Isolation of Xylose Fermentation Strains for Ethanol Production and Xylose Fermentation Research

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Xylose is abundant in lignocellulosic biomass. The fermentation of xylose is still a key problem in cellulose ethanol fermentation. In this research, the xylose fermentation strains were isolated from the soil sample. Strain N6 was chosen for further xylose fermentation experiments. Comparison of its 16S rDNA gene sequence with available sequences in GenBank showed that it was 100 % identical to that of *Bacillus cereus* strain SWFU2816 (Accession No. JN935015.1). Strain N6, which ferments xylose and hexose to ethanol, can produce fuel ethanol. The ability of strain N6 to transform xylose was better than its ability to transform glucose. As a result, the optimal condition for xylose fermentation was determined. The optimum fermentation conditions at 150 rpm for 48 h in a shake flask were as follows: pH 7.0, reducing sugar 70 g L^{-1}, inoculation amount 5 %, and temperature 38 °C. The xylose to ethanol yield coefficient could reach a maximum of 0.26 g g^{-1}. At the same time, the influence of the acid hydrolysis by-products on the isolated strain was studied. Compared to the commonly used saccharomycetes, the tolerance of strain N6 on furaldehyde had greatly improved, which could help ethanol fermentation from lignocellulose materials.

Key words:

Xylose fermentation strain, ethanol fermentation, lignocelluloses, acid hydrolysis by-products

Introduction

As a clean and renewable source of energy, ethanol is a promising alternative to fossil fuels.[1] Especially lignocelluloses, as raw material for ethanol production, have gained great interest during these years.[2] Production of bio-ethanol will help to cope with the over-consumption of fossil fuels and further work for the reduction of carbon dioxide emissions.

Lignocelluloses (crop residue, cellulose waste, municipal solid waste, and so on) as raw material, cost less than starches, thus, larger quantities are acquirable, permitting large-scale production.[3] Lignocellulose materials can be found everywhere in the world, and utilization makes use of waste. During the ethanol fermentation process, lignocellulose materials are hydrolyzed by acids and enzymes. Dilute-acid hydrolysis is a common but good method to quickly produce sugars form lignocelluloses.[4–6] Xylose is the major product of the hydrolysis of hemicellulose from many plant materials. Corn straw, as a plant material, contains two main types of polymers, cellulose and hemicellulose, whose conversion into fermentable sugars is easy. The hydrolyzates of corn straw are composed of xylose, hexose, and other hydrolysis byproducts. Hexose is easily fermented to ethanol but xylose is more difficult to ferment. Xylose utilization is important for successful lignocellulose-ethanol fermentation.[7]

Xylose is abundant in lignocellulosic biomass such as corn stover,[8] and forest industry waste.[9] The fermentation of xylose is still a key problem in cellulose ethanol fermentation. Successful utilization of xylose could improve the efficiency of the biomass-ethanol fermentation process. A number of attempts have been made in obtaining a stable microorganism capable of utilizing xylose. It was reported that several xylose fermentation strains had been separated, such as *Pichia stipites*,[10] *Candida shehatae*,[11] *Pachysolen tannophilus*,[12] some genetically engineered xylose-fermenting yeast[13] and so on. However, these fermentation strains are relatively poor fermentative microorganisms. And the influ-

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ence of the acid hydrolysis by-products on the growth and fermentation of these microorganisms was great.[14] Namely, xylose can be fermented to ethanol by many bacteria and yeasts, but by-product formation or slow xylose conversion limits its economical application for ethanol production.

In this research, the xylose fermentation strains were isolated from the soil sample. The microorganism identification was carried out by common physiological and biochemical experiments. The xylose fermentation performance was measured and the ethanol yield was analyzed to obtain the optimal fermentation conditions. At the same time, the glucose fermentation experiment by the isolated strain was carried out for the comparative analysis of mixed sugar and xylose fermentation. Finally, the influence of the acid hydrolysis by-products on the isolated strain was studied.

Material and methods

Materials

All chemicals of analytical reagent grade were purchased from Beijing Chemical Factory (Beijing, China). All the biochemical reagents were bought form Beijing Biological Technology Factory (Beijing, China). The strains were stored in the tube culture at 4 °C.

Culture media and microorganism culture

The composition of the multiplying culture was as follows: xylose 20 g L^{-1}, peptone 15 g L^{-1}, yeast extract 10 g L^{-1}, ammonium sulfate 1 g L^{-1}, KH_2PO_4 1 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, and calcium chloride 1 g L^{-1}. The composition of solid medium was: xylose 20 g L^{-1}, peptone 15 g L^{-1}, ammonium sulfate 1 g L^{-1}, KH_2PO_4 1 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, yeast extract 10 g L^{-1}, agar 20 g L^{-1} and calcium chloride 1 g L^{-1}. The composition of the liquid fermentation medium was: peptone 15 g L^{-1}, yeast extracts 10 g L^{-1}, ammonium sulfate 1 g L^{-1}, KH_2PO_4 1 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, and a certain amount of xylose. The composition of the screening medium was: xylose 25 g L^{-1}, peptone 15 g L^{-1}, yeast extracts 10 g L^{-1}, ammonium sulfate 1 g L^{-1}, KH_2PO_4 1 g L^{-1}, and $MgSO_4$ 0.5 g L^{-1}. The composition of the TTC (2,3,5 – chlorinated three benzene tetrazole) upper medium was as follows: 2,3,5 – chlorinated three benzene tetrazole 0.5 g L^{-1}, xylose 5 g L^{-1}, and agar 15 g L^{-1}. The composition of the TTC lower medium was: xylose 10 g L^{-1}, peptone 2 g L^{-1}, yeast extract 1.5 g L^{-1}, KH_2PO_4 1 g L^{-1}, $MgSO_4$ 0.4 g L^{-1}, citric acid 0.27 g L^{-1}, and agar 20 g L^{-1}. The composition of the mixed sugar fermentation medium was: peptone 15 g L^{-1}, yeast extracts 10 g L^{-1}, ammonium sulfate 1 g L^{-1}, KH_2PO_4 1 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, and a certain amount of sugar.

Isolation of efficient xylose fermentation bacteria

The soil sample was taken together with rotting leaves near a locust tree on March 26, 2012 (Yanshan University, Qinhuangdao, China). The soil sample was mixed with physiological saline and the solution was placed in the shaker bed with shaking speed of 150 r min^{-1} for 3 h. Then 5 mL suspension liquid was added to the multiplying culture (100 mL) in the conical flask. The flask was put in the incubator at 37 °C for 1–2 days. 1 mL supernatant liquor was diluted for different times and then poured onto the solid culture medium. The culture dish was put in the incubator at 37 °C for 2 days. After cultivation and crossed purification, the strains were stored in the tube culture at 4 °C. The crossed purification on the solid medium was processed until the strains with the same morphology were examined by microscope. The strains were then inoculated in the TTC lower medium and cultured at 37 °C for 1 day. After the cultivation, TTC upper medium was added to the dishes, and the dishes shielded from light, were put in the incubator at 37 °C for 3 h. The colony color was observed. The xylose fermentation strains dark red in colour were picked from the TTC medium and inoculated. The picked strains were inoculated in the fermentation medium, through the measure of ethanol yield, the efficient xylose fermentation strains were isolated.

After cultivation, the strains were stored in the tube culture at 4 °C. The microorganism identification was carried out by common physiological and biochemical experiments. The cells concentration was represented by OD600. The dry cell weight (mg L^{-1}) was measured by the centrifugal drying method.[15]

The genomic DNA of strain was extracted using the precipitation method. The 16S rDNA was amplified using the primers; 16F (AGAGTTTGATCCTGGCTCAG) and 16R (GGTTACCTTGTTACGACTT). PCR amplification was performed under the following conditions; 3 min. at 95 °C; 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 1 min. at 72 °C, plus an additional 5-minute cycle at 72 °C. The automatic sequence was carried out by Beijing Sun Biotech Co. Ltd. The 16S rDNA sequence was checked in GenBank.

Xylose fermentation experiments

In order to reach optimal fermentation conditions, xylose fermentation experiments were carried out. The preserved strains were activated in the culture medium and then inoculated into the fermentation medium with a certain amount of xylose as carbon source in the shake flasks with rotating speed of 150 min^{-1}. After a certain time (48h), the ethanol concentration and residual xylose concentration

were measured, and the ethanol yield was calculated. The effect of temperature, pH, inoculum amount, xylose concentration was considered in this research. At the same time, the glucose fermentation experiments with the isolated strain were carried out for a comparative analysis of xylose and xylose fermentation. In order to improve accuracy, four groups of the same fermentation experiments were carried out under different fermentation parameters. The xylose fermentation efficiency was represented by sugar consumption percentage and sugar to ethanol conversion yield.

The influence of acid hydrolysis by-products on the isolated strain

The inhibitor-supplemented culture was prepared in the present study. In order to investigate the effect of hydrolyzate inhibitors on the isolated strain, a certain amount of inhibitor was added to the culture medium respectively and then the cells concentration was measured under the same culture conditions.

Analytical methods

The reducing sugars were analyzed by the DNS method.[16] The ethanol content (volume) (v/v) in the distilled fluid was determined by the colorimetric method.[17] The pH was measured in a pH meter (PHS-3B, Shanghai Precision & Scientific Instrument Co. Ltd, Shanghai, China). The ethanol yield was the quality of ethanol generated from per gram of the sugar consumption. The optical density (OD) of cell growth at regular intervals was determined at 600 nm using a UVVIS spectrophotometer (TU-1901, Purkinje General Instrument Co. Ltd, Beijing, China) throughout the study. Fourier transform infrared spectroscopy (Nicolet 5700, Thermo-Electron Co. Ltd, Madison, WI USA) was used for the analysis of the component in lignocellulose hydrolysates.

The sugar consumption percentage in the xylose fermentation experiments was calculated as:

$$S_P = (S_1 - S_2)/S_1$$

where S_1 was sugar concentration (g L^{-1}) before fermentation, S_2 was sugar concentration (g L^{-1}) after fermentation.

The sugar to ethanol conversion yield in the xylose fermentation experiments was calculated as:

$$E_r = C_e/(S_1 - S_2)$$

where S_1 was sugar concentration (g L^{-1}) before fermentation, S_2 was sugar concentration (g L^{-1}) after fermentation, and C_e was ethanol concentration (g L^{-1}) after fermentation.

The inhibition rate of toxic compounds on the strain growth in the experiments was calculated as:

$$I_r = (C_2 - C_1)/C_1$$

where C_1 was cell concentration in the standard medium after cultivation, C_2 was cell concentration in the liquid medium containing toxic compounds after cultivation. The cells concentration was represented by OD600.

Results and discussion

The isolation of efficient xylose fermentation strain

Through the multiplication cultivation and plate streaking purification, 7 strains were selected which could use xylose as carbon source for growth. These seven strains were inoculated in the TTC medium, the color of the colony was observed. If the colony was red, the strains could secrete the dehydrogenase and produce ethanol from xylose. Four strains of the isolated seven strains could secrete the dehydrogenase and the colony was red.

Then the four kinds of strains were inoculated in the fermentation medium under the same conditions as for ethanol production. The concentration of residual sugar and ethanol were measured and the ethanol yield was calculated. The pentose fermentation condition was as follows: inoculation amount 2 %; fermentation temperature 35 °C; rotate speed of 150 min^{-1}; pH 7, and initial sugar concentration 50 g L^{-1}. The result is shown in Table 1.

Table 1 – *Ethanol fermentation performance with different strains (numbers in parentheses correspond to the number of measurements used for the determination of the mean values and standard deviations)*

Strain number	Sugar consumption percentage/%	Sugar to ethanol conversion yield/%
Strain N4	70.0 ± 1.27 (4)	19.5 ± 0.2 (4)
Strain N5	31.0 ± 2.64 (4)	5.65 ± 0.62 (4)
Strain N6	78.2 ± 0.57 (4)	23.7 ± 0.31 (4)
Strain N7	48.5 ± 1.36 (4)	11.9 ± 0.47 (4)

As shown in Table 1, in the same conditions, the sugar utilization rate of No. 6 strain was the highest. With the better use of the sugar residue in the alcoholic fermentation broth, fermentation was promoted and the brewing rate was raised. Strain N6 was chosen for further experiments. Comparison of its 16S rDNA gene sequence (with available sequences in GenBank) showed that it was 100 % identical to *Bacillus cereus* strain SWFU2816 (Accession No. JN935015.1). The phylogenetic tree

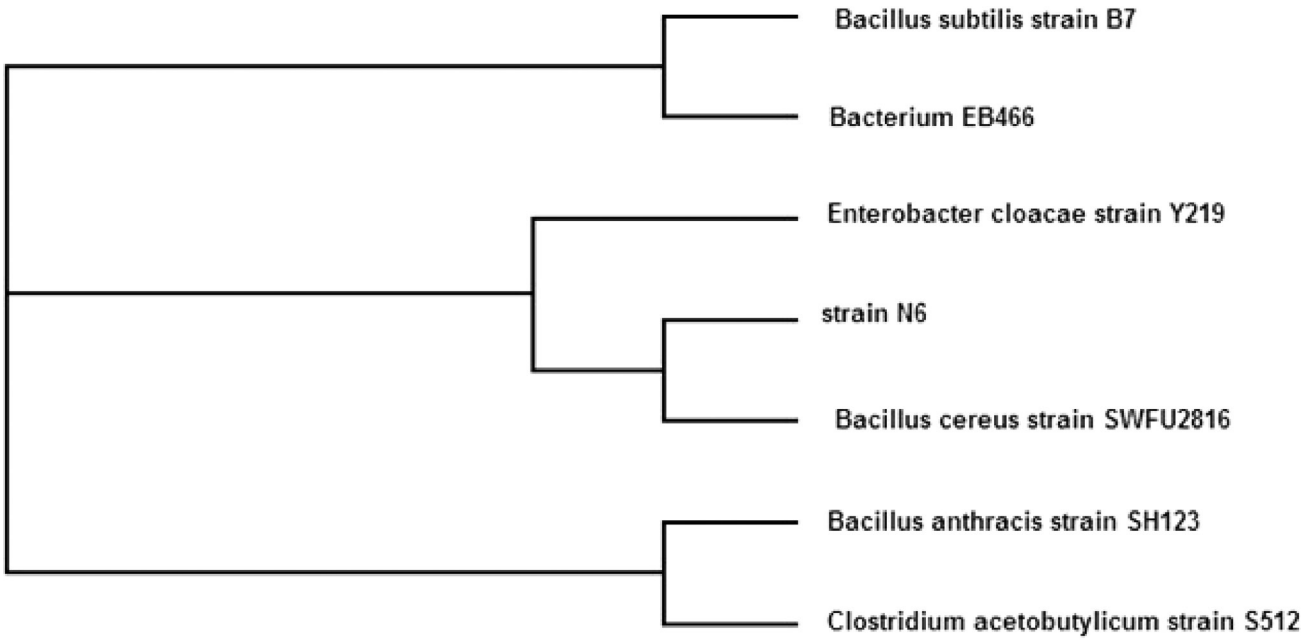

Fig. 1 – *Phylogenetic tree of strain N6*

of the strain is shown in Fig. 1. Ethanol fermentation with *Bacillus cereus* strain SWFU2816 has not been reported in other references.

Ethanol fermentation with the strain N6

There are many factors that influence the ethanol yield and fermentation rate in the fermentation process from sugar with microorganism, such as pH, the sugar content, temperature and types of sugar.

Effect of pH on ethanol fermentation

The pH of the solution has an important influence on the growth of the microorganism. In the process of microorganism growth, the pH may cause a change in the charge of the cell membrane, thereby changing the permeability of the membrane. At the same time, pH may also cause a change in the degree of ionization of various ionic substances required for the growth of the microorganism, thus affecting both the absorption of nutrients, as well as growth and reproduction of microorganisms. On the other hand, the activity of various enzymes involved in the microbial physiological and biochemical activity is influenced by the pH of the intracellular environment. Therefore, pH for bacterial growth has played a pivotal role.

The pH of the fermentation medium had considerable influence on the ethanol yield and fermentation rate in the fermentation process. In order to optimize pH in the ethanol fermentation process, the influence of pH on ethanol concentration is shown in Fig. 2. Residual sugar and ethanol concentration was determined after ethanol fermentation of 48 h. The fermentation conditions were as follows: inoculation amount 2 %; fermentation temperature 35 °C; rotate speed 150 min^{-1}; and initial sugar concentration 50 g L^{-1}. The pH was 3 to 8.

Judging by Fig. 2, a pH of 7.0 was chosen to be a suitable parameter for ethanol fermentation. At pH 7.0, the highest ethanol yield could be obtained. The reason was that the decrease in pH could increase the concentration of acids, which could inhibit yeast cells fermentation. However, higher initial pH would promote senescence of yeast cells and decrease the fermentation rate. At the same time, high pH increased the opportunity of contamination.[10,12]

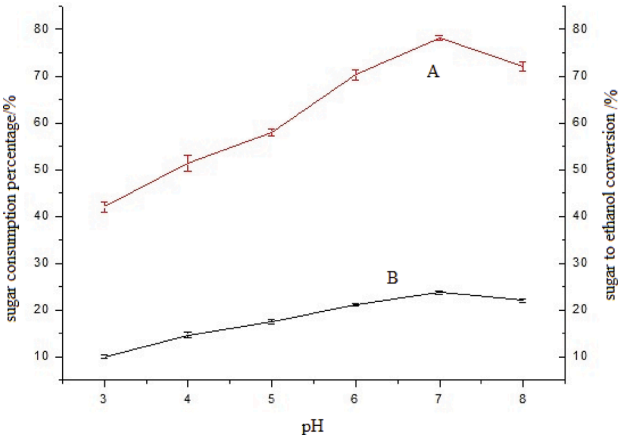

Fig. 2 – *Effect of pH on ethanol fermentation performance: (A) sugar consumption percentage; (B) sugar to ethanol conversion yield*

Effect of temperature on ethanol fermentation

Temperature is an important survival factor for microorganisms. The effect of temperature on sugar fermentation by strain N6 was analyzed. The temperature gradient ranged from 25 °C to 46 °C. The fermentation conditions were as follows: inoculation amount 2 %; pH 7; rotate speed 150 min^{-1}; and initial sugar concentration 50 g L^{-1}.

Residual sugar and ethanol concentration were determined after ethanol fermentation of 48 h. The result is shown in Fig. 3. Temperature affects the activity of the enzyme and changes the rate of enzymatic reaction. Within the appropriate temperature range, if the temperature is increased, the enzymatic reaction rate will increase and lead to a corresponding increase in the rate of metabolism and growth rate of the microorganisms. At appropriate temperature, microorganisms grow and reproduce the fastest. According to the optimum growth temperature, microorganisms can be divided into categories such as psychrophilic bacteria, mesophilic bacteria, and thermophilic bacteria, and so on. The temperature can also affect the fluidity of the cell membrane and the transport of substances, therefore the change in temperature has some effect on the absorption of nutrients and secretion of metabolites. In this research, when the temperature was 38 °C, the sugar and alcohol conversion rate was the highest reaching up to 25 %. According to the reported research, the higher temperature could improve the ethanol productivity of bacteria. In Lacis's research, thermophilic bacteria was obtained through high temperature continuous cultivation and the ethanol yield reached 0.44 g ethanol/g xylose.[18]

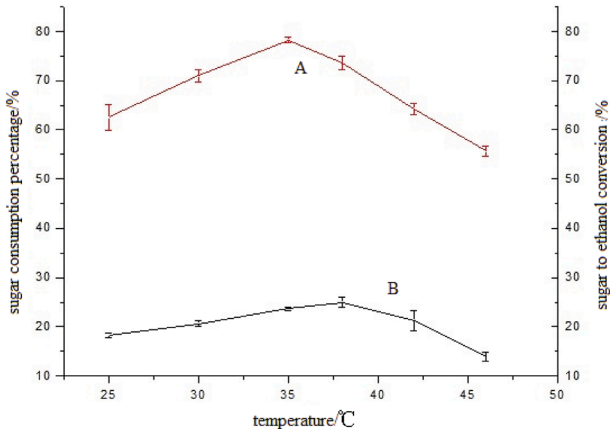

Fig. 3 – *Effect of temperature on ethanol fermentation performance: (A) sugar consumption percentage; (B) sugar to ethanol conversion yield*

Effect of nutrient conditions on ethanol fermentation

Nutrient conditions play an important role in the production of ethanol. The synthetic media with different sugar concentrations were run continuously for ethanol fermentation. Residual sugar and ethanol concentration was determined after ethanol fermentation of 48 h. The fermentation conditions were as follows: inoculation amount 2 %; pH 7; rotate speed 150 min^{-1}; and fermentation temperature 38 °C. The initial sugar concentration was from 20 g L^{-1} to 100 g L^{-1}.

As shown in Fig. 4, with low sugar content, the concentration of ethanol increased but after the highest point, the concentration somewhat decreased. The optimal level of sugar content for microorganism fermentation was nearly 70 g L^{-1}.

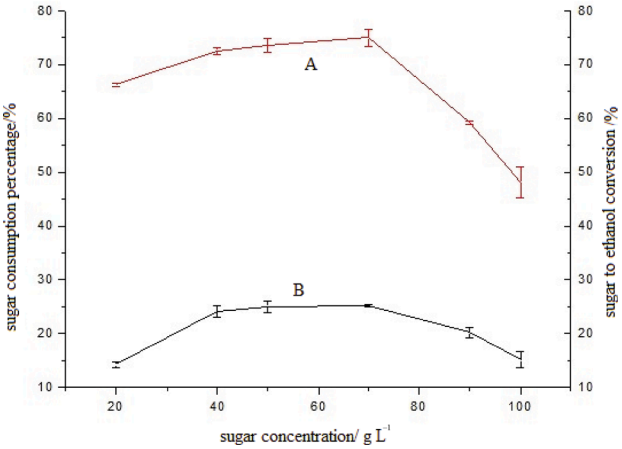

Fig. 4 – *Effect of sugar concentration on ethanol fermentation performance: (A) sugar consumption percentage; (B) sugar to ethanol conversion yield*

In the research, the effect of the types of sugar was considered. About five kinds of sugar combinations of monosaccharide and mixed sugar fermentation culture were prepared. A fermentation solution contained xylose 50 g L^{-1}. B fermentation solution contained glucose 50 g L^{-1}. C fermentation solution contained xylose 25 g L^{-1} and glucose 25 g L^{-1}. D fermentation solution contained xylose 40 g L^{-1} and glucose 10 g L^{-1}, and E fermentation solution contained xylose 10 g L^{-1} and glucose 50 g L^{-1}. Residual sugar and ethanol concentration were determined after ethanol fermentation of 48 h. The fermentation conditions were as follows: inoculation amount 2 %; fermentation temperature 35 °C; rotate speed 150 min^{-1}; and pH 7.

The result is shown in Fig. 5. The ethanol fermentation was best in the fermentation solution with 50 g L^{-1} xylose. The xylose transformation ability of strain N6 was better than the glucose transformation ability of strain N6.

At the same time, the inoculum can affect the fermentation effect and ethanol production. When the inoculum size was 5 %, the ethanol production rate was the highest and reached 26 %.

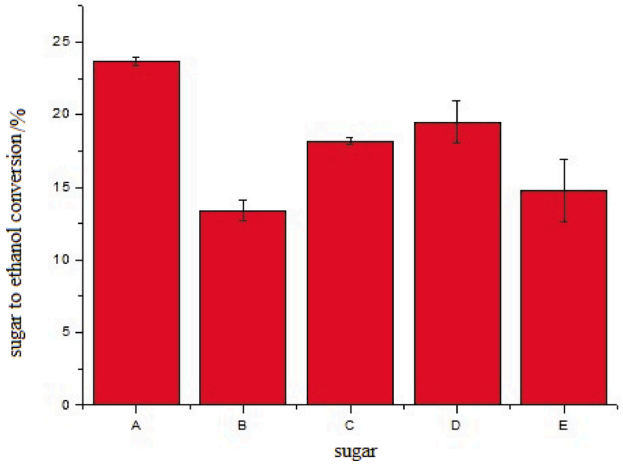

Fig. 5 – *Effect of sugar types on ethanol fermentation performance: (A) fermentation solution contained xylose 50 g L^{-1}; (B) fermentation solution contained glucose 50 g L^{-1}; (C) fermentation solution contained xylose 25 g L^{-1} and glucose 25 g L^{-1}; (D) fermentation solution contained xylose 40 g L^{-1} and glucose 10 g L^{-1}; and (E) fermentation solution contained xylose 10 g L^{-1} and glucose 50 g L^{-1}*

In the research, strain N6, which ferments xylose and hexose to ethanol, can produce fuel ethanol from lignocellulosic hydrolysates. As a result, the optimal conditions for ethanol fermentation were determined. The optimum fermentation conditions at 150 rpm for 48 h in a shake flask were as follows: pH 7.0, reducing sugar 70 g L^{-1}, inoculation amount 5 %, and temperature 38 °C. The ethanol yield coefficient could reach a maximum of 0.26 g ethanol/g xylose. The theoretical yield of ethanol from xylose is given by the equation if the main products are ethanol and CO_2:

$$3 \text{ xylose} \rightarrow 5 \text{ ethanol} + 5 \text{ } CO_2$$

The maximal theoretical yield for complete fermentative xylose conversion is 0.51 g ethanol/g xylose. Therefore, in this research, 51 % of the maximum theoretical yield for complete fermentative xylose conversion was reached, which was close to other reported research. In Kötter's research, in *P. stipitis* cultures the yield of fermentation products was 52 % of the maximum theoretical yield for complete fermentative xylose conversion.[19] And in Li's report, the maximum volume of ethanol by *Candida tropoicalis* was 0.06 g ethanol/g xylose and after domestication, the xylose conversion yield was 0.15 g ethanol/g xylose.[20]

Effect of toxic compounds in lignocellulose acid hydrolysate on strain N6

During acid hydrolysis,[21] pentoses could be degraded to furfural, and the decomposition of hexoses could form 5-hydroxymethylfurfural (5-HMF). 5-HMF could then be degraded, generating formic acid. Acetate is liberated from hemicellulose hydrolysis. The degradation of lignin results in numerous aromatic compounds, such as vanillin[22] and phenol. The concentration of these hydrolysis byproducts depends on the raw materials and hydrolysis conditions.[21] These inhibitors can retard the yeast fermentation and other biochemical reactions. Therefore, knowing their inhibition on microorganisms is very important. Most studies focused on the effect of furfural and acetic acid on fermentation,[23,24] while lacking other inhibitors for comparing effects on fermentation. Thus, the research was scarce on different inhibitors' tolerance toward domesticated strains and undomesticated strains (xylose fermentation). Some research involved hexose fermentation[25,26] but the inhibitor of xylose fermentation was relatively small.

Strain N6 was inoculated into the liquid medium containing an adequate carbon source supplemented with varying initial concentrations of furaldehyde, acetate acid, formic acid, and benzyl phenol. The inhibition rate of toxic compounds on the strain's growth after 24 h and 48 h are shown in Table 2.

Table 2 – *Effect of toxic compounds on strain growth*

The concentration of furaldehyde (V/V):	0.05 %	0.10 %	0.15 %	0.20 %	0.25 %
The inhibition rate (24 h):	0	0	0	19.4 %	40 %
The inhibition rate (48 h):	0	0	0	5.20 %	42.27 %
The concentration of formic acid (V/V):	0.05 %	0.10 %	0.15 %	0.20 %	0.25 %
The inhibition rate (24 h):	5.27 %	45 %	65.2 %	75.5 %	89 %
The inhibition rate (48 h):	10.7 %	29 %	59.2 %	86.0 %	91 %
The concentration of acetate acid (V/V):	0.05 %	0.10 %	0.15 %	0.20 %	0.25 %
The inhibition rate (24 h):	0	0	18.97 %	25 %	40 %
The inhibition rate (48 h):	0	0	18.5 %	48 %	57.8 %
The concentration of phenol (V/V):	0.05 %	0.10 %	0.15 %	0.20 %	0.25 %
The inhibition rate (24 h):	0	51.8 %	64 %	79 %	64 %
The inhibition rate (48 h):	7.5 %	60.1 %	85 %	95.5 %	99 %

Compared to the standard culture, all toxic compounds-supplemented cultures showed an inhibition effect on cell growth. Of the tested inhibitors, furaldehyde had a less toxic effect on the strain. Low concentration (0.05, 0.1, and 0.15 %) could not decrease the growth of strain N6. Formic acid was more effective than furaldehyde. At the initial concentrations of 0.05 % or higher, formic acid induced a decreased cell concentration. When the concentration of formic acid was 0.25 %, the inhibition rate increased to 91 %. Acetate acid was an important inhibition factor. The inhibition rate of strain N6 was 57.8 % when the concentration of furaldehyde in culture solution reached 0.25 %. Phenol played a more powerful role among all the inhibitors. At low initial concentration (0.10 %), phenol was able to inhibit cell growth significantly; moreover, at concentrations above 0.10 %, the toxicity increases. The toxicity order was phenol > formic acid > acetate acid > furaldehyde. The lowest initial concentrations of toxins affecting the growth of the strain were 0.15 % (acetate), 0.05 % (formic acid), 0.20 % (furaldehyde), and 0.05 % (phenol) following the preceding discussion. Compared to the commonly used saccharomycetes, the tolerance of strain N6 toward furaldehyde had greatly improved, which could help the ethanol fermentation from lignocellulose materials. The research of Zhang[14] reported that when the furadehyde concentration reached 0.1 %, the inhibition rate of *P. tannophilus* after 24 h was 94 %.

In order to analyze the effect of toxic compounds on the growth period of strain N6, the growth curves of the strain in the liquid medium containing different toxic compounds (0.15 % acetate, 0.05 % formic acid, 0.20 % furaldehyde, and 0.10 % phenol, respectively) are shown in Fig. 6.

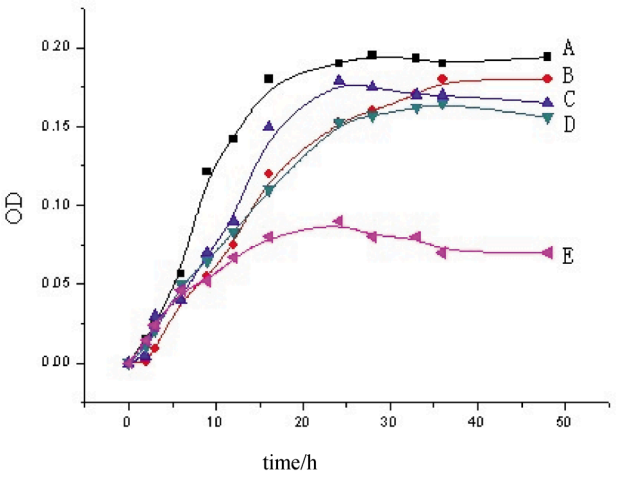

Fig. 6 – *Effect of toxic compounds on the growth curve of the strain: (A) without toxic compounds; (B) 0.20 % furaldehyde; (C) 0.05 % formic acid; (D) 0.15 % acetate; (E) 0.10 % phenol*

The growth curve of strain N6 was measured. The period of logarithmic growth was from 3 h to 24 h, and after 28 h the strain was in the stable phase.

As shown in Fig. 6, from 0–24 h was the fast-growing period of the strain and after 28 h the growth rate slowed down. The addition of furaldehyde slowed down the growth of the strain from 0–30 h (compared to the strain cultivated in the solution without toxic compounds); however, after 36 h, the cell growth rate had increased, indicating the adaptability of the strain to furaldehyde. Acetate acid also contributed to the decrease in growth rate, especially in the period from 0 to 24 h. Formic acid led to the stop of growth during the 24–36 h, and before 24 h the strain was slow-growing. Phenol resulted in the slow growth rate of strains in 0–24 h, and after 24 h the growth rate was slower. Fig. 6 also indicated that 0.10 % of phenol had not killed large numbers of strains, but it would affect the reproduction of the strains.

Conclusions

Xylose is abundant in lignocellulosic biomass. The fermentation of xylose is still a key problem in cellulose ethanol fermentation. Successful utilization of xylose could improve the efficiency of the biomass-ethanol fermentation process.

In this research, the xylose fermentation strains were isolated from a soil sample. Strain N6 was chosen for further experiments. A comparison of its 16S rDNA gene sequence (with available sequences in GenBank) showed that it was 100 % identical to that of *Bacillus cereus strain* SWFU2816 (Accession No. JN935015.1).

Strain N6, which ferments xylose and hexose to ethanol, is capable of producing fuel ethanol from lignocellulosic hydrolysates. The xylose transformation ability of strain N6 was better than its glucose transformation ability. As a result, optimum conditions for xylose fermentation were determined. The optimum fermentation conditions at 150 rpm for 48 h in a shake flask were as follows: pH 7.0, reducing sugar 70 g L^{-1}, inoculation amount 5 %, and the temperature 38 °C. The xylose to ethanol yield coefficient could reach a maximum of 0.26 g/g.

At the same time, the influence of the acid hydrolysis by-products on the isolated strain was studied. The lowest initial concentrations of toxins affecting the growth of the strain were 0.15 % (acetate), 0.05 % (formic acid), 0.20 % (furaldehyde), and 0.05 % (phenol). The toxicity order was phenol > formic acid > acetate acid > furaldehyde. The addition of furaldehyde slowed down the growth of the strain during the period from 0 to

30 h. Acetate acid also contributed to decreased growth rate, especially from 0 to 24 h. Formic acid stopped growth from 24 to 36 h. Phenol resulted in the slow growth rate of strains from 0 to 24 h, and after 24 h the growth rate was slower.

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