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Effects of Aldehydes on the Growth and Lipid Accumulation of Oleaginous Yeast *Trichosporon fermentans*

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ABSTRACT: The effects of five representative aldehydes in lignocellulosic hydrolysates on the growth and the lipid accumulation of oleaginous yeast *Trichosporon fermentans* were investigated for the first time. There was no relationship between the hydrophobicity and the toxicity of aldehyde, and 5-hydroxymethylfurfural was less toxic than aromatic aldehydes and furfural. Binary combination of aromatic aldehydes caused a synergistic inhibitory effect, but combination of furan and aromatic aldehydes reduced the inhibition instead. A longer lag phase was found due to the presence of aldehydes and the decrease of sugar consumption rate, but more xylose was utilized by *T. fermentans* in the presence of aldehydes, especially at their low concentrations. The variation of malic enzyme activity was not related to the delay of lipid accumulation. Furthermore, the inhibition of aldehydes on cell growth was more dependent on inoculum size, temperature, and initial pH than that on lipid content.

KEYWORDS: lignocellulosic hydrolysate, aldehyde, inhibition, lipid production, *Trichosporon fermentans*

INTRODUCTION

Nowadays, lignocellulosic biomass is considered to be the most available, renewable, and green source of sugars that can be converted into liquid biofuel[1] or other valuable chemicals.[2,3] Generally, the utilization of lignocellulosic biomass includes the process of biomass degradation and the subsequent fermentation or bioconversion.[4] Lignocellulosic biomass consists of three main components, namely, cellulose, hemicellulose, and lignin, whose relative proportion depends on the material source.[5] The complete hydrolysis of lignocellulose would produce a variety of soluble, fermentable sugars. These sugars are mainly pentoses (e.g., xylose and arabinose) and hexoses (e.g., glucose and mannose, etc.). The ratio of hexoses to pentoses typically ranges from 1.5:1 to 3:1. Unfortunately, besides sugars, various byproducts, such as aldehydes, organic acids, and alcohols, would also be generated during the dilute acid hydrolysis process. Some of them are produced due to the decomposition of sugars. For example, acetic acid and furfural are from pentoses while 5-hydroxymethylfurfural (HMF) is from hexoses. Aromatic aldehydes, acids, and alcohols, instead, stem from the degradation of lignin.[6] In most cases, these byproducts, known as inhibitors, exert negative effects on the growth, metabolism, and product formation of microbial cells in the fermentation.[7]

Microbial lipids, namely, single cell oils (SCOs), are similar to vegetable oils in fatty acid composition and have been believed to be promising raw materials for biodiesel production.[8−10] In spite of the favorable impacts SCOs might exert, the economic aspect of current SCO production has been restricted primarily by the high medium cost. The adoption of lignocellulosic biomass as a carbon source could be a solution to this problem due to its great availability and low cost. Recently, we reported that the oleaginous yeast *Trichosporon fermentans* can use xylose as a carbon source for lipid formation.[11] Other microorganisms were also proven to be able to grow in the media containing xylose.[12,13]

Table 1. Concentration of Aldehydes Required To Inhibit the Lipid Yield of *T. fermentans*

	inhibitory concn (mM)		
aldehydes	IC_{25}[a]	IC_{50}[b]	log P[c]
furfural	2.1	4.7	0.41
HMF	15.1	37.7	−0.37
4-hydroxybenzaldehyde	3.4	6.6	1.35
syringaldehyde	6.6	8.8	0.99
vanillin	5.3	6.6	1.21

[a] Concentration of 25% inhibition on lipid yield. [b] Concentration of 50% inhibition on lipid yield. [c] The log P data was cited from Zaldivar et al.[16]

These results demonstrate the potential of lignocellulosic biomass as raw material for SCO production.

More recently, our ongoing research found that although *T. fermentans* gave a poor lipid yield on nondetoxified sulfuric acid treated rice straw hydrolysate (SARSH), it grew well with efficient lipid accumulation on the detoxified SARSH,[14] indicating that inhibitors in the lignocellulosic hydrolysate did show great effects on SCO fermentation. Although studies have been made for years on the toxic effects of various inhibitors on ethanologenic bacteria and yeasts,[15,16] there have been few reports about the influence of inhibitors on the lipid accumulation by oleaginous microorganisms.[17−19] In this work, we investigated the inhibitory effects of representative aldehydes in lignocellulosic hydrolysate on the growth and lipid accumulation of *T. fermentans*. Also, the influences of inoculum size and

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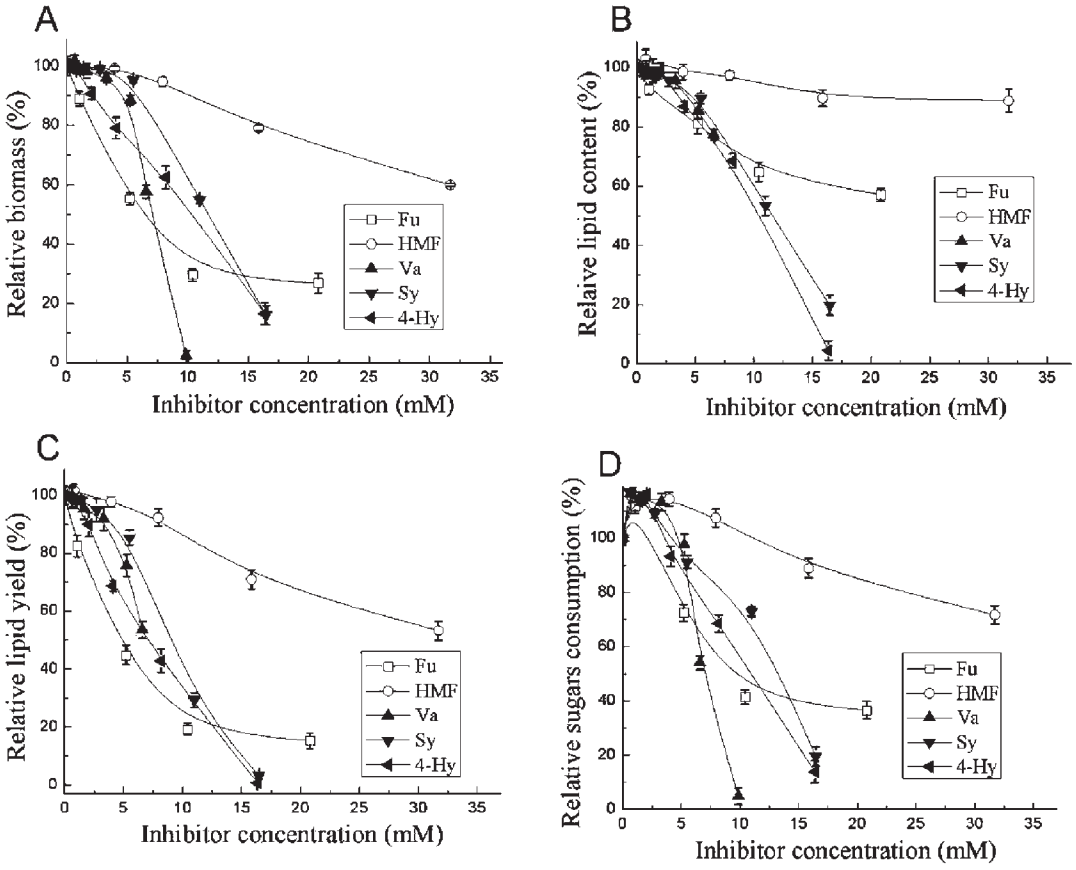

Figure 1. Effect of selected aldehydes on the growth and lipid accumulation of *T. fermentans*: (A) biomass; (B) lipid content; (C) lipid yield; (D) sugar consumption. Abbreviations: HMF, 5-hydroxymethylfurfural; Sy, syringaldehyde; 4-Hy, 4-hydroxybenzaldehyde; Fu, furfural; Va, vanillin.

environmental factors (temperature and initial pH) on the inhibition of aldehydes were examined. In addition, we further studied the effects of aldehydes on the sugar utilization, malic enzyme activity and cell morphology of *T. fermentans*. A mixture of glucose and xylose at a ratio of 2:1 (w/w), which is about the value of lignocellulosic hydrolysates, was selected as the carbon source.

MATERIALS AND METHODS

Microorganism and Chemicals. Oleaginous yeast *Trichosporon 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-Aldrich (USA). 5-Hydroxymethylfurfural (HMF), 4-hydroxybenzaldehyde, syringaldehyde, and vanillin were obtained from Alfa Aesar (U.K.). All other chemicals were from commercial sources and were of the highest purity available.

Medium, Precultivation and Cultivation. The precultivation medium (pH 6.0) contained (g/L) glucose and xylose (ratio 2:1) 20, peptone 10, yeast extract 10. The fermentation medium (pH 6.5) included (g/L) glucose and xylose (ratio 2:1) 100, yeast extract 0.5, peptone 1.8, $MgSO_4 \cdot 7H_2O$ 0.4, KH_2PO_4 2.0, $MnSO_4 \cdot H_2O$ 0.003, $CuSO_4 \cdot 5H_2O$ 0.0001. The preculture was performed in a 250 mL conical flask containing 50 mL of precultivation medium in a rotary shaker at 28 °C and 160 rpm for 24 h. Then, 5% seed culture (2.5 mL) was inoculated to a 250 mL conical flask containing 47.5 mL of fermentation medium and cultivation was carried out in a rotary shaker at 25 °C and 160 rpm for 7 days. Results are expressed as a percentage of the control value without adding the tested inhibitor.

Effect of Aldehydes on Growth and Lipid Accumulation. After precultivation, 2.5 mL of seed culture was inoculated to 47.5 mL of fermentation medium containing various aldehydes. In the absence of inhibitors, biomass, lipid content, lipid yield, and residual sugar concentration after 7 days fermentation were 24.0 g/L, 61.7%, 14.8 g/L, and 15.7 g/L, respectively. IC_{25} and IC_{50}, defined as concentrations of the tested aldehydes that caused 25% or 50% inhibition on the lipid yield, respectively, were measured on the data shown in Table 1. All reported data are the mean of duplicate experiments.

Effect of Inoculum Size, Temperature, and Initial pH on the Inhibition of Aldehydes. The effects of inoculum size, temperature or initial pH on the potency of aldehydes were examined using aldehydes concentrations of IC_{50}. With regard to inoculum size, 5%, 10% and 15% seed culture were inoculated to the fermentation media containing the selected aldehydes. For temperature, the cultures with 5% inoculum size were maintained at 22 °C, 25 °C, and 28 °C, respectively. Fermentation media containing the assayed aldehydes were adjusted to pH 5.5, 6.5, or 7.5 prior to inoculation to test the effect of initial pH. Biomass, lipid content, and lipid yield were all measured after 7 days fermentation. All the data were subjected to a two-way ANOVA after arcsin of the square root transformation of percent data.

Binary Combinations of Aldehydes. Two selected aldehydes were added to the growth medium at concentrations equal to their respective IC_{25}. Cultures were inoculated as described above and incubated for 7 days (5% inoculum size, pH 6.5 and 25 °C). Cultures grown without aldehydes were included as controls.

Effect of Aldehydes on Utilization of Sugars. The sugar consumption was tested by using aldehyde concentrations of IC_{25}. The relative sugar consumption is defined as the ratio of the amount of glucose and

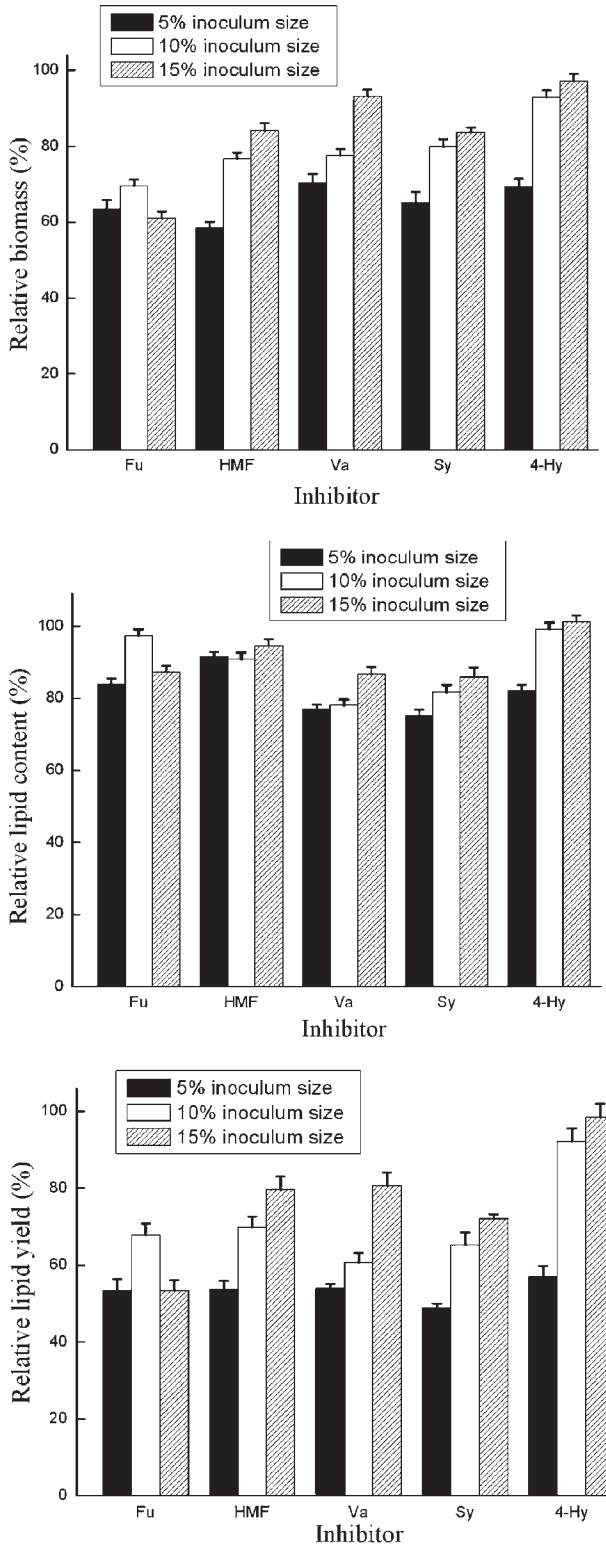

Figure 2. Effect of inoculum size on the inhibition of aldehydes. Each aldehyde was tested at its respective IC_{50}. Cultures were incubated at initial pH 6.5, 25 °C and 160 rpm for 7 days. Results are expressed relative to controls without aldehydes. Biomass, lipid content and lipid yield of cultures in the absence of aldehydes with 5%, 10% and 15% inoculum size were 24.0 g/L, 61.7% and 14.8 g/L; 22.4 g/L, 58.6% and 13.1 g/L; 21.6 g/L, 54.3% and 11.7 g/L, respectively. Abbreviations: HMF, 5-hydroxymethylfurfural; Sy, syringaldehyde; 4-Hy, 4-hydroxybenzaldehyde; Fu, furfural; Va, vanillin.

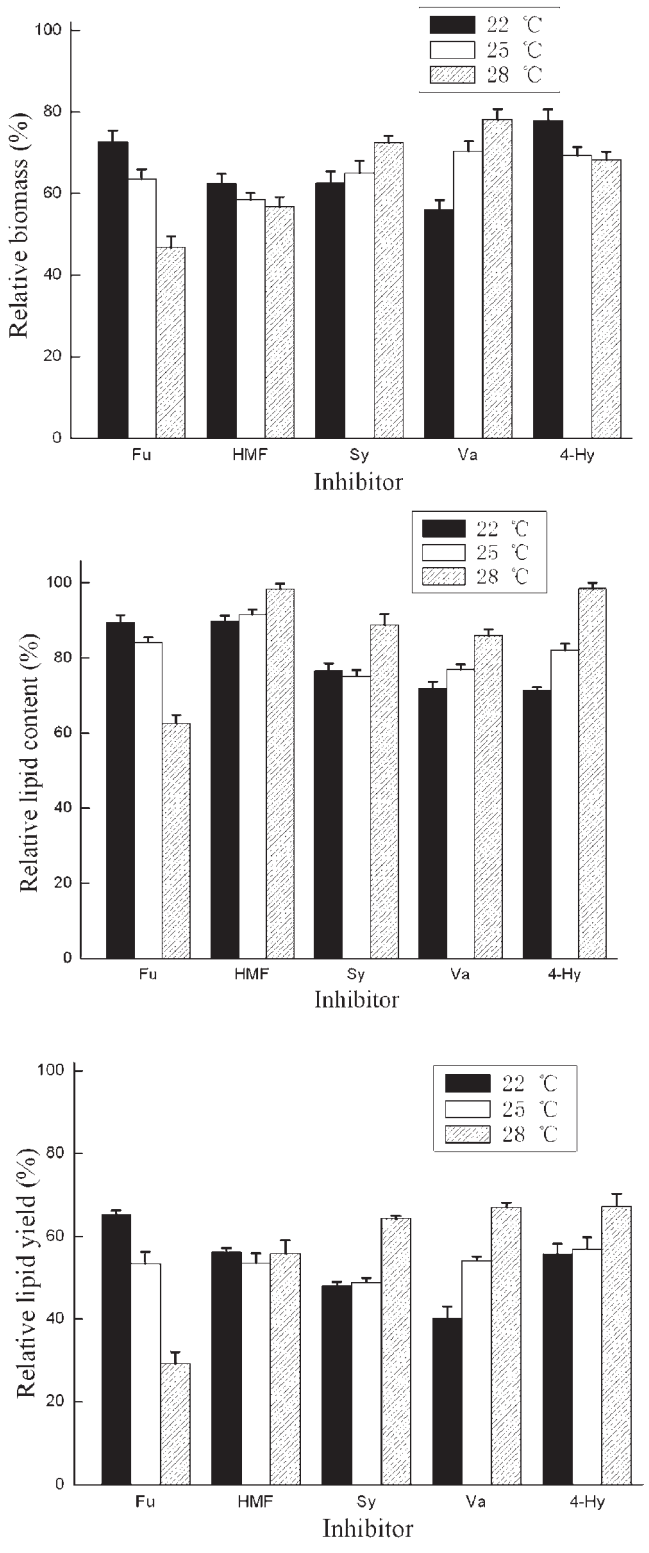

Figure 3. Effect of temperature on the inhibition of aldehydes. Each aldehyde was tested at its respective IC_{50}. Cultures with 5% inoculum size were incubated at initial pH 6.5 and 160 rpm for 7 days. Results are expressed relative to controls without aldehydes. Biomass, lipid content and lipid yield of cultures lacking aldehydes at 22 °C, 25 °C and 28 °C were 19.9 g/L, 55.8% and 11.1 g/L; 24.0 g/L, 61.7% and 14.8 g/L; 23.6 g/L, 58.9% and 13.9 g/L, respectively. Abbreviations: HMF, 5-hydroxymethylfurfural; Sy, syringaldehyde; 4-Hy, 4-hydroxybenzaldehyde; Fu, furfural; Va, vanillin.

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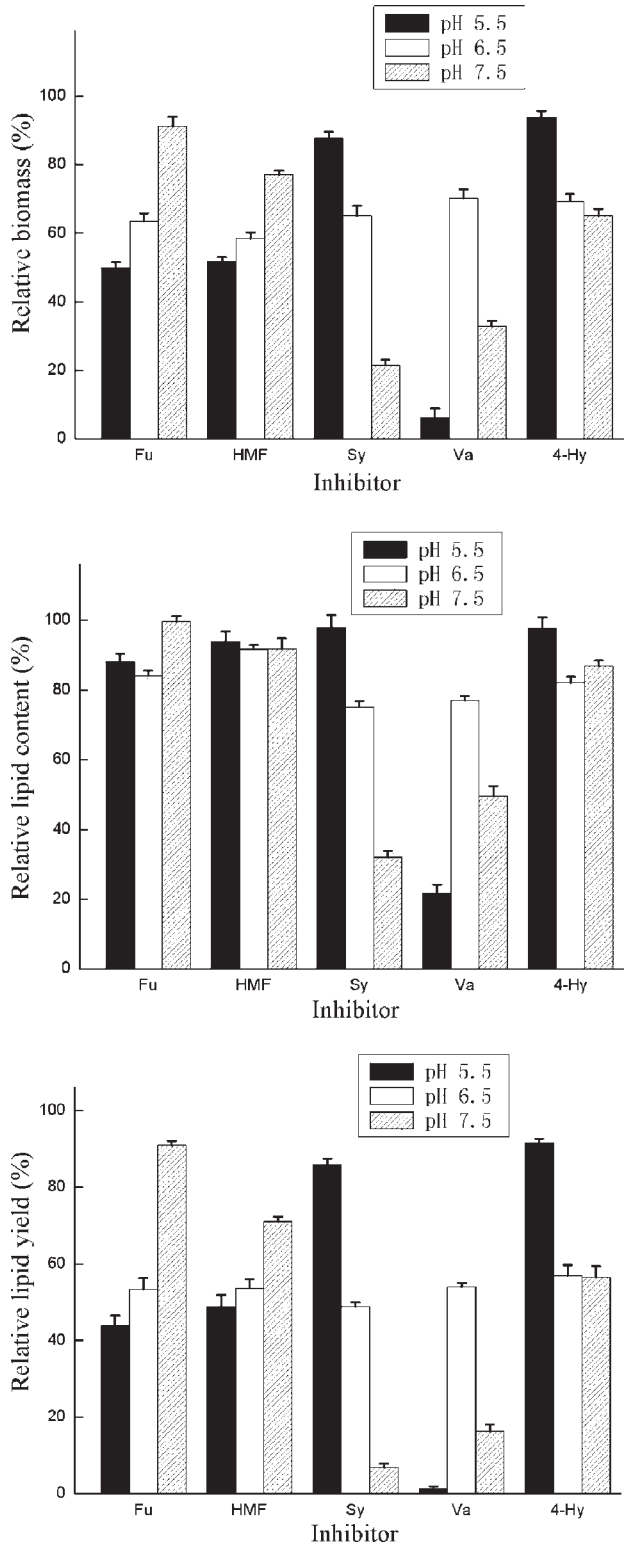

Figure 4. Effect of initial pH on the inhibition of aldehydes. Each aldehyde was tested at its respective IC_{50}. Cultures with 5% inoculum size were incubated at 25 °C and 160 rpm for 7 days. Results are expressed relative to controls without aldehydes. Biomass, lipid content and lipid yield of cultures lacking aldehydes at pH 5.5, pH 6.5 and pH 7.5 were 18.4 g/L, 57.2% and 10.5 g/L; 24.0 g/L, 61.7% and 14.8 g/L; 21.5 g/L, 56.3% and 12.1 g/L, respectively. Abbreviations: HMF, 5-hydroxymethylfurfural; Sy, syringaldehyde; 4-Hy, 4-hydroxybenzaldehyde; Fu, furfural; Va, vanillin.

xylose consumed by the yeast cells grown in the culture medium containing various aldehydes for 7 days to that without inhibitors.

Effect of Aldehydes on Malic Enzyme Activity. Biomass was harvested by centrifugation (4000*g* for 15 min at 4 °C), washed three times with distilled water and then suspended in extraction buffer (50 mM Tris-HCl containing 1 mM $MgCl_2$ and 1 mM DTT, pH 7.5). After being disrupted by a sonic cell disrupter at 300 W for 10 min on ice, the mixture was centrifuged (10000*g* for 10 min at 4 °C) and the supernatant was used immediately for enzyme activity assay.

Malic enzyme activity was detected at 340 nm and 30 °C with a SHIMADZU UV-2550 spectrophotometer (Japan). The reaction was initiated by adding 10 mM sodium L-malate into 3 mL of reaction mixture containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.5 mM $NADP^+$, and 0.04 mL of cell extract. The absorbance of the formed NADPH was recorded after reaction at 30 °C for 3 min. A control experiment, which was performed by following the above procedure except that no sodium L-malate was added, demonstrated that no $NADP^+$—NADPH conversion was detectable. One unit corresponds to the amount of enzyme producing 1 nmol of NADPH per minute at 30 °C.

Analytical Methods. Biomass was harvested by centrifugation and weighed in its lyophilized form.[20] Extraction of lipid from lyophilized biomass was performed according to the modified procedure of Bligh and Dyer.[21] 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 content was defined as the ratio of lipid weight to biomass weight.

The fatty acid profile of the lipid from *T. fermentans* was determined by gas chromatography (GC-2010) with an ionization detector and a DB-1 capillary column (0.25 cm × 30 m, Agilent Technologies Inc., USA) according to the published procedure.[11] D-Xylose and D-glucose were measured by HPLC (Waters Corp., USA) with a RI detector (Waters 2410) and an Aminex HPX-87P column (300 mm × 7.8 mm, Bio Rad Corp., USA) at 85 °C. Deionized water was used as the mobile phase at 0.5 mL/min. Furfural was analyzed by HPLC (Waters Corp., USA) using a UV detector at 220 nm (Waters 2489) and a Xbridge C18 column (Waters Corp., USA), and the mixture of water and methanol (75/25, v/v) was used as the mobile phase at 1.0 mL/min.

RESULTS AND DISCUSSION

Effects of Aldehydes on the Growth and the Lipid Accumulation of *T. fermentans*. Furan derivatives (furfural and HMF) and aromatic compounds (vanillin, syringaldehyde, and 4-hydroxybenzaldehyde) are the two main types of aldehydes in lignocellulosic hydrolysates.[6] Their effects on the growth and lipid accumulation of *T. fermentans* were depicted in Figure 1A—C. Table 1 reports IC_{25} and IC_{50} values relative to the lipid yield. Among the aldehydes examined, HMF was the least toxic, followed by aromatic aldehydes and furfural. It is clear that there was no correlation between the hydrophobicity and the toxicity of aldehyde, which disagrees with the previous reports by Zaldivar et al.[16] and Hu et al.,[18] suggesting that the hydrophobicity of an aldehyde is not the only determinant for its toxicity. Interestingly, the inhibitions by both furan and aromatic aldehydes were weak at their low concentrations (<2 mM), which was also confirmed by measuring the sugar consumption after fermentation for 7 days (Figure 1D). Figure 1D shows that both xylose and glucose were quantitatively consumed by *T. fermentans* when the inhibitor concentration was less than 2.5 mM. The HMF showed the least influence on sugar utilization, which agrees with the observation that HMF displayed the lowest

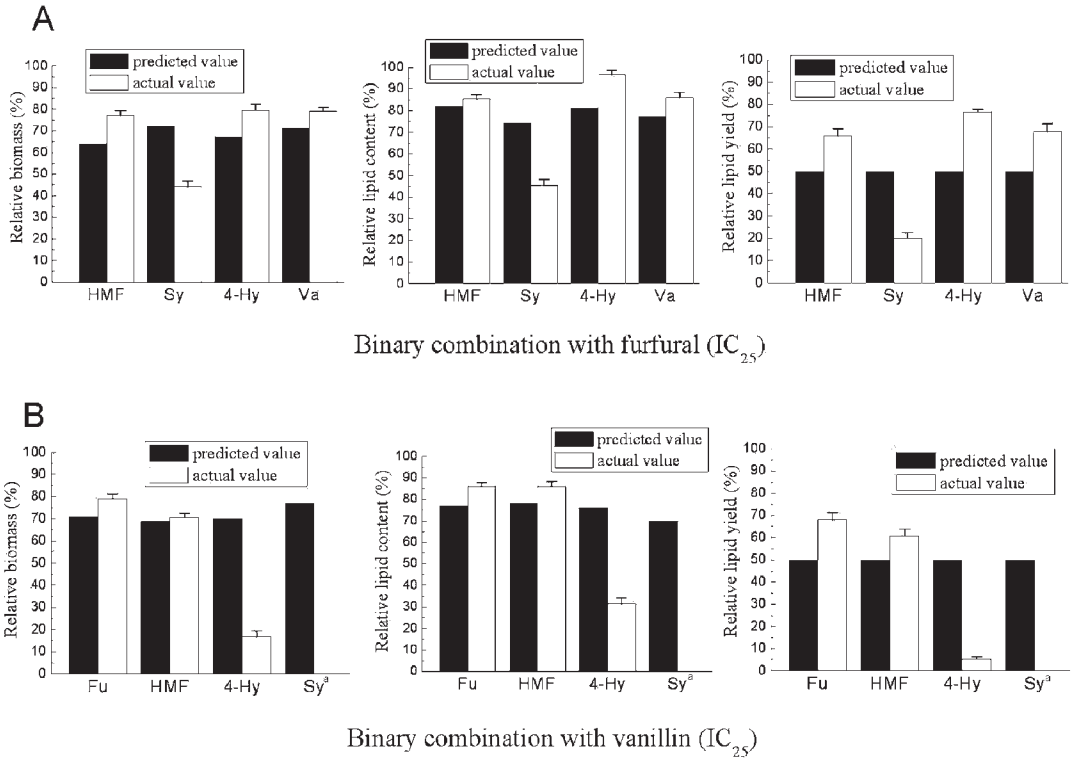

Figure 5. Effect of binary combinations on the growth and lipid accumulation of *T. fermentans*: (A) furfural (IC_{25}); (B) vanillin (IC_{25}). Abbreviations: HMF, 5-hydroxymethylfurfural; Sy, syringaldehyde; 4-Hy, 4-hydroxybenzaldehyde; Fu, furfural; Va, vanillin. Superscript "a" indicates that no growth was observed.

toxicity to *T. fermentans*. Interestingly, many yeast species have the ability to convert aldehydes into less toxic compounds.[22,23] For example, furfural can be reduced to furfuryl alcohol or oxidized to furoic acid. Also, in this work, we could not detect any furfural after fermentation for 7 days in the medium with furfural at its respective IC_{25} or IC_{50} concentration, suggesting that furfural was metabolized by *T. fermentans*.

The effect of the selected aldehydes on the fatty acid composition of lipid from *T. fermentans* was also investigated, and the results showed that there was no significant change in the fatty acid composition of lipid with the variation of aldehyde, and oleic acid, the most abundant one, amounted to about 60% of the total fatty acids, followed by palmitic acid, stearic acid and linoleic acid (data not shown). This supports the report that inhibitors in spruce hydrolysate had little influence on the fatty acid composition of lipid from *R. toruloides* Y4.[18]

Effect of Inoculum Size, Temperature, and Initial pH on the Inhibition by Aldehydes. It is proved that aldehydes can be converted to less toxic compounds by yeast cells and increasing cell density might accelerate the conversion.[23,24] Therefore, raising inoculum size has been considered as a strategy to decrease the effect of inhibitors.[16] Our previous study showed that 5% inoculum size was the optimal for lipid production on medium without inhibitors by *T. fermentans*.[11] In this work, we increased the inoculum size up to 10% and 15% to see its effect on the inhibition by aldehydes. As shown in Figure 2, the toxicity of all aldehydes except furfural was reduced by increasing the inoculum size and especially in the case of 4-hydroxybenzaldehyde. Furfural displayed the minimum inhibition at 10% inoculum size. The results of a two-way ANOVA after arcsin of the square root transformation of all the percent data showed that the interactions between aldehydes and inoculum size were highly significant ($P < 0.01$), and the main effects of inoculum size on the biomass, lipid yield, and lipid content were also highly significant ($P < 0.01$).

Temperature and initial pH also have significant influence on the cell growth and lipid accumulation. In the absence of inhibitors, the optimal temperature and initial pH for lipid production by *T. fermentans* were 25 °C and 6.5, respectively.[11] As can be seen in Figure 3, there is no direct correlation between growth temperature and the inhibitory effect by aldehydes. For example, a higher temperature reduced aromatic aldehydes' inhibition on the lipid yield while it enhanced the inhibitory effect by furfural. Similarly, no relationship between initial pH and the inhibition by aldehydes was found (Figure 4). Initial pH, however, had greater impact on the inhibition than temperature. The initial pH exerted a more significant impact on the inhibition in the presence of aromatic aldehydes than furan derivatives. The inhibition on the cell growth by furan aldehydes decreased with the rise of pH, while lipid synthesis was not affected by the medium's pH. As for aromatic aldehydes, lower initial pH could reduce the inhibitory effect of syringaldehyde and 4-hydroxybenzaldehyde. However, in the medium containing vanillin, the optimal initial pH was 6.5, and lower or higher pH would bring serious inhibition on the cell growth and lipid synthesis. The ANOVA results showed that the interactions between aldehydes and temperature or initial pH were highly significant ($P < 0.01$), and all the main effects of temperature or initial pH on the biomass, lipid content, and lipid yield were highly significant ($P < 0.01$) except that the effect of temperature on the biomass was not significant ($P > 0.05$).

Obviously, inoculum size and environmental factors (temperature and initial pH) also exerted serious influences on the

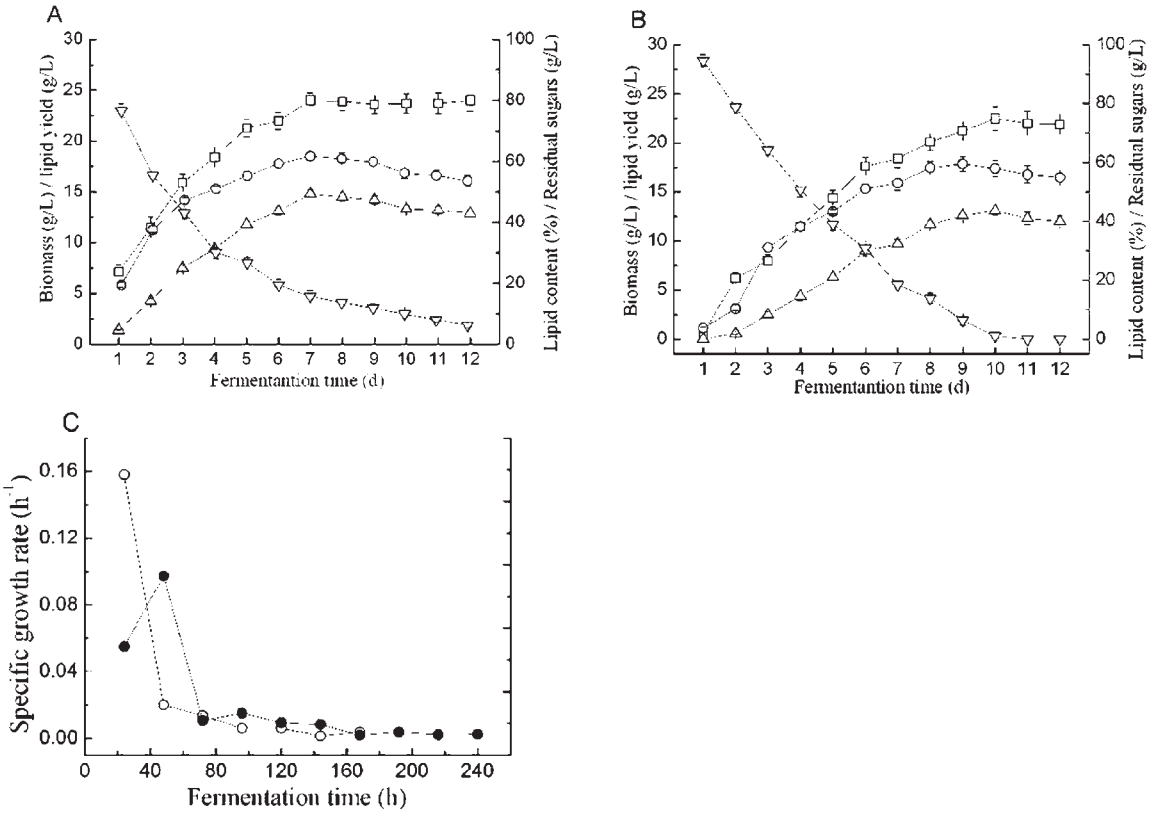

Figure 6. Comparison of lipid fermentation process by *T. fermentans* in the absence or presence of different aldehydes (concentration of different aldehydes (mM): furfural 0.10, HMF 0.79, vanillin 0.47, syringaldehyde 3.36). (A) Control medium with no inhibitors: (□) biomass; (○) lipid content; (△) lipid yield; (▽) residual sugars. (B) Simulated medium with various aldehydes: (□) biomass; (○) lipid content; (△) lipid yield; (▽) residual sugars. (C) Specific growth rate: (○) control medium; (●) simulated medium.

inhibition of aldehydes, and the inhibition could be partly relieved by careful control of the culture conditions.

Effect of Combinations of Aldehydes on Cell Growth and Lipid Accumulation of *T. fermentans*. The lignocellulosic hydrolysates generally contain more than one inhibitor, and the synergistic effect of different inhibitors was complex.[7,25] Therefore, binary combinations of five aldehydes were tested at their respective IC_{25} concentrations (Table 1). Furfural and vanillin, the typical furan and aromatic aldehydes existing in most lignocelluosic hydrolysates, were used for a binary combination with another aldehyde. In the experiments, whenever two aldehydes were combined, the predicted relative biomass, lipid content and lipid yield represent the values after deduction of the summed inhibition on biomass, lipid content and lipid yield by each of the two tested inhibitors at their IC_{25} concentrations. If the actual experimental value exceeded the predicted value, the inhibition was referred to as synergistic. As can be seen in Figure 5A, the binary combination of furfural and syringaldehyde showed a synergistic effect. On the contrary, the binary combination of furfural with the other three aldehydes (HMF, vanillin and 4-hydroxybenzaldehyde) exerted less inhibition than individual inhibitor itself. This phenomenon is different from the previous report on ethanologenic *Escherichia coli*.[16] As depicted in Figure 5B, the binary combinations of vanillin with furan derivatives also gave less toxic effect on cell growth and lipid accumulation, while the combination of vanillin with the other two aromatic aldehydes, especially syringaldehyde, caused a significant synergistic inhibition, suggesting that the binary combination of aromatic aldehydes was more toxic. The serious toxicity caused by binary combination of aromatic aldehydes has also been reported on oleaginous yeast *R. toruloides*[18] and another yeast *Candida guilliermondii* for xylitol production.[26] Thus controlling aromatic aldehydes' concentration at low level is very important for lipid production by *T. fermentans*.

In addition to binary combinations, the fermentation was further performed in the medium containing four aldehydes at their respective concentrations found in concentrated brewer's spent grain hemicellulosic hydrolysate.[27] As shown in Figure 6, the cell growth of *T. fermentans* was clearly inhibited in the early phases of fermentation in the presence of inhibitors as indicated by a lower specific growth rate. Interestingly, although sugars were consumed in the presence of inhibitors, the maximum biomass was still 6.3% lower than the control (22.5 g/L vs 24.0 g/L). However, the difference was not significant ($P > 0.05$). Under these conditions, albeit slightly postponed, the maximal lipid content reached a value similar to that in the control medium (59.5% vs 61.7%) thus suggesting that lipid accumulation was not negatively affected. A similar phenomenon was also observed in oleaginous yeast *R. toruloides*.[18]

Effects of Aldehyde on Sugar Utilization, Malic Enzyme Activity, and Cell Morphology of *T. fermentans*. Lipid accumulation in oleaginous microorganisms includes the sugar assimilation and utilization by glycolysis and then lipid synthesis by a series of enzymes.[28] Previous studies have showed that furan aldehydes can affect the glycolysis and Krebs cycle fluxes and then influence the energy metabolism.[6,16,29] Aromatic aldehydes would damage the membrane integrity via their incorporation

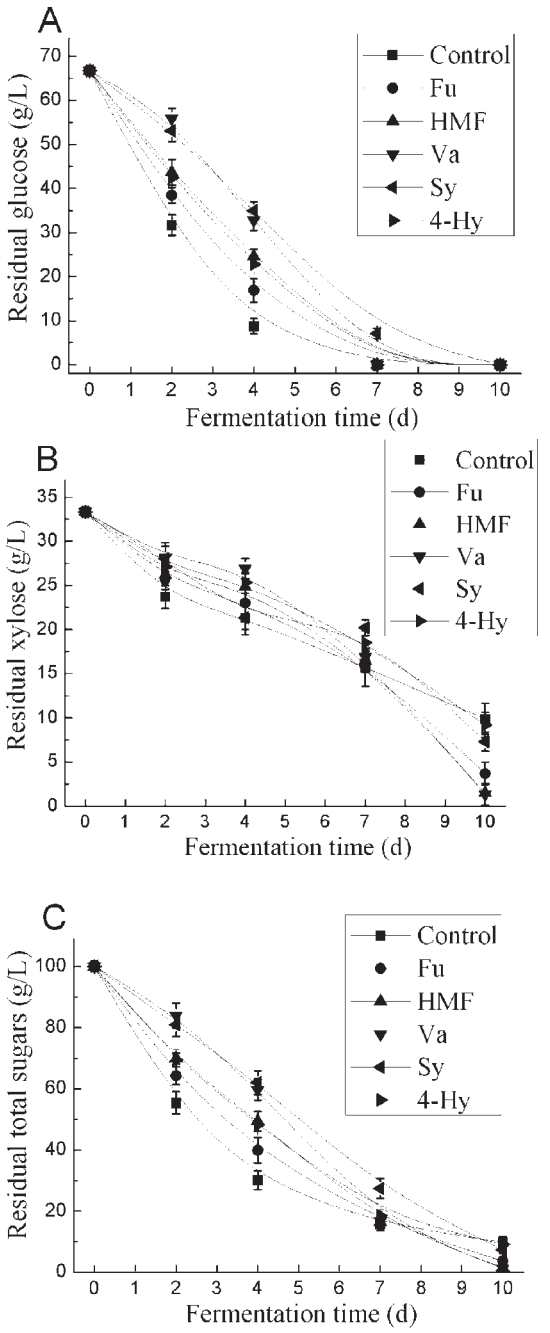

Figure 7. Effect of the selected aldehydes on the sugar utilization of *T. fermentans*: (A) glucose consumption; (B) xylose consumption; (C) total sugars consumption. Abbreviations: HMF, 5-hydroxymethylfurfural; Sy, syringaldehyde; 4-Hy, 4-hydroxybenzaldehyde; Fu, furfural; Va, vanillin.

into the membrane thus affecting cell growth.[6,23] Also, aldehydes in the medium would lead to a longer lag phase for microorganism growth.[6,30] In order to understand the effects of aldehydes on the sugar utilization by *T. fermentans*, the time courses of sugar consumption were further investigated in the media in the presence of aldehydes at their respective IC_{25} concentrations. As shown in Figure 7, the glucose was utilized more slowly in the presence of inhibitors and the decreased glucose consumption rate led to an extension of lag phase. Similarly, xylose utilization was also slower during the first 7 days. It is interesting to note that

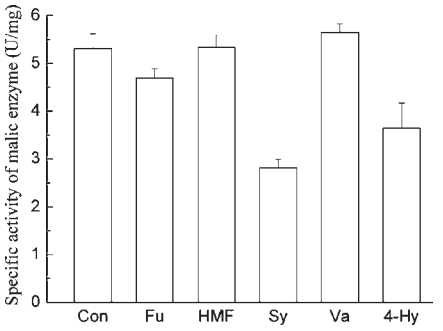

Figure 8. Effects of selected aldehydes on the activity of malic enzyme of *T. fermentans*. Abbreviations: Con, control; HMF, 5-hydroxymethylfurfural; Sy, syringaldehyde; 4-Hy, 4-hydroxybenzaldehyde; Fu, furfural; Va, vanillin.

T. fermentans metabolized xylose more quickly than the control without aldehydes after day 7. Pereira et al.[31] also reported the similar phenomenon that syringaldehyde stimulated the xylose consumption compared with the control without any inhibitor on xylitol bioconversion by *Candida guilliermondii*. And they found that the medium's pH exerted great impact on xylose consumption. Low pH was unfavorable for xylose utilization; however, the presence of syringaldehyde alleviated its negative influence. In our work, we also found the medium's pH decreased during fermentation and the pH of the control was about 4.2 on the seventh day (initial pH 6.5). This partly accounts for the xylose consumption result by *T. fermentans* mentioned above.

Malic enzyme, one of the main enzymes providing a supply of NADPH to microorganisms, is usually considered as the key enzyme for lipid synthesis in oleaginous microorganism.[28,32] The effects of the selected aldehydes at their respective IC_{25} concentrations on the malic enzyme activity were tested after the second day of fermentation when the lipid formation rate reached the maximal in the culture without inhibitors. As can be seen in Figure 8, the malic enzyme activity was inhibited by syringaldehyde, 4-hydroxybenzaldehyde, and furfural, which can well explain the delay of lipid accumulation in the fermentation. However, for HMF and vanillin, there was no significant change in the specific activity of malic enzyme, and even a higher malic enzyme activity was detected, suggesting that the delay of lipid accumulation might be due to other reasons such as the decreased glucose consumption rate and the adaptation of cells to the environment with inhibitors.

Morphological changes were also observed microscopically during cultivation in the presence of inhibitors at their concentrations of IC_{50}. The cell morphology in the medium without inhibitors was typically elliptical. With furfural, vanillin and syringaldehyde, cells appeared elongated as rods or short chains during fermentation. However, little change in cell morphology was observed in the presence of HMF and 4-hydroxybenzaldehyde, suggesting that there was no correlation between the toxicity of aldehydes and the cell morphology.

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