African Journal of Biotechnology Vol. 9(50), pp. 8661-8666, 13 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.833
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Investigating the effects of two lignocellulose degradation by-products (furfural and acetic acid) on ethanol fermentations by six ethanologenic yeast strains

Wu Zheng-yun[1,2], Deng Yu[2], Tan Li[1], Liu Yue-hong[1], Zang Yi-jie[1] and Zhang Wen-xue[1]*

[1]Department of Food Engineering, College of Light Industry and Food Engineering, Sichuan University, Chengdu 610065, China.
[2]Biogas Institute of Ministry of Agriculture, Chengdu 610041, China.

Accepted 2 November, 2010

The effects of two lignocellulose degradation by-products (furfural and acetic acid) on ethanol fermentations by six ethanologenic yeast strains were investigated. It was found that 1.5 g/l of furfural and 4.8 g/l of acetic acid, especially the latter, inhibited cell growth and increased ethanol yield, significantly. On the other hand, different yeast strains have different cell growth rate but very similar ethanol yield. This suggests that more attention should be put on finding strains with relatively high growth rate to enhance the bioethanol productivity. Among the tested yeast strains, 1300 exhibited the highest growth rate, thus can be a promising candidate for mass production of bioethanol. Three important operation parameters: temperature, pH value and inoculum size within investigated range (temperature 28 - 32℃; pH 4.5 - 6.5; and inoculum size 5 - 10%) did not significantly affect ethanol fermentation by strain 1300 with the existence of inhibitors furfural and acetic acid.

Key words: Lignocellulose degradation inhibitor, furfural, acetic acid, bioethanol, yeast.

INTRODUCTION

Bioethanol is one of the most promising sustainable fuels. As a cheap and widespread carbon resource for ethanol fermentation, lignocelluse material received increasing interest in recent years (Chen et al., 2009). A hindrance for further development of lignocelluse-based fermentation, however, is that some inhibitive compounds will be produced during the degradation of lignocelluse material, among which acetic acid and furfural are two representative inhibitors (Chen et al., 2009; Alves et al., 2002; Davis et al., 2005). A lot of efforts have been put on eliminating the negative effect of these inhibitions on fermentation, including removing the inhibitors from the lignocelluse hydrolyzate (de Mancilha and Karim, 2003), screening inhibitor-tolerant strains (Chen et al., 2009), optimizing operation parameters for lignocelluse degradation and fermentation (Carvalheiro et al., 2004).

Furfural and acetic acid are not only two inhibitors, but can also be consumed by the yeast during fermentation (Carvalheiro et al., 2004). The ability of yeast to tolerate furfural was suggested to be directly coupled to the ability to convert furfural to less inhibitory compounds (Horvath et al., 2003). As for acetic acid, it can be utilized as a carbon resource and also leads the variation of pH value during the fermentation. These make the effect of the inhibitors rather complicated. Additionally, the effects of a certain lignocellulose degradation inhibitor for different strains are not always the same (Keating et al., 2006; Larsson et al., 1999), the mechanisms for which was attributed to the different influence on carbon metabolism (Hristozova et al., 2006; Gorsich et al., 2006; Lin et al., 2009) and nitrogen assimilation (Hristozova et al., 2008)

*Corresponding author. E-mail: zwxtl@mail.sc.cninfo.net. Tel: +86-021-85401785. Fax: +86-028-85405137.

Table 1. Factors and levels adopted in the L_9 (3^4) orthogonal test.

Level	Factor		
	Temperature (°C)	pH value	Inoculum size (%)
1	28	4.5	5
2	30	5.5	8
3	32	6.5	10

by the inhibitor. Finding the difference of the response to the inhibitors by different strains is not only helpful for selecting excellent strains for ethanol industry, but also useful for further understanding the mechanisms of the yeasts under stress (Liu et al., 2005).

The purpose of this study, therefore, is to compare the kinetics of ethanol fermentations by six ethanologenic yeast strains with the existence of furfural and aceticacid, find inhibitor-tolerance strain and then investigate the effect on ethanol fermentation by some important operation parameters with the existence of inhibitors.

MATERIALS AND METHODS

Strains

Six yeast strains: *Saccharomyces shochu* LI-E12 (a protoplast fusion strain of Japanese Shochu-producing yeast S20E12 and Chinese liquor-producing yeast LI1-1), *Saccharomyces Sake* Q77 (a Japanese sake-producing yeast), *Saccharomyces* sp. K211 (a ethanolgenic yeast donated by Yalian Co. Ltd.), *Saccharomyces* sp. J-S1 (a Chinese liquor-producing yeast isolated from *Daqu*), *Saccharomyces* sp 1300 (an ethanolgenic yeast purchased) and *Candida* LI8 (a Chinese liquor-producing yeast isolated from a fermentation pit) were used in this study. All these strains exhibited high ethanol productivity in previous assessment.

Cultivations

The reserved yeast strains were first activated by cultivating them on 2% yeast extract peptone dextrose (YPD) medium slope at 28°C for two days. A loop of the activated seed for each strain was then inoculated into 250-ml flasks containing 50 ml of rice starter juice (sugar degree 10°Bx) and cultivated at 28°C and 150 rpm for 24 h.

Fifty milliliters of the culture was then transferred to 450 ml sterilized 10% YPD medium supplemented with 1.5 g/l of furfural and 4.8 g/l of acetic acid, respectively, while the medium containing no furfural and acetic acid was used as the control. Furfural and acetic acid concentrations used in this study were determined referring to previous reported effective ranges of these inhibitors for *Saccharomyces cerevisiae* (Klinke et al., 2003; Martin et al., 2002; Martin et al., 2002). The inoculated flasks was wrapped with fresh-keeping film and cultivated at 28°C until stable stage of cell growth was attained.

A L_9 (3^4) orthogonal test was used for investigating the effect of operation parameters. The factors and levels adopted for the orthogonal test are listed in Table 1. In this test, 10% YPD medium supplemented with 1.5 g/l of furfural and 4.8 g/l of acetic acid was used. Other cultivation parameters are the same as mentioned earlier.

Analytical methods

The cultivated flasks were weighted every day until their weight became almost stable. The gas production was calculated by the decrease of weight compared with the initial flask weight.
After the cultivation, part of the fermented culture was distilled and subjected to ethanol measurement; the other part of the fermented liquor was used to measure the residual sugar and to count the number of cells.

Reduced sugar was analyzed using the dinitrosalicylic acid (DNS) method. The cell number of yeast was counted by using hymetocyte plate method. The ethanol produced was determined directly using an alcoholometer (Liminju Glass Instrument Company, Hejian city, Hebei Province) after the residue was distilled. Ethanol yield was calculated as follows:

$$\text{Ethanol yield (\%)} = \frac{\text{Actual ethanol formed (g)}}{\text{Theoretical ethanol formed (g)}} \times 100 \quad (1)$$

Mathematic model

Considering the sigmoid shape of the gas production curves, logistic model (1) was used to fit the gas production data:

$$X = \frac{X_0 e^{\mu_m t}}{1 - \frac{X_0}{X_f}(1 - e^{\mu_m t})} \quad (2)$$

where X is the gas produced (g) at a certain fermentation time, t is the fermentation time (h), X_0, X_f and μ_m are three kinetic parameters.

Referring to the physical meaning of the logistic model (Goudar et al., 2005), X_0 reflects the initial gas produced (g), X_f represents the final gas produced (g), while μ_m is the maximum (also the initial) specific rate of gas produced (h^{-1}) which can be expressed by:

$$\mu_m = \left(\frac{1}{X} \cdot \frac{dX}{dt}\right)_{\max} \quad (3)$$

As the cell growth and gas production are almost in proportion at the initial stage of ethanol fermentation by yeast (Heitmann et al., 1996), parameter μ_m can be roughly regarded as an equivalent of the maximum specific cell growth of the yeast (h^{-1}).

Statistical analysis

Student Newman-Keuls test and factorial analysis were performed by using SAS 6.12 (SAS Institute Inc. USA).

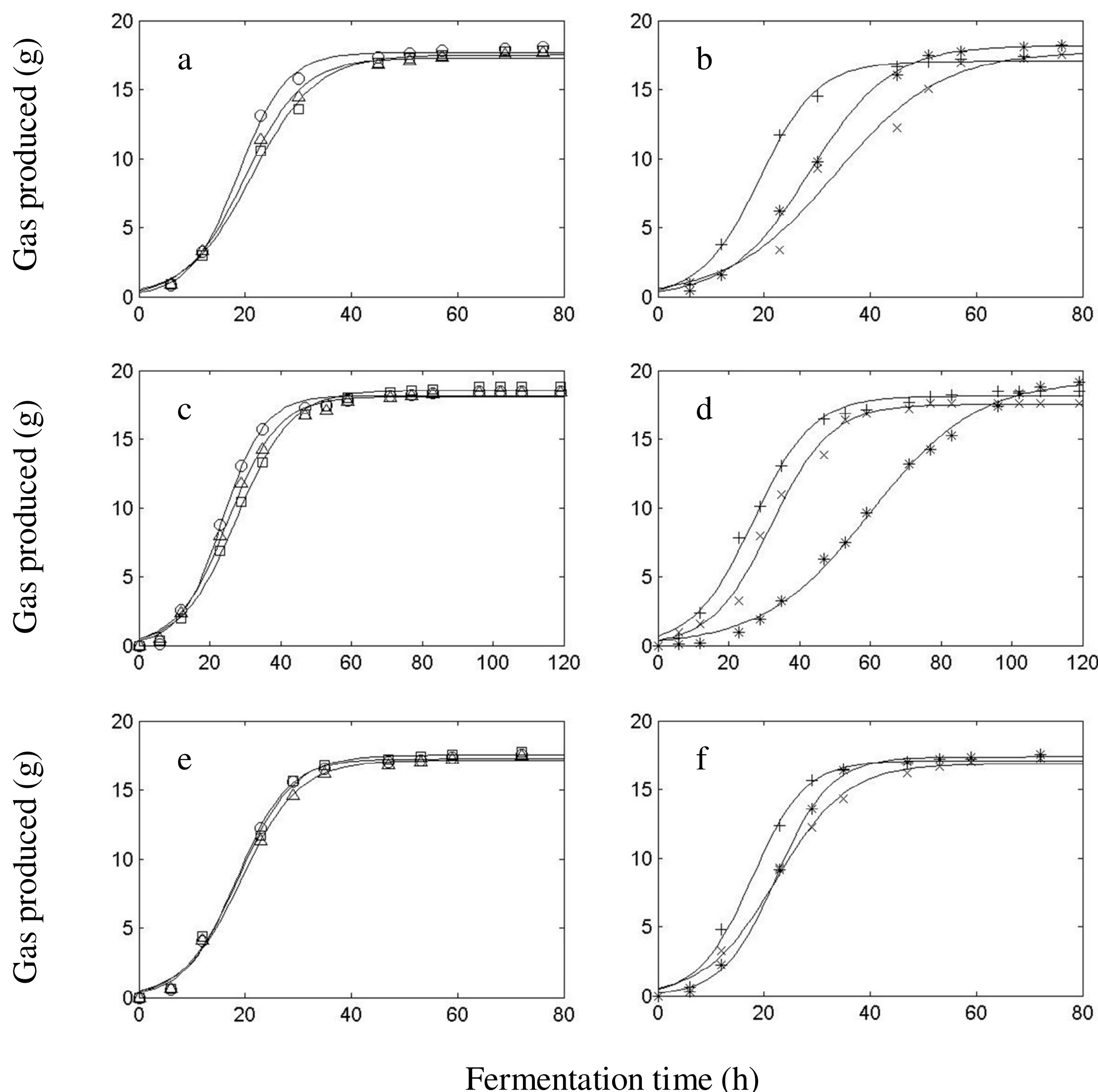

Figure 1. Time courses of gas productions by different yeast strains in 10% YPD medium supplemented with 1.5 g/l furfural (a and b), 4.8 g/l acetic acid (c and d) and the control (e and f). □, LI-E12; , LI8; ○; 1300; *, Q77; +, JS1; ×, K211; curves are results calculated by model.

RESULTS AND DISCUSSION

Ethanol fermentations by different yeast strains in medium supplemented with furfural and acetic acid

Figure 1 demonstrates the time courses of gas production by different yeast strains in medium supplemented with inhibitors and the control. On the whole, the gas produced increased with the extending of cultivation time, and tended to be level off finally. By fitting experimental data, parameters of logistic model (1) were estimated for each cultivation, respectively. All curves were fitted satisfactorily by the model (Figure 1).

The maximum specific growth rate, final gas produced, ethanol yield, cell number and residual sugar of different yeast strains in medium with and without inhibitors are summarized in Table 2. The first two indexes were obtained by model fitting as describe earlier, while the latter three indexes were directly assessed after the cultivations. For different strains and inhibitors, all these indexes varied in a rather complicated manner.

A factorial analysis was performed to understand the effects on these indexes by inhibitor and strain (Table 3). The result shows that the maximum specific growth rate and final gas produced was significantly affected by both inhibitor and strain; while the ethanol yield and residual sugar were mainly influenced by inhibitor. The variation of cell number, however, can be attributed neither to the inhibitor nor the strain.

According to Tables 2 and 3, both furfural and acetic

Table 2. The maximum specific growth rate, final gas produced, ethanol yield, cell number and residual sugar of different yeast strains in medium with inhibitors and the control.

Inhibitor supplemented	Strain	Maximum specific growth rate (μ_m, h^{-1})	Final gas produced (X_f, g)	Ethanol yield (%)	Cell number ($\times 10^8$)	Residual sugar (g/100ml)
Furfural (1.5 g/l)	LI-E12	0.16 ± 0.02^{abcde}	17.52 ± 0.12^{e}	74.06 ± 0.21^{f}	1.72 ± 0.04^{n}	0.28 ± 0.01^{abc}
	LI8	0.18 ± 0.02^{abcd}	17.30 ± 0.01^{f}	77.86 ± 0.01^{c}	1.84 ± 0.01^{m}	0.27 ± 0.01^{abc}
	1300	0.22 ± 0.01^{a}	17.71 ± 0.01^{d}	73.43 ± 0.03^{g}	4.44 ± 0.02^{a}	0.26 ± 0.01^{abc}
	Q77	0.13 ± 0.01^{cde}	18.19 ± 0.01^{c}	69.63 ± 0.03^{m}	3.44 ± 0.01^{d}	0.25 ± 0.01^{bc}
	JS1	0.18 ± 0.03^{abcd}	17.06 ± 0.01^{h}	74.70 ± 0.05^{e}	2.76 ± 0.01^{h}	0.28 ± 0.01^{abc}
	K211	0.10 ± 0.01^{ef}	17.78 ± 0.03^{d}	74.70 ± 0.03^{e}	1.74 ± 0.01^{n}	0.27 ± 0.03^{abc}
Acetic acid (4.8 g/l)	LI-E12	0.13 ± 0.01^{cde}	18.57 ± 0.05^{b}	76.59 ± 0.01^{d}	1.94 ± 0.01^{l}	0.35 ± 0.01^{a}
	LI8	0.14 ± 0.01^{cde}	18.11 ± 0.03^{c}	74.70 ± 0.04^{e}	3.04 ± 0.02^{f}	0.30 ± 0.01^{abc}
	1300	0.17 ± 0.01^{abcd}	18.17 ± 0.01^{c}	79.76 ± 0.02^{b}	1.26 ± 0.01^{p}	0.32 ± 0.01^{abc}
	Q77	0.07 ± 0.01^{f}	19.32 ± 0.03^{a}	74.70 ± 0.03^{e}	1.38 ± 0.03^{o}	0.33 ± 0.03^{ab}
	JS1	0.12 ± 0.01^{def}	18.14 ± 0.01^{c}	81.03 ± 0.03^{a}	3.78 ± 0.03^{c}	0.31 ± 0.03^{abc}
	K211	0.12 ± 0.01^{def}	17.55 ± 0.03^{e}	72.80 ± 0.03^{h}	2.32 ± 0.01^{i}	0.34 ± 0.01^{ab}
Control	LI-E12	0.20 ± 0.01^{abc}	17.38 ± 0.01^{f}	70.90 ± 0.03^{k}	3.14 ± 0.01^{e}	0.25 ± 0.01^{bc}
	LI8	0.18 ± 0.01^{abcd}	17.14 ± 0.01^{gh}	68.37 ± 0.01^{n}	2.16 ± 0.01^{j}	0.22 ± 0.02^{c}
	1300	0.21 ± 0.01^{ab}	17.25 ± 0.04^{fg}	72.16 ± 0.01^{i}	2.06 ± 0.03^{k}	0.24 ± 0.01^{bc}
	Q77	0.19 ± 0.01^{abcd}	18.07 ± 0.03^{c}	70.90 ± 0.04^{k}	2.90 ± 0.10^{g}	0.24 ± 0.01^{bc}
	JS1	0.20 ± 0.01^{abc}	17.10 ± 0.04^{h}	70.58 ± 0.03^{l}	3.88 ± 0.03^{b}	0.22 ± 0.03^{c}
	K211	0.15 ± 0.01^{bcde}	16.89 ± 0.06^{i}	71.53 ± 0.03^{j}	2.88 ± 0.04^{g}	0.26 ± 0.03^{abc}

For the same column, there are significant differences among values with different superscripts ($p < 0.05$). All experiments were carried out in triplicate.

Table 3. Factorial analyses of the effects on the maximum specific growth rate, final gas produced, ethanol yield, cell number and residual sugar by inhibitor and strain.

Factor	Maximum specific growth rate	Final gas produced	Ethanol yield	Cell number	Residual sugar
Inhibitor	++	++	++	-	++
Strain	+	++	-	-	-

++, $p < 0.01$; +, $p < 0.05$; -, $p > 0.05$.

acid, especially the later, significantly decreased the specific cell growth rate and increased the final gas produced, the residual sugar and ethanol yield. The decrease of cell growth rate by these inhibitors has been reported by many researchers (Couallier et al., 2006; Gutierrez et al., 2002; Helle et al., 2003; Duarte et al., 2005). However, the significant enhancement of ethanol yield by the inhibitors was seldom reported so far. A lot of researchers observed that ethanol yield were unaffected (Keating et al., 2006; Gutierrez et al., 2002; Diaz et al., 2009) or even decreased (Larsson et al., 1999; Helle et al., 2003) by these inhibitors.

Table 3 also indicates that for ethanol fermentation by different strains, the maximum specific growth rate and the final gas produced were significantly different; while their ethanol yields were rather similar. The latter might be explained by fact that the ethanol yield was determined more by the glucose concentration in the medium than by the strain applied. As the ethanol yields are similar for different strains, the ethanol productivity will then depend mainly on the cell growth rate (higher growth rate results in shorter fermentation period, Figure 1). Therefore, in screening yeast strain for bioethanol production with the existence of inhibitors, it seems that more attention should be put on finding strains with relatively high growth rate to enhance the bioethanol productivity.

Among the tested strains, 1300 demonstrated comparatively high specific growth rate when different inhibitors existed. This character is useful for ethanol production based on cellulose material. Therefore, this strain was used in further investigating the effects on ethanol fermentation by three important operation parameters, namely temperature, pH value and inoculum size.

Table 4. The effects of temperature, pH value and inoculum size on ethanol fermentation by yeast strain 1300 using orthogonal test.

Level of factor			Maximum specific growth rate (μ_m, h^{-1})	Final gas produced (X_f, g)	Ethanol yield (%)
Temperature	pH	Inoculum size			
1	1	1	0.13	17.13	70.90
1	2	2	0.15	16.45	63.93
1	3	3	0.17	16.15	68.37
2	1	2	0.17	17.06	76.59
2	2	3	0.16	17.05	69.00
2	3	1	0.16	16.63	67.73
3	1	3	0.18	17.30	72.16
3	2	1	0.17	16.78	72.16
3	3	2	0.16	16.67	71.53

Table 5. The effect of maximum specific rate, final gas produced and ethanol yield on the fermentation of strain 1300 by adding furfural and acetic acid simultaneously when compared with those in the fermentations in which each inhibitor alone and the control is added.

Treatment	Maximum specific growth rate	Final gas produced	Ethanol yield
Control	↓	-	-
Adding acetic acid	-	↓	↓
Adding furfural	↓	↓	-

↓, significantly decreased ($p < 0.05$); -, no significant difference ($p > 0.05$).

Investigating the effects of temperature, pH value and inoculum size on ethanol fermentation by strain 1300 using orthogonal experimental test

An orthogonal test was performed to find out how temperature, pH value and inoculum size affects ethanol fermentation by 1300 cultivated in medium with furfural and acetic acid. For all treatments, the gas production curves were similar to those shown in Figure 1 (details not shown). Table 4 summarized the treatments and corresponding maximum specific growth rate, final gas produced and the ethanol yield. These indexes were obtained using the same method as mentioned previously. Factorial analysis shows that none of the three operation parameters had significant effect on these indexes ($p > 0.05$) within the investigated range.

We also tried to find whether there are any differences between the fermentation with the existence of the combined inhibitors and the fermentation with the existence of each inhibitor, respectively. Variation analyses of the maximum specific rate, final gas produced and ethanol yield for the fermentation by strain 1300 under different conditions were performed (Table 5). The result shows that the maximum specific growth rate is significantly lower in treatment with the combined inhibitor than the control (treatment with neither furfural nor acetate acid) and the treatment with furfural; but was similar to the treatment with acetate acid. This again verified that in comparison with furfural, acetic acid has a more important reason for the decrease of cell growth rate. The final gas produced in the treatment with the combined inhibitor was similar to the control but significantly lower than the treatment with either furfural or acetate acid alone. The ethanol yield of the treatment with the combined inhibitor is similar to the control and the treatment with furfural, but significantly lower than that with acetic acid. This implies that even acetate acid can significantly increase ethanol yield, the effect may be lessen by the existence of furfural. Previous research (Hu et al., 2009) demonstrated that inhibition effects caused by many lignocellulose degradation inhibitors including acetate and furfural, generally were additive. Our result, however, suggests that interaction effects on ethanol fermentation by these two inhibitors may also need to be considered in some cases.

Conclusion

The results of this study revealed that the two lingocellulose degradation by-products: furfural and acetic acid, especially the latter, inhibited cell growth and increased ethanol yield, significantly. On the other hand, different yeast strains have different cell growth rate, but very similar ethanol yield, suggesting that more attention should be put on finding strains with high growth rate to enhance the bioethanol productivity. Among the tested yeast strains, 1300 exhibited the highest specific growth

rate, thus can be a promising candidate for mass production of bioethanol. The three important operation parameters: temperature, pH value and inoculum size within investigated range do not have significant effect on ethanol fermentation by strain 1300 with the existence of inhibitors: furfural and acetic acid.

ACKNOWLEDGEMENTS

This work was financially supported by Science and Technology Bureau of Sichuan province, China (International Science and Technology Cooperation Program No. 07HH015-009), Ministry of Science and Technology of the People's Republic of China (International Science and Technology Cooperation Program No. 2009-DFA60890) and Open Fund from Biogas Institute of Ministry of Agriculture, People's Republic of China (2009002).

REFERENCES

Alves LA, Vitolo M, Felipe MGA, Silva JBDE (2002). Xylose reductase and xylitol dehydrogenase activities of *Candida guilliermondii* as a function of different treatments of sugarcane bagasse hemicellulosic hydrolysate employing experimental design. Appl. Biochem. Biotechnol. 98: 403-413.

Carvalheiro F, Duarte LC, Medeiros R, Girio FM (2004). Optimization of brewery's spent grain dilute-acid hydrolysis for the production of pentose-rich culture media. Appl. Biochem. Biotechnol. 113: 1059-1072.

Chen X, Li ZH, Zhang XX, Hu FX, Ryu DDY, Bao J (2009). Screening of oleaginous yeast strains tolerant to lignocellulose degradation compounds. Appl. Biochem. Biotechnol. 159: 591-604.

Couallier EM, Payot T, Bertin AP, Lameloise ML (2006). Recycling of distillery effluents in alcoholic fermentation-Role in inhibition of 10 organic molecules. Appl. Biochem. Biotechnol. 133: 217-237.

Davis L, Jeon YJ, Svenson C, Rogers P, Pearce J, Peiris P (2005). Evaluation of wheat stillage for ethanol production by recombinant *Zymomonas mobilis*. Biomass Bioenergy, 29: 49-59.

de Mancilha IM, Karim MN (2003). Evaluation of ion exchange resins for removal of inhibitory compounds from corn stover hydrolyzate for xylitol fermentation. Biotechnol. Progr. 19: 1837-1841.

Diaz MJ, Ruiz E, Romero I, Cara C, Moya M, Castro E (2009). Inhibition of *Pichia stipitis* fermentation of hydrolysates from olive tree cuttings. World J. Microbiol. Biotechnol. 25: 891-899.

Duarte LC, Carvalheiro F, Neves I, Girio FM (2005). Effects of aliphatic acids, furfural, and phenolic compounds on *Debaryomyces hansenii* CCMI 941. Appl. Biochem. Biotechnol. 121: 413-425.

Gorsich SW, Dien BS, Nichols NN, Slininger PJ, Liu ZL, Skory CD (2006). Tolerance to furfural-induced stress is associated with pentose phosphate pathway genes ZWF1, GND1, RPE1, and TKL1 in *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 71: 339-349.

Goudar CT, Joeris K, Konstantinov KB, Piret JM (2005). Logistic equations effectively model mammalian cell batch and fed-batch kinetics by logically constraining the fit. Biotechnol. Progr. 21: 1109-1118.

Gutierrez T, Buszko ML, Ingram LO, Preston JF (2002). Reduction of furfural to furfuryl alcohol by ethanologenic strains of bacteria and its effect on ethanol production from xylose. Appl. Biochem. Biotechnol. 98: 327-340.

Heitmann T, Wenzig E, Mersmann A (1996). A kinetic model of growth and product formation of the anaerobic microorganism *Thermoanaerobacter thermohydrosulfuricus*. J. Biotechnol. 5: 213-223.

Helle S, Cameron D, Lam J, White B, Duff S (2003). Effect of inhibitory compounds found in biomass hydrolysates on growth and xylose fermentation by a genetically engineered strain of S-cerevisiae. Enzyme Microb. Technol. 33: 786-792.

Horvath IS, Franzen CJ, Taherzadeh MJ, Niklasson C, Liden G (2003). Effects of furfural on the respiratory metabolism of *Saccharomyces cerevisiae* in glucose-limited chemostats. Appl. Environ. Microbiol. 69: 4076-4086.

Hristozova T, Angelov A, Tzvetkova B, Paskaleva D, Gotcheva V, Gargova S, Pavlova K (2006). Effect of furfural on carbon metabolism key enzymes of lactose-assimilating yeasts. Enzyme Microb. Technol. 39: 1108-1112.

Hristozova T, Gotcheva V, Tzvetkova B, Paskaleva D, Angelov A (2008). Effect of furfural on nitrogen assimilating enzymes of the lactose utilizing yeasts *Candida blankii* 35 and *Candida pseudotropicalis* 11. Enzyme Microb. Technol. 43: 284-288.

Hu CM, Zhao X, Zhao J, Wu SG, Zhao ZBK (2009). Effects of biomass hydrolysis by-products on oleaginous yeast *Rhodosporidium toruloides*. Bioresour. Technol. 100: 4843-4847.

Klinke HB, Olsson L, Thomsen AB, Ahring BK (2003). Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production of *Saccharomyces cerevisiae*: wet oxidation and fermentation by yeast. Biotechnol. Bioeng. 81: 738-747.

Larsson S, Palmqvist E, Hahn-Hagerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvebrant NO (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb. Technol. 24: 151-159.

Lin FM, Qiao B, Yuan YJ (2009). Comparative Proteomic Analysis of Tolerance and Adaptation of Ethanologenic *Saccharomyces cerevisiae* to Furfural, a Lignocellulosic Inhibitory Compound. Appl. Environ. Microbiol. 75: 3765-3776.

Liu ZL, Slininger PJ, Gorsich SW (2005). Enhanced biotransformation of furfural and hydroxymethylfurfural by newly developed ethanologenic yeast strains. Appl. Biochem. Biotechnol. 121: 451-460.

Martin C, Fernandez T, Garcia R, Carrillo E, Marcet M, Galbe M, Jonsson LJ (2002). Preparation of hydrolysates from tobacco stalks and ethanolic fermentation by *Saccharomyces cerevisiae*. Enzyme Mierob. Technol. 18: 857-862.

Martin C, Galbe M, Wahlbom CF, Hahn-Hagerdal B, Jonsson LJ (2002). Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising *Saccharomyces cerevisiae*. Enzyme Microb. Technol. 31: 274-282.