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ISOMERIZATION AND HYDROLYSIS REACTIONS OF IMPORTANT DISACCHARIDES OVER INORGANIC HETEROGENEOUS CATALYSTS

RAJESH SHUKLA, XENOPHON E. VERYKIOS, AND RAJAKKANNU MUTHARASAN
Department of Chemical Engineering, Drexel University, Philadelphia, Pennsylvania 19104 (U.S.A.)
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ABSTRACT

Isomerization and hydrolysis reactions of cellobiose, maltose, and lactose were investigated over various minerals and synthetic zeolite catalysts. Zeolites of type A, X, and Y are the most active for such reactions. Product distributions were determined from batch experiments and are compared with those obtained under homogeneous alkaline conditions. Product distributions indicate that reaction routes consist of parallel hydrolysis and isomerization of the disaccharides to their corresponding ketoses, followed by hydrolysis of the ketoses. Approximately 10–13% of the disaccharide reacted is not accounted for in the product distribution, indicating that degradation reactions occur, probably in the alkaline broth of the mixtures.

INTRODUCTION

It is well known that such inorganic compounds as silica, silicates, and aluminosilicates catalyze isomerization, hydration, dehydration, hydrogenation, cyclization, and various other reactions. Production of ketoses by the action of sodium or potassium aluminate, and rearrangement of aldoses by strongly basic resins, are also well known[1,2]. Furthermore, certain minerals such as amphibolite, anthophyllite, muscovite, biotite, pulogopite, and crocidolite also catalyze dehydrogenation, polymerization, and hydrolysis reactions[3]. It has been demonstrated[4], as early as 1957, that these minerals also possess enzyme-like activity. This idea has led to the concept of pseudoenzymes[5], which was applied to the oxidative, polymerizing, and hydrolytic properties of these minerals. Recently, a low level of β-glucosidase-like activity was attributed to the same minerals[3].

Enzymic hydrolysis of biomass is an important area of current interest, related to the production of specialty chemicals and chemical intermediates, and to the development of new energy sources. Problems related to enzymic hydrolysis include the short half-life of the available enzymes, the expenses associated with the growth of the cellulase-producing organism, and the narrow operating ranges under which they can be utilized. Another major difficulty, especially severe with currently available cellulase enzyme-systems, is the occurrence of cellobiose in-

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hibition[6]. As a result, rates of cellulose hydrolysis are significantly decreased, leading to long residence-times in the vessel and low glucose concentrations. A remedy to this difficulty would be to add an enzyme to hydrolyze cellobiose as it is formed. Nevertheless, most cellobiases require a low pH, inhibiting the action of cellulase whose pH optimum[6] is ~7.2.

The present investigation studies the enzyme-mimicking capabilities of various minerals and synthetic aluminosilicates (zeolites) to hydrolyze disaccharides–cellobiose, maltose, and lactose. The objective is the identification of surface sites responsible for such activity, which would enable the synthesis of materials having optimum performance-characteristics in terms of activity and selectivity for desired products. Such solid inorganic materials could be used by themselves as catalysts for hydrolysis of disaccharides or in conjunction with enzymic systems. For example, the problem of cellobiose inhibition could be alleviated by adding an inorganic catalyst capable of hydrolyzing cellobiose to the enzyme system. The surface of the catalyst would offer the conditions required for cellobiose hydrolysis, whereas the bulk of the broth would be at the optimum pH for cellulase activity.

EXPERIMENTAL

Materials. — The minerals tested for hydrolytic activity with cellobiose, lactose, and maltose are of the amphibole (amphibolite, hornblende, actinolite, tremolite, and anthophyllite) and mica (muscovite and biotite) groups. They were selected because of their promising β-glucosidase-like activity in Siegal's[5] study, and were obtained from the Department of Geology, University of Pennsylvania. Zeolites of types A and X were selected for characterizing their hydrolytic activity. The zeolites were procured from PQ Corporation and Zeochem. The various disaccharides were obtained from Sigma Chemicals.

Procedure. — Batch experiments were performed for determining enzyme-like activity of various minerals and zeolites under conditions for hydrolysis of cellobiose, lactose, and maltose. Sugar solutions of appropriate concentration were placed in 250-mL flasks, which were then inserted in a constant-temperature bath. When the solution reached the desired temperature, an appropriate amount of the catalyst was added and the flasks were sealed. The slurry was stirred with magnetic stirrers. Samples of 2 mL were periodically withdrawn with hypodermic syringes and analyzed. Sugar concentrations were varied between 2 and 30% (w/v), the temperature between 70 and 85°, and catalyst loading between 0.1 and 0.3 g of catalyst/mL of sugar solution.

Carbohydrate analysis. — Samples obtained from the reactors were first filtered through 0.22-μm filter paper (Millipore Corporation), and then diluted to appropriate concentrations, and 10 μL of the sample was injected onto a liquid chromatograph (Spectra Physics, SP8000) equipped with an auto injector (Micromeritics, Model 725), a carbohydrate column (Biorad-HPX-87C), and a refractive-index detector (Waters 401). Peak identification was accomplished by injection of

standards and concentrations were determined by using calibration constants based on peak areas. Identification of various disaccharides was also confirmed by using an oligosaccharide column (Biosil Amino 5S).

RESULTS AND DISCUSSION

(a) Screening experiments. — Initial screening experiments were conducted in order to identify the most promising solids for hydrolysis of cellobiose, lactose, and maltose; batch reactors were used in which the disaccharide concentration was 4% by weight, the catalyst loading 0.3 g/mL, and the temperature 85°. Activities, determined as percent of disaccharide reacted over a period of 10 h, are shown in Table I. These results clearly demonstrate that, although some of the minerals exhibit hydrolytic activity towards disaccharides, the activity exhibited by synthetic zeolites is significantly higher. For this reason, further work concentrated on these and other zeolites.

(b) Cellobiose hydrolysis. — Hydrolysis of cellobiose was investigated over zeolite Na-X at a temperature of 70°, initial reactant concentration of 1.0%, and a catalyst loading of 0.1 g/mL. Conversion profiles under these conditions are shown in Fig. 1. Total conversion is based on the amount of cellobiose consumed by the reactions. Conversion into cellobiulose, glucose, and fructose is based on the amount of these products formed. Fig. 1 shows that a very large fraction of the reaction occurs within the first 0.5 h, and all reactions essentially terminate after one h.

The pH profile of the reaction broth is also shown in Fig. 1. Upon addition of the catalyst to the sugar solution, the pH rises to 11.3 and then drops rapidly, as

TABLE I

RESULTS OF ACTIVITY SCREENING OF VARIOUS MINERALS AND ZEOLITES

Catalysts	*Percent of sugar reacted in 10 h*		
	Cellobiose	*Lactose*	*Maltose*
Amphibollite	1.5	6.8	0
Anthophyllite	0.3	0.2	0
Hornblend	0.1	0.2	0
Actinolite	0.1	0.2	0
Tremolite	0.1	0.2	0
Muscovite	1.4	0	0
Biotite	0.8	0	0
Zeolite Na-X	56.8	63.0	67.2
Zeolite 13X	77.7	63.6	40.0
Zeolite 3A	51.5	53.8	52.3
Zeolite 4A	42.3	67.8	69.8

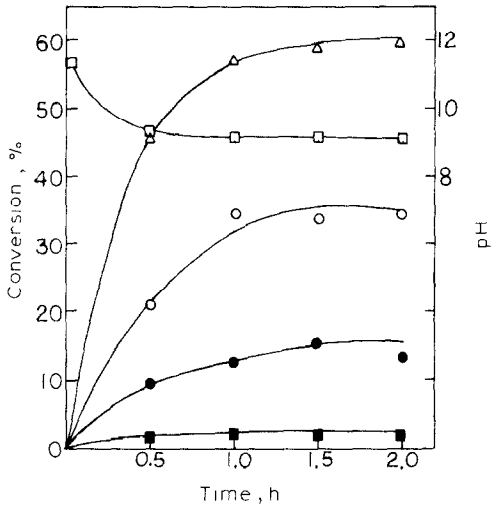


Fig. 1. Conversion and pH profiles for hydrolysis of cellobiose over zeolite Na-X. —△— Total conversion; conversion into: —○— cellobiulose, —●— glucose, —■— fructose; —□— pH.

the reaction proceeds, stabilizing at a value of 9.0. The decrease in pH is probably due to dissociative adsorption of water molecules on the surface of the zeolite, or through leaching of ionic components from the solid. As it is well known that sugars react in an alkaline environment[5,7,8], experiments were conducted under identical pH conditions, using NaOH to adjust the initial pH of the broth to 11.3. Reaction was performed for 2 h, and the final pH of the mixture was 8.0. The results obtained from these experiments are compared in Table II with those obtained with zeolite Na-X under identical conditions. Yields are defined as the fraction of disaccharide converted into the particular product divided by the total amount of disaccharide reacted. Although the total conversion of cellobiose is approximately the same in both cases, the distribution of products is widely different. A considerably larger fraction of sugar is lost to degradation products in the alkaline hydrolysis than it is in the hydrolysis by zeolite. Results obtained with the zeolite Na-Y are also shown in the same Table. In this instance, the pH of the mixture is lower and total conversion of the disaccharide is significantly lower over the same time-period. The high yield of cellobiulose indicates that, in addition to the isomerization reaction, hydrolysis of the keto-disaccharide is also much slower.

(c) Maltose hydrolysis. — Hydrolysis of maltose was investigated over zeolites Na-X, Na-Y, and 4A at a temperature of 85°, initial reactant concentration of 4.0%, and a catalyst loading of 0.3 g/mL. Conversion profiles obtained with the zeolites Na-X and 4A are shown in Figs. 2 and 3, respectively. On both catalysts, total conversion is ~70% within a period of 2 h. The major product formed is maltulose, followed by glucose and fructose. A small amount of mannose is also formed when the reaction is performed over Na-X. An interesting aspect of these results is the apparent maximum in the conversion into maltulose.

TABLE II

COMPARATIVE RESULTS OF CELLOBIOSE HYDROLYSIS WITH Na-X, AND Na-Y, AND NaOH UNDER IDENTICAL CONDITIONS

	Na-X	*Na-Y*	*NaOH*
Initial pH	11.3	10.0	11.3
Total conversion, %	60	19	62
$Y_{cellobiulose}$, %[a]	58	75	32
$Y_{glucose}$, %	25	11	6
$Y_{fructose}$, %	4	3	0
$Y_{degr\ prod}$, %	13	11	62

[a]Y denotes yield.

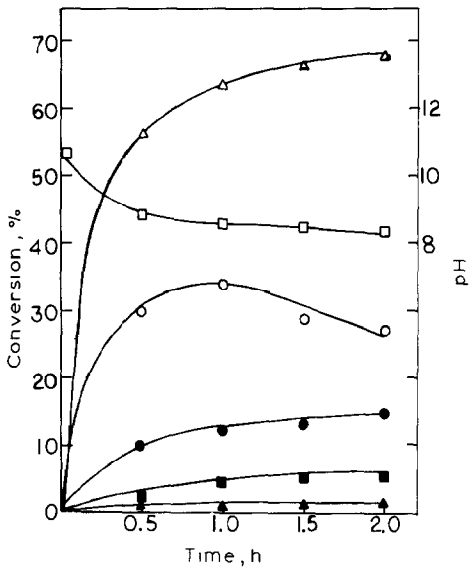


Fig. 2. Conversion and pH profiles for hydrolysis of maltose over zeolite Na-X. —△— Total conversion; conversion into: —○— maltulose, —●— glucose, —■— fructose; —▲— mannose; —□— pH.

Hydrolysis experiments were conducted on maltose under identical conditions by adjusting the pH of the initial broth to 10.6, equivalent to that obtained with the zeolites Na-X and 4A. Comparative results are shown in Table III. As with the hydrolysis of cellobiose, total sugar conversion under homogeneous alkaline conditions is slightly higher than in the presence of solids. Nevertheless, the product distribution is widely different, indicating that reaction routes in the presence of zeolites might be different. Results of maltose hydrolysis with the Na-Y zeolite are also included in Table III. The observations made in the hydrolysis of cellobiose also apply for maltose. Interestingly, the difference in initial pH of the mixtures produces no measurable differences in the relative activities of the various

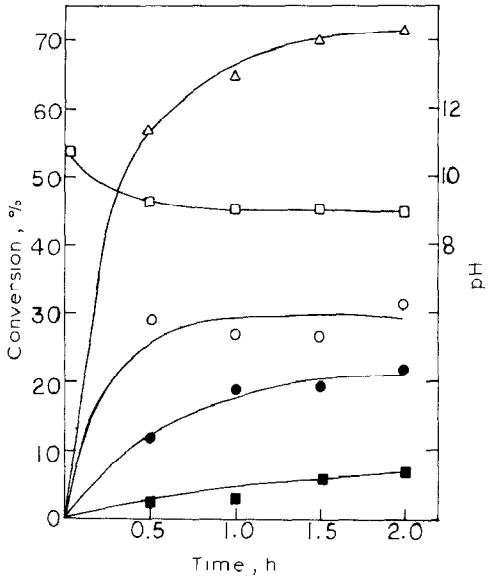


Fig. 3. Conversion and pH profiles for hydrolysis of maltose over zeolite 4A. —△— Total conversion; conversion into: —○— maltulose, —●— glucose, —■— fructose; —□— pH.

zeolites, as shown in Tables II and III. This result indicates that the lower activity exhibited by zeolite Na-Y is probably due to its lower intrinsic activity rather than to lower pH.

(d) Lactose hydrolysis. — Lactose hydrolysis was investigated over zeolites 3A, 4A, 5A, Na-X, and Na-Y, at a temperature of 85°, initial reactant concentration of 4.0%, and catalyst loading of 0.3 g/mL. Results obtained with all type A zeolites were approximately the same. These zeolites differ only in their average pore-diameter, which is indicated by their number. This observation indicates that the internal surface-area of these catalysts is probably not utilized, because of their narrow pore-openings, which do not permit passage of the lactose molecule whose dimensions are 7.9 × 8.4 Å. Representative conversion-profiles obtained with the zeolites 4A, Na-Y, and Na-X are shown in Figs. 4, 5, and 6, respectively. Lactose

TABLE III

COMPARATIVE RESULTS OF MALTOSE HYDROLYSIS WITH Na-X, 4A, Na-Y, AND NaOH

	Na-X	*4A*	*Na-Y*	*NaOH*
Initial pH	10.6	10.6	10.0	10.6
Total conversion, %	68	72	19	75
$Y_{maltose}$, %	54	42	72	36
$Y_{glucose}$, %	22	31	14	9
$Y_{fructose}$, %	9	10	4	0
$Y_{mannose}$, %	4	0	0	0
$Y_{degrad\ prod}$, %	11	17	10	55

conversions of 50–65% are obtained within the first 2 h of reaction. In all cases, conversion into lactulose proceeds through a maximum at a time period of 1–2 h, whereas conversions of galactose, glucose, and tagatose increase monotonically with time.

To determine the effects of the alkalinity of the medium on hydrolysis of lactose, experiments were conducted under identical conditions by adjusting the pH to 10, identical to that obtained with the Na-X zeolite. Comparative results shown in Table IV indicate that the activity obtained with the zeolites is significantly higher than that obtained with homogeneous alkalinity at identical pH. Furthermore, the product distribution over the zeolites is very different from that of the homogeneous reaction.

(e) Reaction paths. — A common characteristic of the conversion (or, equivalently, concentration) profiles shown in Figs. 1–6 is the fact that the concentration of the ketodisaccharides–cellobiulose, maltulose, and lactulose–passes through a maximum. On the other hand, the concentrations of glucose and fructose (with cellobiose and maltose) and galactose, glucose, and tagatose (with lactose) increase monotonically with time. Furthermore, monosaccharide concentrations increase with time, even after total disaccharide conversion essentially terminates, whereas the concentration of glucose is consistently higher than that of fructose.

These profiles indicate that zeolites catalyze two types of reaction, namely hydrolysis and isomerization. Monosaccharides are probably formed by hydrolysis of the original disaccharide as well as by hydrolysis of the ketodisaccharide. The product mixture from cellobiose, consists of cellobiulose, glucose, and fructose; maltose gives maltulose, glucose and fructose. Cellobiose and maltose are both

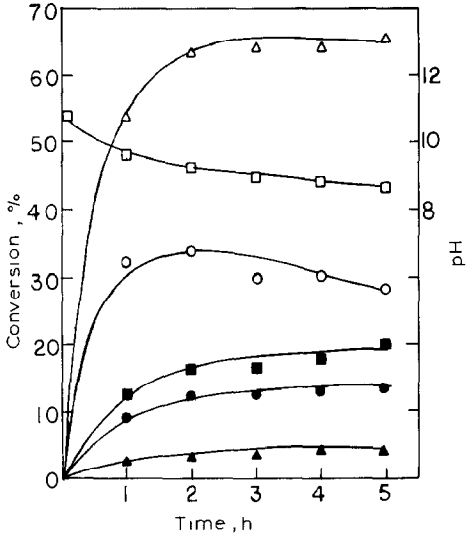


Fig. 4. Conversion and pH profiles for hydrolysis of lactose over zeolite 4A. —△— Total conversion; conversion into: —○— lactulose, —■— galactose, —●— glucose, —▲— tagatose; —□— pH.

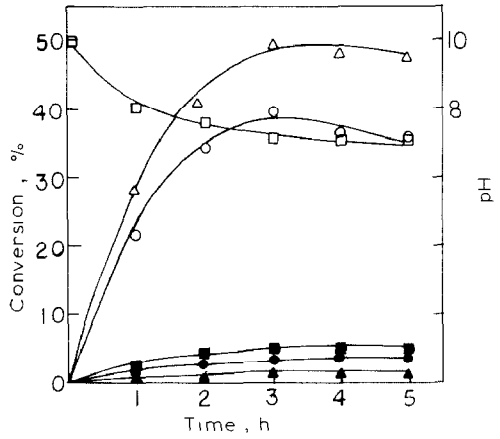


Fig 5. Conversion and pH profiles for hydrolysis of lactose over zeolite Na-Y (Symbols as in Fig. 4)

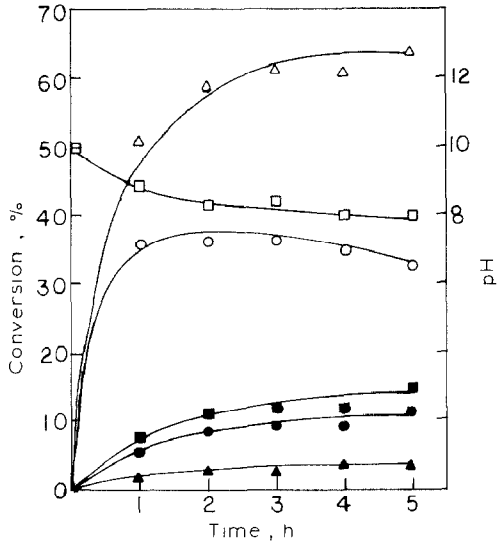


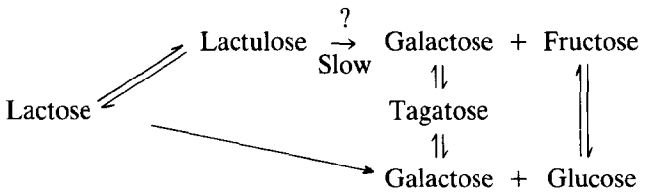
Fig. 6. Conversion and pH profiles for hydrolysis of lactose over zeolite Na-X (Symbols as in Fig. 4.)

glucodisaccharides, and hence the keto disaccharide is unique. Examination of the product distribution obtained by reaction of lactose over zeolites provides additional insight into possible reaction-routes. Figs. 4–6 show that the rate of formation of lactulose at short reaction times is very rapid and approximately equal to the rate of lactose conversion. The absence of fructose in the mixture indicates that hydrolysis of lactulose to galactose and fructose is either very slow (conversion of $<1\%$, which is the detectability limit of our analysis) or nonexistent. The galactose and glucose found in the mixture result from direct hydrolysis of lactose. Tagatose is formed by isomerization of galactose. These reaction steps, including the possibility of isomerization between glucose and fructose, may be summarized as follows:

TABLE IV

COMPARATIVE RESULTS OF LACTOSE HYDROLYSIS WITH 4A, Na-Y, Na-X, AND NaOH

Initial pH	*4A* *10.4*	*Na-Y* *10.0*	*Na-X* *10.0*	*NaOH* *10.0*
Total conversion, %	65	50	64	31
$Y_{galactose}$, %	31	10	23	3
$Y_{lactulose}$, %	52	80	61	30
$Y_{glucose}$, %	20	8	17	3
$Y_{tagatose}$, %	6	2	6	1
$Y_{degr\ prod}$, %	0	3	2	63



In the homogeneous alkaline system, hydrolysis of the disaccharide is either insignificant or the hydrolysis products degrade rapidly. Thus the alkalinity of the mixture, within the time frame of these studies, appears to have a weak hydrolytic activity. Isomerization activity seems, however, to be significant. The fact that a very large fraction of the reacted disaccharide is unaccounted for in the mixture under alkaline (NaOH) conditions indicates that a large fraction of the isomerization and/or hydrolysis products are degraded rapidly under these conditions. This might imply that the intrinsic product-distribution obtained over the zeolite catalysts might be different than that observed in this study, because of degradation reactions in the bulk of the reaction broth, which was shown to be alkaline. It should be noted that 10–13% of the disaccharide reacted is not accounted for in the product distribution obtained over the zeolite catalysts (Tables II–IV). In the homogeneous alkaline reactions, 55–62% of the reacted disaccharide is not accounted for. This fraction has undergone degradation to products that cannot be detected or identified by l.c. analysis.

SUMMARY AND CONCLUSIONS

Hydrolysis and isomerization reactions of cellobiose, maltose, and lactose over synthetic zeolite catalysts of type A, X, and Y, were investigated and shown to possess significant activity towards these reactions in the temperature range of 70–85°. Product distributions obtained with the zeolite catalysts were significantly different from those obtained in homogeneous alkaline environments. From these preliminary results, it was concluded that the reactions probably involve parallel hydrolysis and isomerization of the disaccharides to their corresponding ketoses,

followed by hydrolysis of the latter. Degradation products are believed to be formed mostly in the alkaline broth of the mixtures and to a lesser degree on the surface of the zeolite catalysts.

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