T. fermentans* among these typical inhibitors. These results show that the complex effect of combination of many inhibitors on the growth and lipid accumulation of oleaginous microorganisms could be evaluated in a relatively simple way by RSM.

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