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Conversion of Biomass to Ethanol

Isomerization of Xylose over HY Zeolite

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ABSTRACT

Xylose, a pentose indigestible to most yeasts, was converted to ethanol by a two-step isomerization and fermentation. HY zeolite was used to catalyze the isomerization of xylose, and the xylulose produced was directly used as the carbon source in ethanol fermentation. Zeolite catalysts offer pH compatibility with yeast fermentation and the ability to carry out isomerization at higher temperature where equilibrium xylulose concentration is higher. Initial rate studies indicate that xylose consumption follows pseudo-first-order kinetics, with a specific rate constant of 6.2×10^{-4} L/solution/g zeolite/h.

Index Entries: Xylose; xylulose; zeolite; isomerization; fermentation.

INTRODUCTION

In order to improve the efficiency of production of fuel ethanol from cellulosic biomass, the conversion of hemicelluloses has to be considered. Hemicelluloses can be hydrolyzed easily by dilute acid (*1*), and the resulting hydrolysates contain about 70–80% xylose. For many microorganisms, the fermentation of xylose to produce ethanol is difficult. On the other hand, xylulose, the keto-isomer of xylose, can easily be converted to ethanol by many yeasts (2). Isomerization of xylose to xylulose

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can be accomplished by either xylose isomerase or acid-base catalysts. Both xylose isomerase and homogeneous acid-base catalysts suffer from the problem of pH incompatibility with yeast fermentation. The adjustment of pH is required between isomerization and fermentation. To avoid this problem, zeolites are introduced as new catalysts for isomerization.

Zeolites are crystalline, hydrated aluminosilicates of group I and group II elements (3). The three-dimensional crystal structure of zeolites contains pores, cages, and channels of uniform size on a molecular scale. These structural characteristics endow zeolites with unique shape selectivity, which makes zeolites one of the favored catalysts for highly selective hydrocarbon reactions, such as cracking, isomerization, and alkylation in the petrochemical industry.

Although much attention has been paid to heterogeneous gas or non-aqueous-phase adsorption and reactions involving zeolites, only a few applications of zeolites in aqueous solution of sugars have been reported. Zeolites were applied successfully for the separation of glucose and fructose in aqueous solution for making high-fructose syrup (4). UOP developed the process known as Sarex for the separation of fructose from glucose using CaY zeolite as the adsorbent (5). This processes depends on the molecular sieving property of Y zeolite and the perferred interaction between calcium ion and fructose.

Shukla and coworkers (6) studied the application of zeolites as catalysts in aqueous sugar solution for the isomerization and hydrolysis reactions of disaccharides. They examined several zeolites for catalytic activity in the isomerization and hydrolysis of cellobiose, maltose, and lactose solutions, and concluded that zeolites of type A, X, and Y are the most active catalysts for these reactions. They suggested that the reaction proceeded by parallel hydrolysis and isomerization of disaccharides to their corresponding ketoses, followed by hydrolysis of those ketoses. However, their reactions were conducted under alkaline conditions, where isomerization occurred even without the presence of zeolites. Their controlled experiment using sodium hydroxide as catalyst obtained similar cellobiose conversion (62%), but less hydrolysis and isomerization products compared to reactions over NaX zeolite at similar initial pH. Although zeolite seemed to alleviate the degradation of the sugars, similar cellobiose conversion rates obtained with and without zeolite were hard to explain, and therefore the role of zeolite in this reaction system was not clear.

Jow et al. (7) studied the dehydration of molten D-fructose to levulinic acid over LZY zeolite at 140°C. Under their experimental conditions, the concentration of fructose declined with time, accompanied by an increase in levulinic acid concentration and peaks in glucose and 5-hydroxymethylfurfural (HMF) concentrations. They concluded that the dehydration of D-fructose over LZY zeolite was similar to that in homogeneous acid solution but with lower selectivity for HMF. Recently, Lourvanij and Rorrer

(*8*) studied the reactions of aqueous glucose solutions over HY zeolite. Several acid-catalyzed reactions were identified in their work, including isomerization of glucose to fructose, partial dehydration of glucose to HMF, rehydration and cleavage of HMF to formic acid and 4-oxopentanoic acid, and carbonization. The lowered selectivity for HMF in the solution phase of aqueous glucose/HY zeolite system obtained by Lourvanij and Rorrer agrees with the observations of Jow et al. Both suggested that molecular sieving by zeolites might play a role in affecting product distribution. In Lourvanij and Rorrer's study, they proposed that the pyranose ring was opened first by the acidic site located on the outer surface of zeolite, and the acyclic glucose molecules then diffused into the pores of zeolite, where the subsequent acid-catalyzed reactions occurred. Because of the greater size of HMF formed inside the supercages of Y zeolite, HMF was trapped inside the zeolite, and this led to the low concentration of HMF in the solution phase.

In this work, we studied the conversion of xylose catalyzed by HY zeolite in aqueous solution at various initial xylose concentrations, zeolite loadings, and reaction temperatures. The interaction between xylose and HY zeolite is discussed. Fermentation was carried out using the reaction broth as the carbon source in yeast fermentation to produce ethanol.

METHODS

Materials

Xylose and lyxose were purchased from Sigma. Xylulose, in the form of 0.67*M* aqueous solution, was a gift from an industrial source. The purity of xylulose was checked by HPLC and NMR spectroscopy. The HY zeolite used was the LZY-72 zeolite in powder form from UOP. The other chemicals used were reagent-grade.

Reaction Procedure

For reaction temperatures above 100°C, xylose isomerization over HY zeolite was performed in a temperature-controlled high-pressure reactor (Model 4561, Parr Instruments). In this 300-mL reactor, 150 mL of xylose solution was mixed with 5–15 g of zeolite catalyst. This reactor was then sealed and placed into a heating mantle. The reaction mixture was well mixed with an agitation rate of 650 rpm, and its temperature was monitored by a J-type thermocouple. Because of the vigorous agitation, the liquid film contacting the reactor wall above the slurry phase was believed to be thin and negligible. Time zero was defined when the reaction mixture reached the set temperature. It took about 12 min to reach 100°C and 18 min for 160°C. The pressure inside this reactor ranged from 15 to 90

psig, depending on the reaction temperature. When the reaction temperature was equal to or lower than 100°C, a 500-mL round-bottom flask was employed with condenser in a temperature-controlled oil bath.

Analytical Methods

HPLC

Monosaccharides and ethanol were analyzed by high-performance liquid chromatography, using Aminex carbohydrate HPX-87C (Bio-Rad Co.) as the analytical column, and 80°C deionized water as the mobile phase. The flow rate was 0.6 mL/min. A refractive index monitor (L-3350, Hitachi Ltd.) and a UV detector (L-4000, Hitachi Ltd.) set at 214 nm were used for chromatographic peak detection. The chromatograms were integrated by integrators (D-2500, Hitachi Ltd.). The concentrations of monosaccharides and ethanol were calculated by comparing with references of known concentration. The determination of sugar concentration in the initial rate experiments and fermentation was carried out by HPLC.

NMR Spectroscopy

Liquid state ^{13}C NMR spectra were obtained using a Gemini 200 spectrometer from Varian Instruments equipped with a 10-mm probe operating at 50.3 MHz. For quantification purposes, the pulse delay time was set to be five times greater than the longest T_1 relaxation time. The product concentrations in the solution phase for long reaction time experiments were determined by ^{13}C NMR spectroscopy. The composition of the mixture was determined by peak height of resolved peaks and comparison with standards of known composition. Integrals were determined to $\pm 5\%$.

Ethanol Fermentation

Schizosaccharomyces pombe was used to produce ethanol in this work. In the growth phase, cells were produced in 20 mL of 50 g/L glucose, 20 g/L becto-peptone, and 10 g/L yeast extract solution for 30 h, in a controlled environment incubator shaker set at 200 rpm, 33°C. In the fermentation phase, cells were resuspended in 20 mL of the 1:1 mixture of reaction broth and the YEP medium containing 40 g/L becto-peptone and 20 g/L yeast extract. The reaction broth was obtained from xylose solution initially at 1.33 mol/L catalyzed by HY zeolite at 130°C. The composition of reaction broth is 0.93 mol/L xylose, 0.20 mol/L xylulose, and 0.087 mol/L lyxose. Fermentation proceeded in a 50-mL Erlenmeyer flask sealed with rubber stopper in the aforementioned shaker. Samples were taken periodically, and the solution composition was analyzed by HPLC.

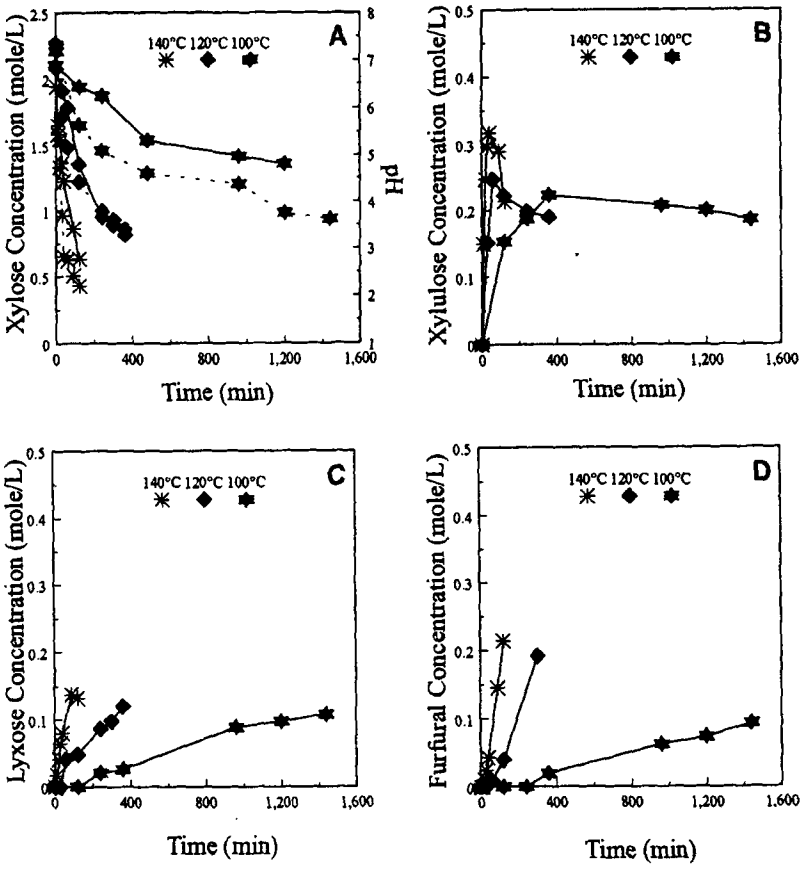


Fig. 1. Time-courses of xylose reaction over HY zeolites at different temperatures. (**A**) xylose consumption (solid lines) and solution pH (dashed lines); (**B**) xylulose production; (**C**) lyxose production; and (**D**) furfural production.

RESULTS

Conversion of xylose was catalyzed by HY zeolite in a batch reactor of 300 mL with agitation rate of 650 rpm. Xylose solutions (150 mL at concentration ranges from 0.33–2.0*M*) were mixed with 5 g of HY zeolite for reactions carried out at 100–160°C. Samples were quenched in ice, and then the reaction solution was separated from the catalyst for analysis. On initial contact with the zeolite, the solution was approximately neutral. Then the solution pH decreased gradually with reaction time as a result of acid production (*see* Fig. 1A, dashed lines). The solution and zeolite were initially colorless and white, respectively. They turned amber, brown, and eventually deep brown as time proceeded. X-ray powder diffraction patterns of the HY zeolites before and after reactions were compared, and no significant structural disintegration was observed after reactions.

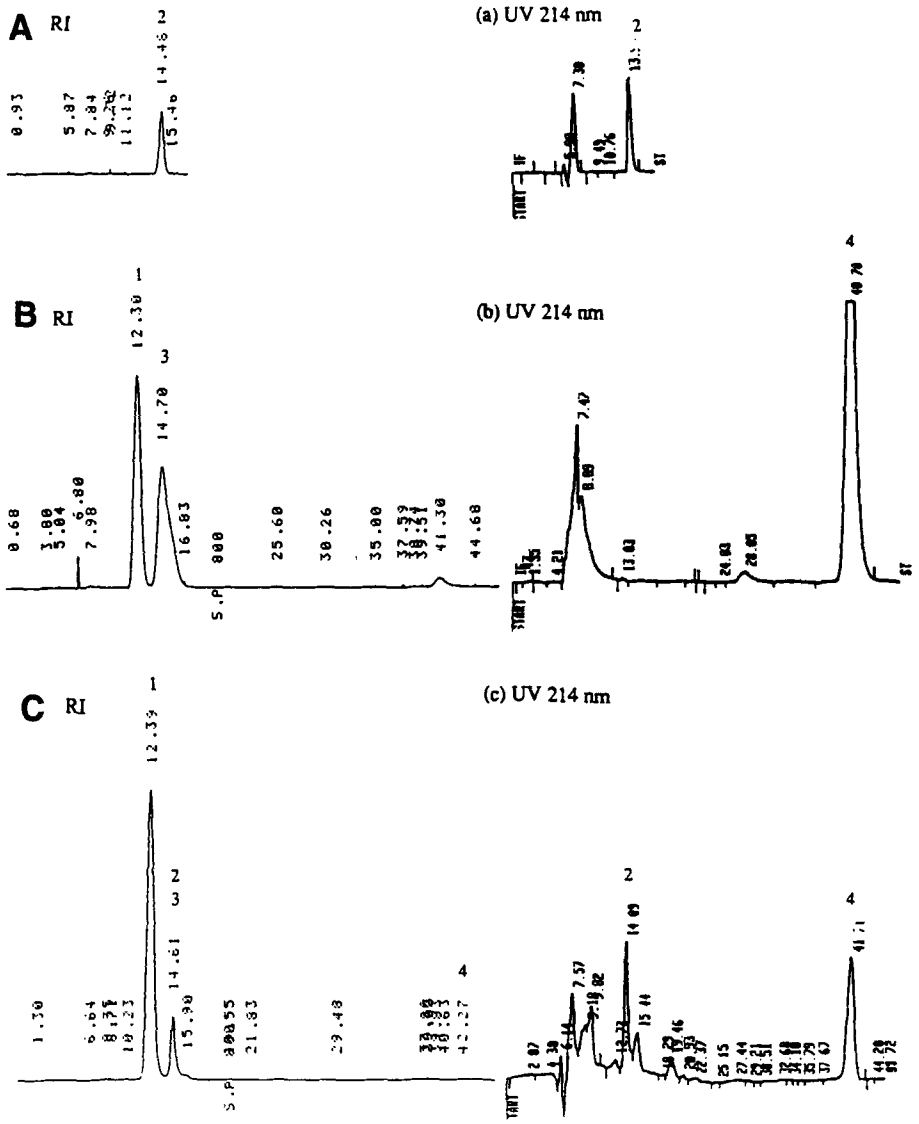


Fig. 2. Chromatograms obtained from A refractive index monitor and UV at 214 nm. (**A**) A xylulose standard, 6.8 m*M*; (**B**) a three-component standard containing 33.3 m*M* xylose, 33.4 m*M* lyxose, and 0.037 m*M* furfural; and (**C**) the reaction broth obtained from the following conditions: initial xylose concentration = 2.0*M*, catalyst loading = 100 g/L, temperature = 100°C, time = 300 min. Peak identification: 1. xylose; 2. xylulose; 3. lyxose; and 4. furfural.

HPLC Analysis

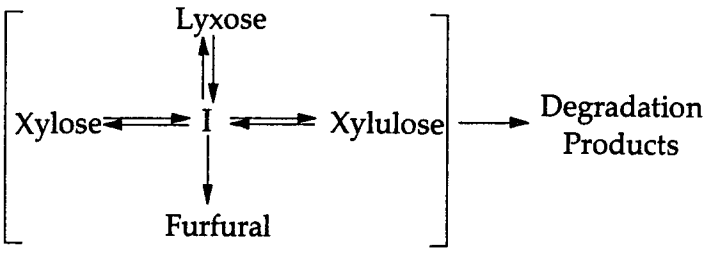
In a typical analysis of the liquid phase of the reaction system (*see* Fig. 2), three peaks were detected by refractive index detection. These three peaks and several peaks of short retention time were observed by UV detector at 214 nm. By injecting the standard solutions of xylose,

xylulose, lyxose, and furfural separately into the same LC column, we found that xylose and furfural were well separated from the other compounds, but xylulose and lyxose were eluted at very similar retention times. A mixture of xylulose and lyxose remained unseparated after chromatography. However, even though all of these four compounds give comparable response on the RI monitor, only xylulose and furfural can be detected at 214 nm in the UV. Therefore, we can distinguish xylulose from lyxose by comparing the chromatograms obtained with UV and RI detectors. The unidentified peaks of low retention time exhibited high UV absorption, but low RI response. Because their detection responses to both detectors are similar to furfural, they are likely to be related to furfural, possibly the condensation products of furfural, with sugars, sugar derivatives, furfural itself, or furfural derivatives. Since no better analytical column was available for xylulose–lyxose separation, the concentration of xylulose was determined from the UV chromatogram, and the concentration of lyxose was calculated from the RI response and xylulose concentration. The concentration of xylose was quantified by RI and the concentration of furfural was determined by UV.

In order to confirm the products in the xylose–HY zeolite reaction system, ^{13}C NMR spectroscopy was employed. The NMR spectra for the samples taken at various reaction times at 140°C confirmed that the four major solution components were xylose, xylulose, lyxose, and furfural. Several minor peaks appearing at the low field (19–28 ppm) in the NMR spectra may correspond to the hydrocarbon-like compounds produced from a series of dehydrations and alkylations.

Isomerization

When catalyzed by zeolites, xylose produced two isomers: xylulose, the keto-isomer, and lyxose, the C2 epimer of xylose. Since xylose was consumed steadily (*see* Fig. 1), xylulose accumulated rapidly to its maximum and then decreased as a result of further degradation. Lyxose was also produced, but at a slower rate. Furfural, which was not detected in the early stages, became one of the major products as reaction proceeded. The interconversion between xylose, xylulose, and lyxose under zeolite catalysis is similar to that between glucose, fructose, and mannose in acidic solution (*9*). The simplest pathway for the reversible interconversion among xylose, xylulose, and lyxose with the enediol as common intermediate follows the Lobry de Bruyn-Alberda van Ekenstein transformation (*10*). This intermediate also exists in the route of dehydration of xylose to furfural, as confirmed by Feather et al. (*11*), who worked with acidified, tritiated water in xylose conversion. These reactions presumably proceed by a collection of reversible protonation–deprotonation steps. Because of the similar product distribution, it is likely that the same intermediates may exist with zeolite catalysis.



(1)

where I represents the reaction intermediate.

Although the formation rate of xylulose is much higher than the other products, xylulose accumulation slows down, and eventually its concentration starts to drop owing to the approach to equilibrium and further reactions. Apparently, long reaction times do not enhance xylulose production. Consequently, short reaction times may be desired to optimize xylulose production.

Temperature Effect

At 40°C, the xylose/xylulose equilibrium is 85:15 (*12*). Increasing the temperature improves the equilibrium concentration of xylulose. However, the stability of the enzyme, xylose isomerase, declines. Therefore, enzymatic isomerization is usually carried out at <70°C. When using zeolites, we are able to catalyze the xylose reactions at higher temperatures. The time-courses of xylose conversion in the temperature range of 100–140°C are shown in Fig. 1. The consumption of xylose appears to follow first-order kinetics. The rate constants for xylose consumption were calculated for different reaction temperatures (*see* Fig. 3), assuming a pseudo-first-order kinetics. The activiation energy was estimated according to the Arrhenius equation over the temperature range of 80–160°C and found to be 92.8 kJ/mol. This value is smaller than the literature value for xylose disappearance in sulfuric acid solution (134 kJ/mol) (*13*), but it is much greater than that for a diffusion-controlled process, ruling out external diffusion limitations and supporting the hypothesis that reaction is the rate-limiting step in this heterogeneous catalytic reaction. The maximum concentration of xylulose increases with reaction temperature (*see* Fig. 1B), but the increment is limited owing to the instability of xylulose under reaction conditions. Because of the thermal stability of xylulose and its limiting equilibrium concentration, it is recommended that the xylulose, once produced, be removed from the reaction system to improve xylulose production.

To investigate the thermal stability of xylose, controlled experiments on xylose solutions without zeolites were conducted at 100 and 140°C. A 35-h incubation of xylose solution at 100°C changed the colorless solution to light yellow, but no residue formation or xylose degradation was observed. However, at 140°C, 20% of the initial xylose (1.33 mol/L) disap-

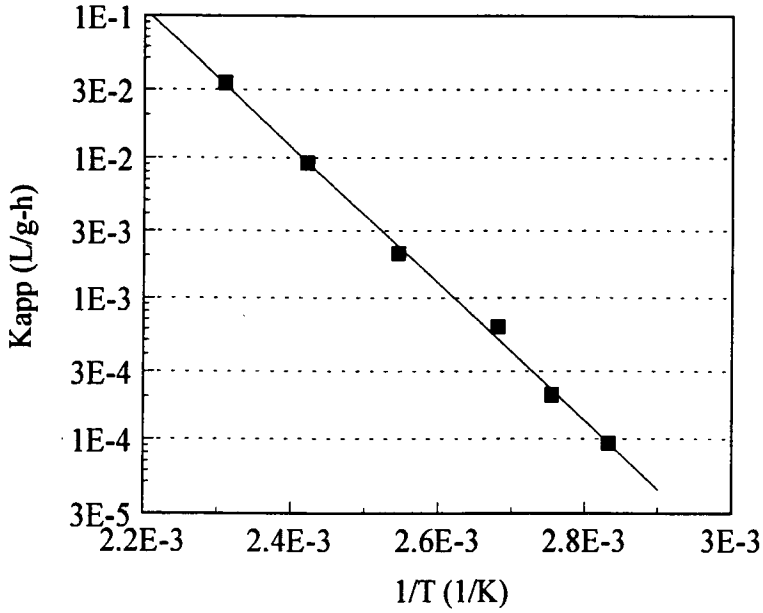


Fig. 3. Arrhenius plot for xylose consumption. Reaction conditions: 150 mL of 0.33*M* xylose solution were mixed with 15 g (for 80 and 90°C) or 5 g (for 100–160°C) of HY zeolite. The activation energy calculated from least-square regression is 92.8 kJ/mol, with r^2 = 0.998.

peared after 2 h, accompanied by formation of an amber color in solution, the production of furfural, and the formation of black, resin-like residues.

The stability of HY zeolite was also examined by comparing the X-ray diffraction patterns of HY zeolites before and after a 2-h reaction at 130°C. The results showed no significant difference between the diffraction patterns, indicating the good stability of HY zeolite.

Catalyst Loading and Initial Xylose Concentration

Based on the assumption of pseudo-first-order reaction kinetics, we can estimate the apparent consumption rate constant from initial rate data. The initial rate data were collected for up to 15% xylose conversion, where the degradation of xylose and deposition of coke onto zeolite were negligible. Table 1 summarizes the estimated consumption rate of xylose at 100°C with different initial xylose concentrations and zeolite loadings. The rate constant does not change with the initial xylose concentration, and is proportional to the catalyst loading for the range of initial xylose concentrations and zeolite loadings examined. With the rate constant defined per gram of zeolites in each liter of sugar solution, the specific rate constant should not vary with sugar concentration and catalyst loading under first-order kinetics. The average specific rate constant calculated from 13 initial rate experiments is $(6.2 \pm 0.5) \times 10^{-4}$ L solution/g zeolite/h.

Table 1
Kinetic Constants for Xylose Consumption Calculated from Initial Rate Experiments (up to 15% of Xylose Conversion)

Zeolite loading, g zeolite/l solution	Xylose concentration, M	Reaction rate constant, 1/h	Specific rate constant, L solution/g zeolite/h
100	2.14	6.7×10^{-2}	6.7×10^{-4}
	2.01	6.4×10^{-2}	6.4×10^{-4}
	1.37	6.0×10^{-2}	6.0×10^{-4}
	0.66	6.9×10^{-2}	6.9×10^{-4}
	0.33	6.8×10^{-2}	6.8×10^{-4}
	0.34	6.6×10^{-2}	6.6×10^{-4}
66.7	1.30	3.5×10^{-2}	5.2×10^{-4}
	0.70	4.2×10^{-2}	6.3×10^{-4}
	0.35	4.0×10^{-2}	6.1×10^{-4}
33.3	2.04	1.9×10^{-2}	5.6×10^{-4}
	1.36	2.0×10^{-2}	5.9×10^{-4}
	0.65	2.1×10^{-2}	6.2×10^{-4}
	0.34	2.0×10^{-2}	5.9×10^{-4}

[a]Reaction temperature = 100°C.

Ethanol Fermentation

Ethanol fermentation from the reacted xylose–xylulose broth was carried out in shaker flasks. The fermentation medium, containing 93.7 g/L xylose, 5.1 g/L xylulose, and 1.1 g/L lyxose, was consumed to produce ethanol. Both xylose and xylulose were utilized as the carbon source. At the end of a 40-h fermentation, all of the xylulose and 3.7 g/L of xylose disappeared, and 2.2 g/L of ethanol were produced (*see* Fig. 4). However, attempted fermentation of xylose by the same yeast did not succeed. The reason for the different xylose utilization in the media with and without xylulose is not clear. Although the xylulose concentration was low because isomerization was stopped at the early stage to avoid the decrease in xylulose selectivity with time, a process of alternate isomerization and fermentation may be adopted in the future to increase the utilization of xylose and, consequently, the production of ethanol.

DISCUSSION

Zeolites are solid acid catalysts that enable aldose–ketose isomerization in solution of pH 4–7 by providing local acidity within the catalyst phase. According to the structural formula of HY zeolite, $[H_{31}(AlO_2)_{31}(SiO_2)_{161}]\cdot 260\ H_2O$, there is 1.9 mmol of protons/g zeolite, which is equivalent to 4.78 mol H^+/L pore volume. For a reaction system with 100

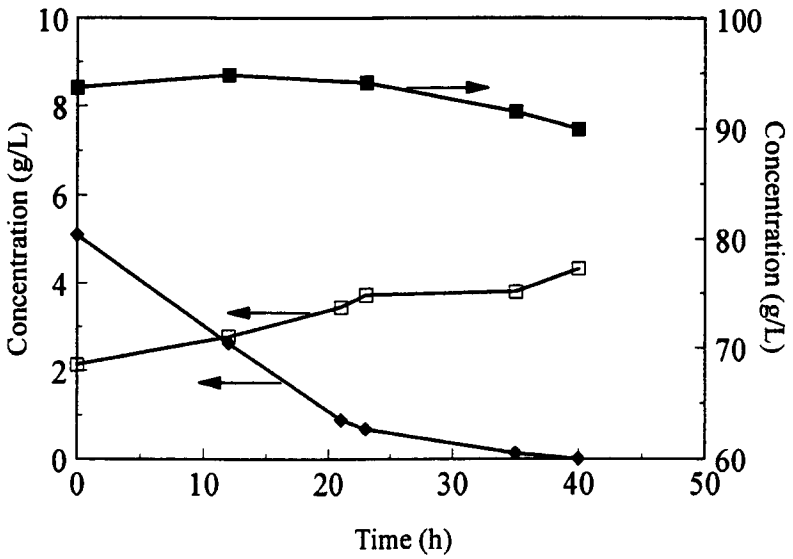


Fig. 4. Ethanol fermentation using reacted xylose–xylulose broth as a carbon source. Medium composition: 93.7 g/L xylose, 5.1 g/L xylulose, 1.1 g/L lyxose, 20.0 g/L becto-peptone, and 10.0 g/L yeast extract. ■: xylose; ◆: xylulose; □: ethanol.

g zeolite/L solution, the total number of protons is equal to that in 0.19*M* homogeneous acid solution. Although there is only a little xylose conversion in 0.19*M* hydrochloric acid solution, local acidity may explain why HY zeolite has stronger catalytic activity than homogeneous acid.

Comparison between 5.0*M* (close to the local proton concentration in zeolite phase) hydrochloric acid and 100 g/L of zeolite was also made at 100°C to investigate if the shape selectivity plays a role in xylose conversion. In hydrochloric acid solution, xylose was converted to furfural and resin-like residues quickly, but no isomerization occurred (*14*). The reactions of xylose catalyzed by zeolite included isomerization and dehydration. The difference may be because of steric hindrance inside the zeolite cages and channels that inhibit the polymerization between furfural, sugars, and their derivatives.

The accessibility of the internal surface of zeolite to sugar molecules affects reaction rate. HY zeolite has the pore size of 7.4 Å and the kinetic diameter of 8.4 A (*3*). The diameter of a xylose molecule, calculated from X-ray crystal data (*15*), is 6.27 A. When considering van der Waals radii, the longest dimension becomes 7.47 A, which is small enough to enter the zeolite pores. Further support for the entrance of xylose into the cages of HY zeolites comes indirectly from the chromatographic study of glucose and fructose (which are bulkier than xylose because of the one more CH_2OH group attached at C5 on the pyranose rings) sorption onto NaX and KX zeolite crystals (*16*). From the band broadening of small

pulses of sugar solution, they obtained the same value ($\sim 10^{-9}$ cm^2/s) for the intracrystalline diffusivities for both glucose and fructose in NaX and KX zeolite crystals, regardless of whether the sugar is strongly or weakly adsorbed. This value is much smaller than the diffusivity of those sugars in a free solution (6.7×10^{-6} cm^2/s at 25°C). Zeolite X has the same pore aperture as zeolite Y. Therefore, one would expect the intracrystalline diffusivity in Y zeolite to be of similar order of magnitude. An unpublished incipient wetness study of the interaction between glucose and xylose with HY zeolite by solid-state NMR spectroscopy by the authors also indicates the entrance of glucose molecule into HY zeolite. In the incipient wetness study, 1.6*M* glucose solution with a volume equivalent to 50% of the pore volume of HY zeolite was loaded into calcined HY zeolite and showed strong glucose binding. With 75 and 100% pore volume loadings of glucose solution, the spectra appear to consist of at least two components of similar chemical shifts superimposed, a relatively mobile, liquid-like species, and a motionally constrained species, as well as bound sugars.

Finally, comparison between isomerization using HY zeolite and zylose isomerase showed that the HY zeolite is not as selective as isomerase. However, the enzyme is more sensitive to reaction environment (such as solution pH, temperature, and the presence of inhibitors) than the zeolites tested. Thus, zeolites can be considered as an attractive alternative for xylose isomerization catalysis.

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REFERENCES

1. Mehlberg, R. (1979), Ph.D. Thesis. Purdue University, Indiana.
2. Skoog, K. and Hahn-Hägerdal, B. (1988), *Enzyme Micro. Technol.* **10,** 66–80.
3. Breck, D. W. (1974), *Zeolites: Molecular Sieves, Structure, Chemistry and Use,* John Wiley, New York.
4. Ruthven, D. M. (1988), *Chem. Eng. Prog.* **84(2),** 42–50.
5. Chang, C. H. (1987), US Patent, US4692514A.
6. Shukla, R., Verykios, X. E., and Mutharasan, R. (1985), *Carbohydr. Res.* **143,** 97–106.
7. Jow, J., Rorrer, G. L., Hawley, M. C., and Lamport D. T. A. (1987), *Biomass* **14,** 185–194.

8. Lourvanij, K. and Rorrer, G. L. (1993), *I&EC Res.* **32,** 11–19.
9. Feather, M. S., and Harris, D. W. (1975), *J. Am. Chem. Soc.* **97,** 178–181.
10. Speck, J. C. (1958), *Adv. Carbohydr. Chem.* **13,** 63–103.
11. Feather, M. S., Harris, D. W., and Nichols, S. B. (1972), *J. Org. Chem.* **37(10),** 1606–1608.
12. Hsiao, H.-Y., Chiang, L.-C., Chen, L.-F., and Tsao, G. T. (1982), *Enzyme Microb. Technol.* **4,** 25–31.
13. Root, D. F., Saeman, J. F., and Harris, J. F. (1959), *Forest Prod. J.* **May,** 158–165.
14. Lee, C. Y., Ruaan, R. C., Wen, J., Thomas, S. N., Delgass, W. N., Grutzner, J. B., and Tsao, G. T. (1993), *Proc. Sem. Biochem. Eng.* **1993,** 137–140.
15. Hordvik, A. (1971), *Acta Chem. Scand.* **25(6),** 2175–2182.
16. Ching, C. B., Hidajat, K., and Uddin, M. S. (1989), *Sep. Sci. Tech.* **24,** 581–597.