A Monte Carlo Analysis of Acid Hydrolysis of Glycosidic Bonds in Polysaccharides

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Kinetic experiments for the hydrolytic depolymerization of cellobiose and amylose were performed in acidic medium. In the case of amylose, only the production rate of glucose was monitored. Then, a Monte Carlo procedure was developed to simulate long-chain saccharide acid hydrolysis using the kinetic information obtained from the model compound, cellobiose. This stochastic procedure, which is applicable to the homogeneous acid hydrolysis of highly amorphous polysaccharides of sufficient degree of polymerization, provides estimated curves for the time evolution of all intermediate products. Literature data on the hydrolysis of oligoxylans were also compared to the results obtained from the Monte Carlo procedure. The depolymerization of linear polysaccharides, which should normally be viewed as a nonhomogeneous Markov chain, could be considered as a homogeneous Markov chain, provided that the chain is visited at specifically determined time intervals.

Introduction

Cellulose, the most abundant organic substance on Earth, is a linear homopolymer with general formula $(C_6H_{10}O_5)_n$, having the anhydrocellobiose moiety as the repeating unit. This unit itself originates from the combination of two glucose molecules. This polymer is linked by $\beta(1\rightarrow4)$ glycosidic bonds (Sjöström, 1981), and it shows a strong tendency to form hydrogen bonds within the same chain and between neighboring chains. Thus, the formed structures are fibrilar, making cellulose a very cohesive, tenacious molecule, hardly soluble in almost any solvent. Nevertheless, in a given fiber there are both crystalline and amorphous zones (Ladisch, 1989), the proportions of which depend on the origin of cellulose, as well as on its pretreatment. The amorphous regions are especially important because they are more likely to react with a particular chemical.

Cellulose conversion into glucose is a key step for the production of several specialty and intermediate chemicals presently synthesized from petroleum fractions as, for example, polyethylene, polystyrene, and synthetic rubber (Sjöström, 1981; Fengel and Wegener, 1983). Hydroxymethylfurfural and sorbitol are other important products that can be prepared from glucose. The former is a potentially interesting intermediate to be used in the synthesis of polyesters, polyamides, polycarbonates, epoxy resins and nylon, whereas sorbitol can be used as a sweetener, a humectant agent, an emulsifying agent in fine chemicals or in the manufacture of vitamin C (Wright, 1974). Sorbitol could also be transformed into polyalcohols of lower molecular weight (glycerol, ethylene glycol, propylene glycol, and butanediol) or deoxygenated into hydrocarbons.

Glucose formation from a solid substrate such as cellulose requires a sequential process (Ladisch, 1989), including a reduction of polysaccharide length to oligosaccharides that are soluble in an aqueous environment and finally the hydrolysis of the formed oligosaccharides.

The present work is part of an experimental program aimed at investigating and modeling the hydrolysis of pretreated cellulose in the presence of various acid catalysts, both homogeneous and heterogeneous, by using cellobiose as a model compound. The molecule of cellobiose itself is made up of two anhydroglucose units with heterocyclic structure of the pyranose form.

To this end, the homogeneous catalytic hydrolysis of cellobiose in sulfuric acid was performed under various conditions to estimate the values of the pseudofirst-order constants and thus the activation energy for rupture of the $\beta(1\rightarrow4)$ glycosidic

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bond. Hydrolysis of other polysaccharides was also performed, and with the aid of a computer-oriented simulation procedure it was investigated whether the results observed for cellobiose hydrolysis could be extended to other polysaccharide substrates, amylose being taken as the first example. Amylose was chosen in the present study to verify the adequacy of the simulated procedure, because a highly amorphous cellodextrin exhibiting an elevated degree of polymerization (DP) is not readily available.

Theory

Saccharide hydrolysis processes

Hydrolysis of cellulose is generally considered as a first-order reaction with respect to the concentration of $\beta(1\rightarrow4)$ bonds between β-anhydroglucose monomers (Sharples, 1968; Ladisch, 1989). These linkages are readily hydrolyzed by acids but are relatively stable in alkali because of the different hydrolytic mechanisms involved in these two media (Pazur, 1970). It is worth mentioning that the hypothesis of an A-2 mechanism, in which the water molecule addition is the limiting step, to describe acid hydrolysis of glycoside linkages has been discarded (Moiseev et al., 1976).

Due to its structure, two main practical difficulties are encountered in the commercial hydrolytic degradation of cellulose. First, the presence of crystalline regions results in a considerable reduction of the reaction rate compared with that for other polysaccharides, such as starch. If the process is carried out with very dilute acids, the course of cellulose decomposition is always complicated by the fact that it is insoluble in the hydrolyzing medium. On the other hand, if concentrated acids are used, the neutralization process becomes expensive. The second problem is that the drastic conditions necessary to produce significant saccharification of cellulose also result in the undesirable secondary decomposition of glucose (Sharpless, 1968) in such products as furfurals and levoglucosan.

Processes based on the two extremes of these conditions have been developed, though major importance has been given to the low-temperature, concentrated-acid method (Fengel and Wegener, 1983). Earlier works, however, have shown that temperatures as high as 180°C, with 0.08 M sulfuric acid, are potentially feasible and that under these conditions cellulose hydrolysis is in fact favored relative to sugar degradation (Saeman, 1945).

Hydrolysis by enzymes is also feasible, but for cellulose there are obstacles due to the expenses associated with the growth of the cellulase-producing organism, the narrow operating ranges under which they can be utilized, and the occurrence of cellobiose inhibition (Shukla et al., 1985). Additionally, a cellulase enzyme is about five times bigger than the acid catalyst, thus penetrating more slowly in the macroscopic structure of cellulose (Ladisch, 1989).

Amylose, the minor component of starch (20%-30%), is generally assumed to be a linear polymer of α-D-glucose linked $\alpha(1\rightarrow4)$ and to contain a minute proportion of ester phosphate groups of the order of 1 per 2,400 glucose residues (Peat et al., 1952). Its chains occur as a helix in its solid state and sometimes also in solution; although the polymer is assumed to be linear, this appears to be true for only part of the amylose, the remainder being lightly branched (Manners, 1989). This was confirmed by Hizukuri et al. (1981), who demonstrated that amylose from different sources contains an average of 2-8 branch points per chain. Thus, the number of $\alpha(1\rightarrow6)$ bonds that form interchain linkages may be considered much smaller than that of $\alpha(1\rightarrow4)$ links.

Hydrolytic decomposition of a polysaccharide

Different kinds of processes have been considered (Simha, 1941; Sillén, 1943; BeMiller, 1967) to model the depolymerization of linear chain molecules:

1. The rate of disintegration is the same for all bonds, independent of their position in the chain.
2. There is a preferred breaking at the ends of the chain (one or both ends) and a common hydrolysis rate for all other bonds.
3. There is a progressive change in the rate of disintegration as a function of the distance from the ends of the chain.

The details of the process that controls acid hydrolysis of linear homogeneous $(1\rightarrow4)$-linked polysaccharides are rather controversial. In the acid hydrolysis of cellulose, Ladisch (1989) reports that the two end bonds of the chains react approximately twice as fast as those in the interior, although the chemical bonds are all of the same $\beta(1\rightarrow4)$ type. On the other hand, in the case of acid hydrolysis of maltotriose and maltohexaose, the kinetics were described by one rate constant for the bond at the nonreducing end of the chain, identical to that of maltose, which was 1.8 times higher than that for the other bonds (Weintraub and French, 1970). In the study of cellotriose in acidic medium, Feather and Harris (1967) observed that the hydrolysis rate at the nonreducing end bond is 1.5 times faster than at the reducing end bond and that this value is smaller than that observed for cellobiose.

It is clear that hydrolysis of glucans does not proceed by simple first-order kinetics, although the glycosidic bonds present in a chain of the polymer are all of the same type. It is then said that the cleavage is not completely random, and it is generally considered that the size of the aglycone exerts no effect on the rate of hydrolysis, that is, there is no change in the first-order kinetic constant with the DP of the saccharide (Moelwyn-Hughes, 1929; Heyraud, 1981).

In the present study, the observations reported by Heyraud (1981) for the case of maltodextrins will be adopted, and it will be considered here that the bond of the nonreducing side reacts 1.7 times faster than the other bonds. This value for the ratio between the kinetic constants at the reducing and at the nonreducing ends would show some minor change with temperature, because the rupture of these two kinds of bond does not possess exactly the same activation energy. Nevertheless, the experimental errors involved in the determination of the activation energies reported by Weintraub and French (1970) do not permit to distinguish different values for each of them. As an approximation, it may be considered that the average value for the ratio between the kinetic constants at the nonreducing and at the reducing ends is 1.7 for all temperatures in the range 75°C-150°C.

Also, from existing literature (Moelwyn-Hughes, 1929; Wolfrom et al., 1963; Heyraud, 1981), the kinetic constants for the hydrolysis of $\alpha(1\rightarrow4)$ glycosidic bonds are approximately 2.5 times as large as those for the rupture of their $\beta(1\rightarrow4)$ homologues. It is, therefore, acceptable to use rate constants determined for cellobiose multiplied by a constant factor as a

first rough estimate for the rate constants of the hydrolytic rupture of nonreducing end bonds in $\alpha(1\rightarrow4)$-bonded saccharides, such as amylose. It is indeed believed that this procedure yields a more reliable estimate of the activation energy of the hydrolysis of $\alpha(1\rightarrow4)$ bonds than the experimental values reported in the literature for the acid hydrolysis of maltose.

Simulating the hydrolytic decomposition of a polysaccharide

Glucose concentration as a function of time obtained in the experiments of amylose hydrolysis was compared to the data simulated by means of a Monte Carlo method.

To illustrate the use of this method in association with the stochastic approach to model polymer decomposition, consider the random reaction trajectory of the linear oligomer represented as an example in Figure 1. This 16-monomer unit is interconnected by 15 reactive bonds, which either could be split or remain unaltered at all subsequent time steps. The simulated decomposition of the arbitrary polymer advances by allowing finite time intervals Δt to elapse in series. After the passage of each Δt, one or more reactions may occur and the result then describes the new state of the polymer by the series of products in Figure 1. Supposing irreversible reaction for this case, this procedure is repeated until the final reaction time is attained or all bonds fragmented. This whole procedure is repeated a fixed number of times to produce the final composition results obtained by averaging all the previous calculations.

The time evolution of this chemically reacting system takes the form of a Markovian random walk in the two-dimensional space of the populations of both kinds of bonds. The Markovian assumption is equivalent to specifying that the physical

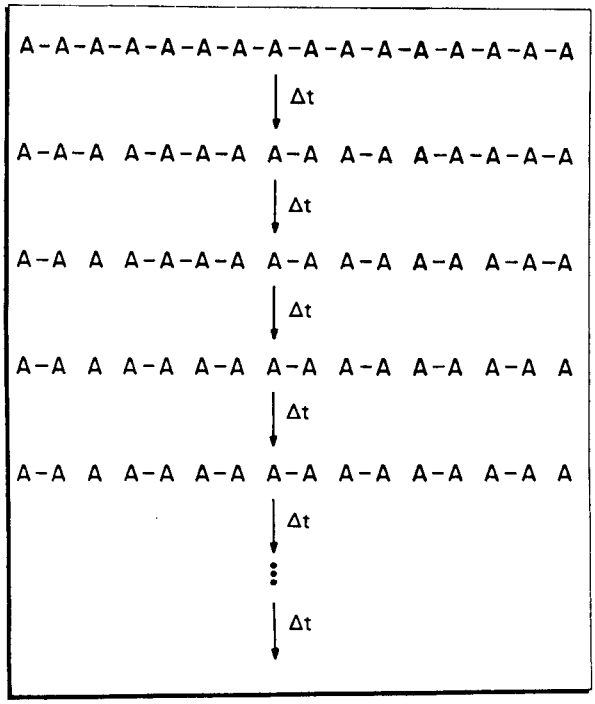

Figure 1. Depolymerization scheme.

system has an extremely limited memory, that is, given the "present" of the process, the "future" is independent of its "past" (Wolff, 1989). Since there is no direct way to establish whether a given system is truly Markovian, for most cases the ultimate test of the Markov model is its validity in representing actual systems, a property that few real systems possess (Seinfeld and Lapidus, 1974).

This more flexible method of description of the time evolution of the concentration of intermediate products will then avoid utilization of the systems of deterministic kinetic equations developed in other works (Simha, 1941; Sillén, 1943) or the solution of a lengthy ordinary differential equations (ODE) system. The developed simulation was thus based on an interpretation of chemical kinetics using stochastic instead of deterministic mathematics. In this approach, *reaction rate constants* are viewed as *reaction probabilities per unit time* (Gillespie, 1976) rather than as a measure of actual reaction rates. The justification for the validity of the stochastic approach can be found in the works of Oppenheim et al. (1969) and Kurtz (1972), and fundamental aspects in statistics can be consulted in other references (Lowry, 1970; Seinfeld and Lapidus, 1974; Ross, 1980; Trivedi, 1982).

To numerically simulate the hydrolysis of linear polysaccharides using a Monte Carlo technique, two distinct approaches were used to develop different FORTRAN procedures (Pinto, 1991). The first one was adapted from Gillespie's classic work (1976) on simple molecules and the second one has been used by McDermott et al. (1990) to simulate lignin degradation. Although the subject of chemical reaction kinetics stochastic modeling has been treated in many theoretical publications (McQuarrie, 1967; Gillespie, 1977; Turner, 1977; López-Serrano et al., 1980; McDermott and Klein, 1986; Train and Klein, 1988), very little emphasis has been given to the confrontation with actual experimental kinetic data.

In Gillespie's approach, the epoch of occurrence of a given reaction, chosen at random among the reactions occurring simultaneously in the system, is calculated, time is advanced, and the whole process repeated until the final reaction time is attained. Although not explicitly stated in the original reference, the independent random variable *time* was considered to be governed by an exponential *probability density function*. It is known that if the times between occurrence of events follow an exponential distribution, then the corresponding process is Poissonian (Parzen, 1962; Karlin and Taylor, 1975, 1981; Ross, 1980; Trivedi, 1982). Thus, reaction observation was implicitly supposed to be an homogeneous Poisson process and the event times were properly simulated. The fundamental assumption of this is that the reactant species is envisaged to be so abundant that its amount is not practically time-dependent (van Kampen, 1981).

Concerning the second approach used to simulate the hydrolysis process, it is reasonable to assume that, over each small interval of time Δt, the probability of observing an irreversible first-order reaction of type $B\rightarrow P$, with kinetic constant, k, is approximated by the product $k\Delta t$. If Δt is sufficiently small, the probability of two or more reactions occurring in the interval of duration Δt may be neglected. The probability of m reactions taking place in the interval Δt, now divided in n equal time subintervals, is approximately given by the corresponding expression of a binomial *probability mass function* (Trivedi, 1982), Eq. 1.

$$b\left(m, n, \mathrm{k}\frac{\Delta \mathrm{t}}{\mathrm{n}}\right) = \binom{n}{m}\left(k\frac{\Delta t}{n}\right)^{m}\left(1-k\frac{\Delta t}{n}\right)^{n-m} \qquad (1)$$

Equation 1 describes a sequence of n-independent Bernoulli trials during a fixed Δt with the probability for reaction or "success" on each trial, p, equal to $k\Delta t/n$. It is also known that the Poisson *PMF*, given by Eq. 2, is a convenient approximation to the binomial *PMF* when n is large and p is small. [An acceptable rule is to use the Poisson approximation for the binomial probabilities, if $n \geq 20$ and $p \leq 0.05$ (Trivedi, 1982).]

$$f(m, k\Delta t) = e^{-k\Delta t}\frac{(k\Delta t)^m}{m!} \qquad (2)$$

The polymer chain itself does not have an individual transition probability, but it can be conceived as a group of parallel subsystems, each one being composed of a single bond. In the case of a system consisting of two reactive species $B1$ and $B2$, whose numbers $X1(t)$ and $X2(t)$ are $x1$ and $x2$, respectively, and considering the existence of two rate constants, k_1 and k_2, for the material comprised initially in one chain of given *DP*, the probability of no reaction in an interval of length Δt is:

$$f_i(0, k_i\Delta t) = e^{-k_i\Delta t} \quad i = 1, 2 \qquad (3)$$

and the individual *transition probabilities* can be defined (McDermott and Klein, 1986) from Eq. 3 as:

$$\mathrm{Prob}_i = 1 - e^{-k_i\Delta t} \quad i = 1, 2 \qquad (4)$$

The whole process seems to have been approximated to a Poissonian one with rate k_i by utilization of Eq. 4. It should be recalled that Eq. 4 is derived from Eq. 2, which may be simply considered as a more convenient form of Eq. 1 due to its capability of being linearized. In addition, Δt is constant and elapses in series, not obeying the exponential *probability distribution function*, thus invalidating the condition of a Poissonian process.

In the first developed Monte Carlo simulation procedure algorithm, which uses Gillespie's approach, once the epoch of occurrence of a reaction is calculated, an arbitrary bond is selected for hydrolysis. Its type (nonreducing or reducing) and location on the chain are determined by using random, or more properly quasirandom, numbers of values bounded by zero and unity that are generated by computational means. The status of the chain is updated, time is advanced, and the whole procedure repeated until a desired time is attained or all bonds fragmented. In the second algorithm (Figure 2), an arbitrary *reducing* end bond is selected for hydrolysis. Then, the transition probability, given by the corresponding form of Eq. 4, is compared to a *quasirandom* number. Reaction does occur if the transition probability for the bond being tested is smaller than the picked random number. All reducing bonds are verified for reaction, the direction of browsing of the chain being also determined randomly. The state of the molecule is updated and then the *nonreducing* bonds are verified for a reaction in a similar fashion. This simulates the ability of the whole molecule to present more than a reactive site at a time and it also prevents the nonreducing bonds of remaining unreactive during

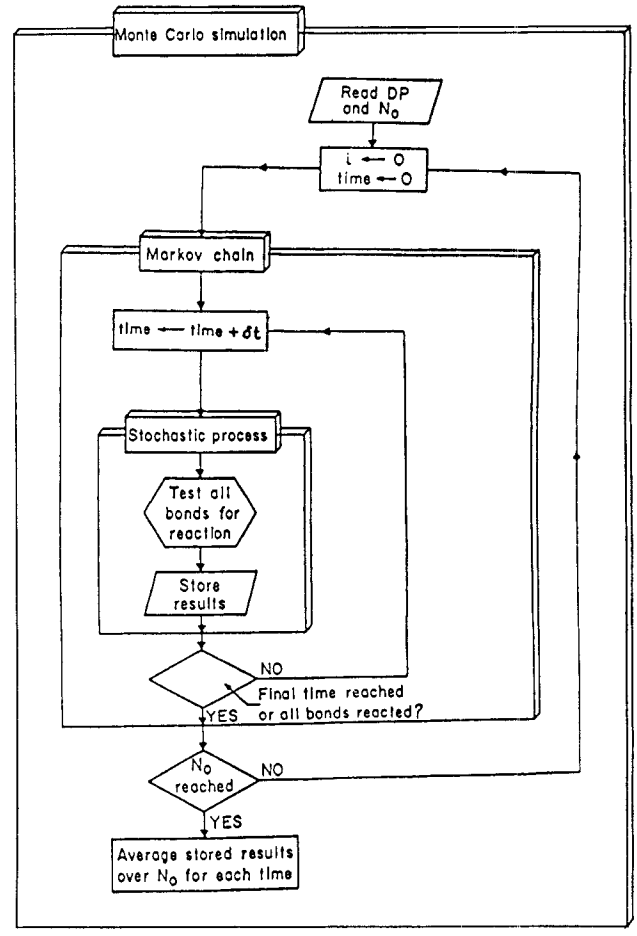

Figure 2. Depolymerization simulation algorithm.

the remaining part of the Δt in which it was formed (McDermott et al., 1990). Due to the *memoryless* property of the Markov process itself, the process can be stopped at any time $t + \Delta t$. At this point, the location of the Markov chain in the time space is determined, the new state is defined and the whole procedure is repeated until all bonds are fragmented or the final reaction time attained, when a Markov chain will have been mapped through time. It is clearly understood that the hydrolysis of nonreducing bonds was considered actually a primary process that feeds into the secondary process of reducing-bond scission.

Since the reaction of a single-polymer chain would not provide statistically significant results in the random situations described, numerous polymers (number N_0) of same chain length were reacted in a similar fashion and the results averaged.

In the way these procedures were conceived, the intermediate information obtained after treatment of each single polymer molecule is temporarily stored in a memory location. This information is composed mainly of the *DP*'s of the products at each reaction time, as well as their number. These values have to be averaged to supply the final results, which include the *DP*, the molecular weight, the mass, and the molar and weight fractions of the formed products, as well as the number average and the mass average *DP*'s at defined reaction times specified by the user as multiples of Δt.

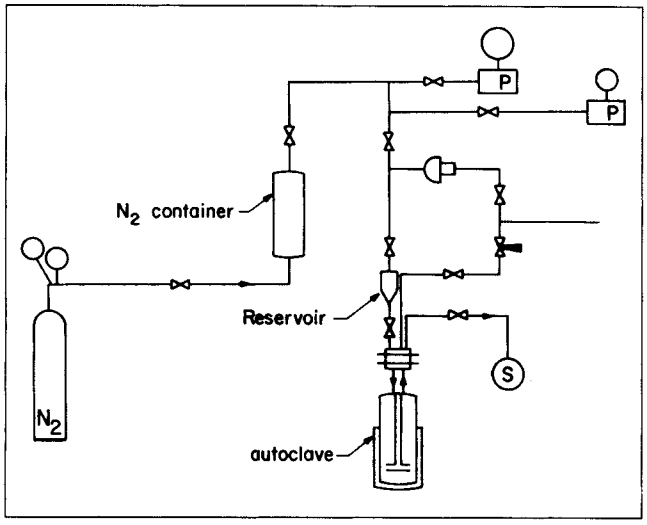

Figure 3. Representation of the experimental set-up.

Table 1. Reaction Conditions of Batch Catalytic Hydrolysis in Sulfuric Acid Solutions

Exp.	Substrate*	Temp. °C	$[H^+]$ mol/L	S_0 g/L
1	*C*	100.5	0.05	5.01
2	*C*	110.3	0.05	5.01
3	*C*	110.3	0.05	10.00
4	*C*	134.3	0.05	10.00
5	*C*	125.0	0.05	5.00
6	*C*	90.4	0.10	10.01
7	*C*	125.3	0.10	5.00
8	*C*	109.6	0.10	10.01
9	*C*	124.7	0.10	10.00
10	*C*	125.0	0.05	10.01
11	*C*	100.2	0.05	10.01
12	*C*	100.0	0.10	5.00
13	*C*	100.1	0.10	10.00
14	*A*	110.2	0.10	5.01
15	*A*	100.7	0.10	3.00
16	*A*	90.3	0.10	3.00

*C = cellobiose, A = amylose

Experimental Studies

The experiments were conducted in a 750-mL steel autoclave (Aminco) equipped with a magnetic stirrer (Figure 3).

For the cellobiose experiments, 675 mL of a sulfuric acid (Aldrich Chemicals Co.) solution of appropriate concentration was loaded into the reactor. The reactor was then purged with nitrogen and heated using an internal 500-W cartridge placed inside a stainless steel sheath, which was coated with a high thermally-conductive resin (OB-200, Omega Engineering) to avoid exposure of the extremely hot metallic surfaces to the acid. The heater was operated through a temperature controller and programmer (Lindberg M211), and additional power was supplied during the warm-up period by a 1.2-kW external source. Reaction temperature deviation from the set-point value was less than 0.6°C in all experiments.

In a typical run, 50 mL of a concentrated aqueous cellobiose (Aldrich Chemicals Co.) solution (145 g/L) was kept in a vertical closed conical reservoir installed in series between the reactor and a nitrogen container. Once the set-point temperature was attained in the previously loaded solution, the liquid was forced inside the reaction vessel. This procedure avoided reactant conversion during the heating-up period, thus allowing a proper definition of time zero (t_0) in kinetic determinations.

In the case of experiments with amylose [Sigma Chemicals, number average *DP* of 630 (Arbin et al., 1981), amylopectin-free], an analogous procedure was followed, but the carbohydrate was initially present in the reactor and the concentrated acid solution placed in the conical reservoir instead.

Samples were collected regularly by opening a microvalve connected to a capillary tube immersed in the reacting solution and discarding a quantity equivalent to the dead volume of this sampling system, the value of which was estimated to be inferior to 0.5 mL.

Samples were neutralized without delay with solid barium carbonate ($BaCO_3$, Anachemia) (Wentz et al., 1982) or calcium carbonate ($CaCO_3$), centrifuged and the supernatant filtered through a 0.45-μm nylon filter unit (Cole-Parmer).

All samples were immediately analyzed by high-performance liquid chromatography (HPLC) using an Aminex HPX-87P Pb form column (BioRad, 300×7.8 mm) equipped with a Carbohydrate Analysis ion-exclusion Micro-Guard precolumn (BioRad). The mobile-phase (filtered, degased HPLC-grade water from Fisher Scientific) was delivered through a pump (Perkin-Elmer 3B) at a flow rate of 0.6 mL·min^{-1}. The column temperature was maintained at 85.0±0.2°C by means of an insulated heating system connected to a temperature controller (model D921K; Omega). The injector (model 7125, Rheodyne) was equipped with a 20-μL sample loop. A differential refractometer (LC-25; Perkin-Elmer) was used as detector with a 3392A integrator (Hewlett-Packard) to record the signals. Calibration curves for glucose and cellobiose were constructed periodically, and xylose (Aldrich) was used as an internal standard in all injections.

Simulation procedures were run on an IBM 4381 (C.T.I., Université Laval), and calculations for statistical analysis were performed using the Statistical Analysis Software-SAS (SAS Institute Inc., Cary, NC) on the same computer.

Results and Discussion

Hydrolysis of cellobiose

Cellobiose hydrolysis was performed in the temperature range from 90°C to 135°C in the presence of dilute sulfuric acid (0.05 N and 0.10 N). Table 1 summarizes the conditions for this set of experiments, for which the only observed product was glucose. The mass balance was typically within an average value of 5% error at all experimental times, thus indicating that there was no degradation of the formed glucose. A graphical representation of the logarithm of cellobiose concentration as a function of time is given for selected experiments (Figure 4), and the straight line through all experimental points indicates that pseudofirst-order kinetics was verified, namely:

$$[C] = [C]_0 \, e^{-k_2' t} \tag{5}$$

The values of k_2' for this set of experiments calculated by regression of the linearized form of Eq. 5:

$$\ln[C] = \ln[C]_0 - k_2' t \tag{6}$$

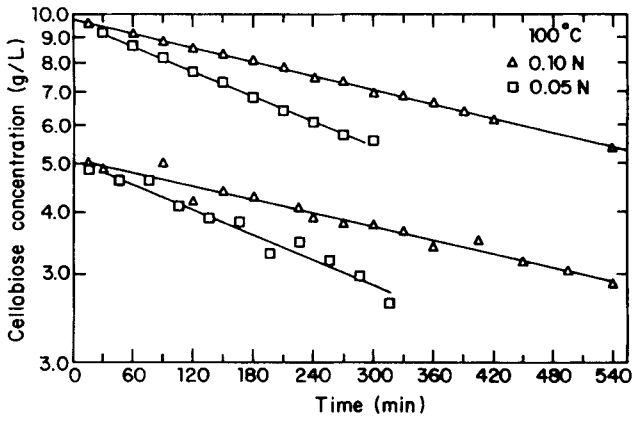

Figure 4. Cellobiose concentration as a function of time for cellobiose hydrolysis (100°C, H_2SO_4 solutions).

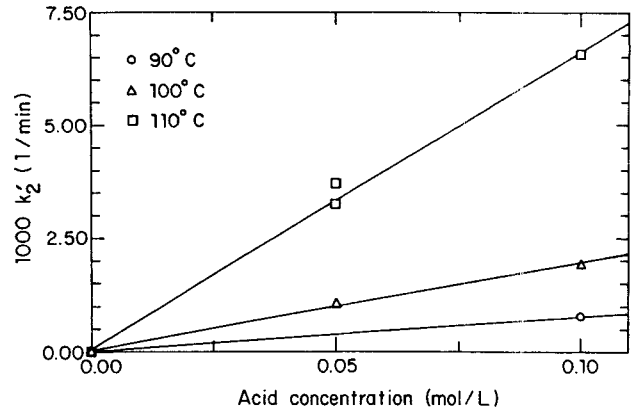

Figure 5. Dependence of the pseudofirst-order constant on acid concentration for cellobiose hydrolysis experiments.

are reproduced in Table 2.

Figure 5 shows the experimental values of the pseudofirst-order kinetic constant k_2 as a function of the acid concentration. In this figure, the points at the origin are actual experimental observations, since no cellobiose conversion was obtained in pure water. The observation of a linear relationship between k_2' and the value of the acid concentration allows to define a rate constant k_H as:

$$k_2' = k_H[H^+] \tag{7}$$

The values obtained for the second-order rate constant k_H are presented in Table 2, together with an estimate (σ) for the error associated with its evaluation. This error is calculated as the quotient of the estimated value of the standard deviation and the value of k_H itself. The error on the value of the acid concentration was considered negligible.

The Arrhenius plot for k_H:

$$k_H = A \exp\left[-\frac{E}{RT}\right] \tag{8}$$

is shown in Figure 6. The numerical calculations necessary to estimate the energy of activation were performed by the SAS software employing a weighed linear least-squares method (Caulcutt and Boddy, 1983). The weight for each experimental point was calculated as $w = (\sigma/\sigma_M)^{-1}$.

The observed value for the activation energy involved in the rupture of a $\beta(1\rightarrow4)$ glycosidic bond of 135 kJ/mol is comparable within a 2%–8% deviation to those obtained in other works (Moelwyn-Hughes, 1929; Senju and Shimizu, 1952; Wolfrom et al., 1963; Timell, 1964; Lindberg, 1964; Bobleter et al., 1986), which are in the range 124 kJ/mol–138 kJ/mol.

The small difference can be due to the fact that a weighed regression technique was employed here to estimate the parameters in the linearized form of Eq. 8.

Table 2. Pseudofirst- and Second-Order Rate Constants for Cellobiose Hydrolysis in Sulfuric Acid Solutions

Exp.	$k_2' \times 10^3$ min^{-1}	$k_H \times 10^2$ $L \cdot mol^{-1} \cdot min^{-1}$	σ %	w
1	1.09	2.19	7.66	0.36
2	3.26	6.51	2.90	0.95
3	3.71	7.42	3.17	0.87
4	43.0	86.0	4.15	0.66
5	17.7	35.3	3.60	0.77
6	0.788	0.788	7.38	0.37
7	32.5	32.5	2.90	0.95
8	6.54	6.54	2.65	1.04
9	29.8	29.8	3.67	0.75
10	15.7	32.3	1.02	2.70
11	1.10	2.19	1.51	1.82
12	1.95	1.95	6.57	0.42
13	1.92	1.92	2.06	1.34

Hydrolysis of amylose

Similar hydrolysis experiments were carried out with amylose, and the detailed conditions are summarized in Table 1. Products of *DP* ranging from 1 to 630 are obtained, but only glucose concentration has been quantified experimentally. Figure 7 shows the batch-reactor experimental data of glucose concentration evolution with time. An induction period, the length of which decreases as temperature increases, is observed on all curves. From Figure 7 it can be noted that glucose concentration attains a plateau in approximately 8 hours at 110°C, even though the conversion into glucose is not com-

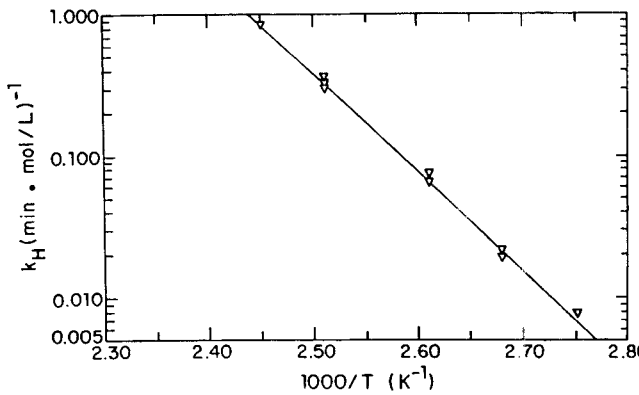

Figure 6. Arrhenius plot for cellobiose hydrolysis experiments (weighed linear regression).

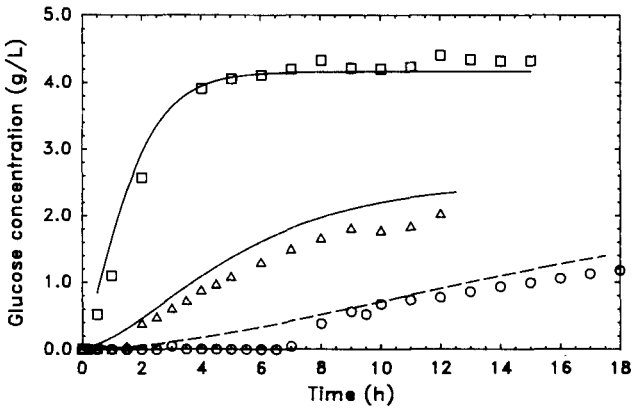

Figure 7. Glucose concentration as a function of time for amylose hydrolysis (0.10 M H_2SO_4): experimental and simulation results.

○, 90°C; △, 100°C; □, 110°C

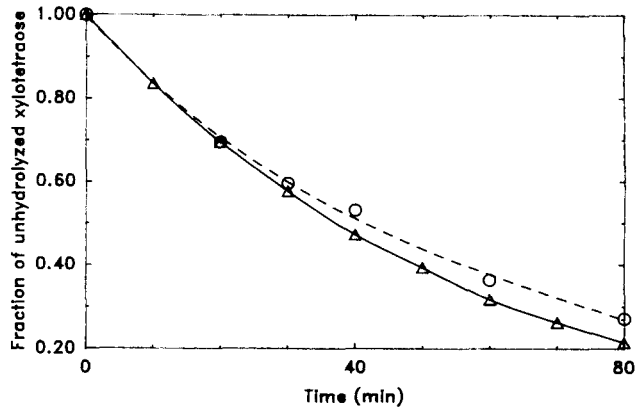

Figure 8. Hydrolysis of xylotetraose (100°C, 0.025 M H_2SO_4): experimental and simulation results.

○, — — —, Kamiyama and Sakai (1979); △, ———, simulation ($M=2$, $k_2=0.0084$ min^{-1})

plete. Kainuma and French (1971) have also observed a certain resistance of amylose to hydrolysis, even if it gave initially an amorphous X-ray diffraction pattern.

Ziderman and Weiss (1979), having hydrolyzed amylose for 18 hours, believe that it is the supramolecular organization of amylose structure that renders it stable to further major degradation in dilute acid. Indeed, amylose in solution undergoes retrogradation, a phenomenon caused by the alignment of linear chains which leads to hydrogen bond formation between chains until insoluble particles consisting of intertwined double helices are formed (Kennedy et al., 1987). The possibility of some type of incipient retrogradation, in which aggregates are not sufficiently large to precipitate from solution but nevertheless set up weak crystalline centers (Banks and Greenwood, 1975) must be considered. In addition, the fact that even though the degree of organization is low, the intermolecular forces may be such that they protect part of the amylose against acid hydrolysis should be factored in. To determine the extent of this stabilization of amylose, experiment 14 was selected because conversion into glucose was there virtually constant once a maximum was attained, an indication of accomplishment of hydrolysis. A material balance for glucose on this region has shown that about 25% of the total mass of amylose initially present in the reactor remained unreactive. This value was subtracted from the initial concentration in all simulation runs.

The corresponding values of the kinetic constants, used in the first simulation procedure and in calculating the transition probabilities as given by Eq. 4 for the second stochastic procedure, are related to the cellobiose results by Eqs. 9 and 10.

$$\frac{k_2}{k_1}=M \tag{9}$$

$$\frac{k_2'}{k_2}=S \tag{10}$$

Concerning the simulation results obtained by use of Gillespie's variable time approach, it was noted that the calculated rate of glucose formation was in poor agreement with the values observed experimentally. This is due to the fact that one and only one bond was fragmented at each time, a situation wholly dissimilar to the actual one where various active centers are capable of being attacked by the acid. In an attempt to overcome this situation, at each time a reaction occurred, different random numbers were generated to describe the fraction of the population of each type of bond that subsisted and also their location in the current state of the chain. The results were rather unsatisfactory for any reasonable arbitrary values of the constants M and S.

Figure 7 shows the curves calculated using the second stochastic procedure described in the Theory section. These calculations were also repeated for arbitrary values of the constants M and S, and Figure 7 represents the curves obtained by setting $M = 1.7$ and $S = 2.5$. It is remarkable that the three curves could be fitted using these same two values which are precisely the ones reported in the literature for the ratio k_2/k_1 and k_2/k_2', respectively (Moelwyn-Hughes, 1929; Wolfrom et al., 1963; Heyraud, 1981). From these results it must be stressed that the kinetic behavior of amylose could be simulated with a satisfactory precision using exclusively kinetic information (k_2') obtained in the hydrolysis of cellobiose experiments in conjunction with reported data of M and S for maltose.

The method used here is then appropriate to simulate polymer hydrolysis within the limits considered here, that is, a chain length of the order of 10^3 (that will give $n \geq 20$) and k_i (for $i=1,2$) and Δt such that their product $k_i \Delta t$ is inferior to 0.05.

To verify the generality of the method, data reported by Kamiyama and Sakai (1979) for the acid hydrolysis of xylooligosaccharides of DP ranging from 3 to 5 were compared to the Monte Carlo simulation results. Although the method presented in this article should be used for high degrees of polymerization, since it is under these conditions that statistical techniques should work, the lack of experimental data for the acid hydrolysis of long-chain linear saccharides has compelled the use of these data for comparison.

Figure 8 displays the experimental results by Kamiyama and Sakai (1979) and those obtained by simulating the acid hydrolysis of xylotetraose. These results were obtained by setting the constant $M=2$, very close to the range of values (1.73–1.91) proposed by Kamiyama and Sakai (1979). The value of k_2' used in the Monte Carlo simulation was proposed by Kamiyama and Sakai (1979).

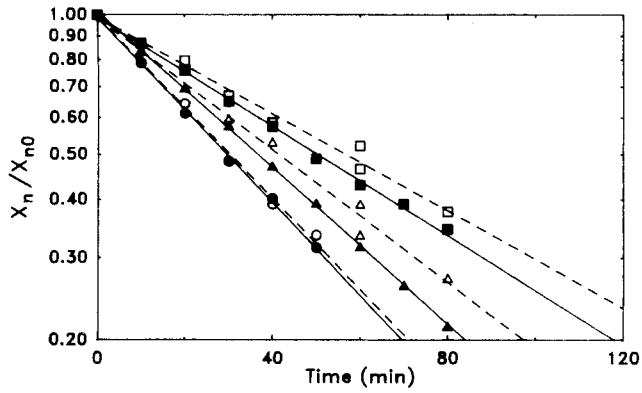

Figure 9. Hydrolysis rates of xylo-oligosaccharides (100°C, 0.025 MH_2SO_4, $k_2 = 0.0084\ min^{-1}$): experimental and simulation results.

— — — Kamiyama and Sakai (1979)
——— simulation
o $DP = 5$, Kamiyama and Sakai (1979)
• $DP = 5$, simulation ($M = 1.9$)
△ $DP = 4$, Kamiyama and Sakai (1979)
▲ $DP = 4$, simulation ($M = 2$)
□ $DP = 3$, Kamiyama and Sakai (1979)
■ $DP = 3$, simulation ($M = 2$)

The simulation results shown in Figure 9 were obtained using similar values of M. It can be noted that as expected the agreement between experimental results obtained by simulation and by direct measurement of hydrolysis rates is improved as the DP gets bigger.

Complementary information concerning the distribution of products is shown in Figures 10 and 11, from which it is seen that concordance between results is good, even though simulated rates of hydrolysis are systematically higher than the experimental values.

Conclusions

Utilization of the kinetic information obtained from experiments with a model compound was successfully applied to the simulation of the homogeneous acid hydrolysis depolymerization of a $\alpha(1\rightarrow4)$-bond-linked polysaccharide, namely amylose. It was verified that the method of constant time increments produced the most adequate results.

Utilization of a predictive stochastic Monte Carlo technique permitted to foresee the time evolution of product distribution upon substrate depolymerization. An excellent quantitative agreement is evidenced between the results reported here for the simulation of the temporal variation of glucose concentration and experimental results at all studied reaction temperatures. Similar information available for the intermediate products can be used as a good approximation to the actual behavior of such chemically reacting systems, thus constituting a simpler and more versatile alternative to the solution of a long ODE set necessary to describe the system in a deterministic manner. In the present case, for example, the stochastic procedure was used in place of the resolution of 630 simultaneous ODE.

The Monte Carlo simulation associated with a Markov chain was therefore found to be a helpful and flexible connection between a model system (dimer hydrolysis) and the conversion of a macromolecular substrate.

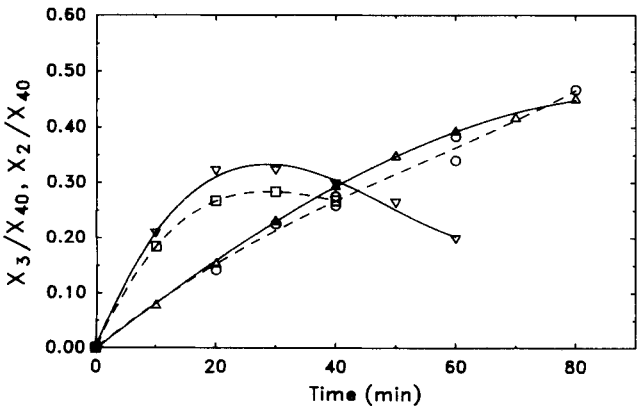

Figure 10. Degradation of xylotetraose to lower oligosaccharides at 100°C: experimental and simulation results.

— — — Kamiyama and Sakai (1979)
——— simulation
□ X_3/X_{40}, 0.05 M H_2SO_4 Kamiyama and Sakai (1979)
▽ simulation ($M = 2$, $k_2 = 0.019\ min^{-1}$)
o X_2/X_{40}, 0.025 M H_2SO_4 Kamiyama and Sakai (1979)
△ simulation ($M = 2$, $k_2 = 0.0084\ min^{-1}$)

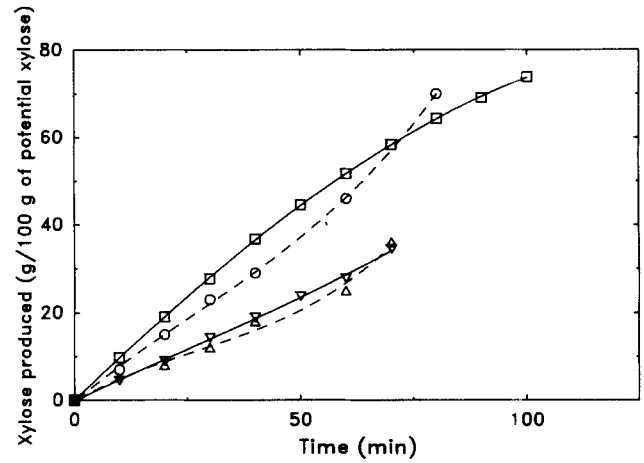

Figure 11. Cleavage of xylo-oligosaccharides into xylose at 100°C: experimental and simulation results.

— — — Kamiyama and Sakai (1979)
——— simulation
o xylotetraose, 0.05 M H_2SO_4 Kamiyama and Sakai (1979)
□ simulation ($M = 2$, $k_2 = 0.019\ min^{-1}$)
△ xylotriose, 0.025 M H_2SO_4 Kamiyama and Sakai (1979)
▽ simulation ($M = 2$, $k_2 = 0.008\ min^{-1}$)

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Notation

A = frequency factor, $L \cdot mol^{-1} \cdot min^{-1}$
b = binomial probability mass function
B = reacting species
$B1$ = reducing bond of a chain
$B2$ = nonreducing bond of a chain
$[C]$ = cellobiose concentration, $g \cdot L^{-1}$

E = energy of activation, $kJ \cdot mol^{-1}$
f = Poisson probability mass function
k = first-order kinetic constant, min^{-1}
k_1 = pseudofirst-order kinetic constant for reducing bonds, min^{-1}
k_2 = pseudofirst-order kinetic constant for nonreducing bonds, min^{-1}
k_2' = pseudofirst-order kinetic constant for nonreducing bonds, min^{-1}
k_H = second-order kinetic constant for nonreducing bonds, $L \cdot mol^{-1} \cdot min^{-1}$
m = integer number
M = ratio k_2/k_1
n = integer number
N_0 = number of Monte Carlo simulations
p = probability of "success" in a binomial distribution
P = product species
Prob = transition probability value
R = universal constant of gases ($=8.314\ J \cdot mol^{-1}K^{-1}$)
S = ratio k_2'/k_2
S_0 = initial concentration of substrate
t = time, min
T = temperature, K^{-1}
V = volume, L
w = weight given to each point in the least-squares method
x = current number of B molecules in V
$x1$ = number of reducing bonds of a polymeric chain in V
$x2$ = number of nonreducing bonds of a polymeric chain in V
$X1(t)$ = number of reducing bonds $B1$ in V at time $t(=x1)$
$X2(t)$ = number of nonreducing bonds $B2$ in V at time $t(=x2)$
X_n = concentration of xylo-oligosaccharide of $DP = n$ ($mol \cdot L^{-1}$)
X_{n0} = concentration of xylo-oligosaccharide of $DP = n$ at time zero ($mol \cdot L^{-1}$)

Greek Letters

Δt = constant time increment, min
σ = standard deviation, %
σ_M = average value for σ

Subscripts

0 = initial state
i = type of reaction 1 or 2

Registry numbers

amylose	[9005-82-7]
barium carbonate	[513-77-9]
calcium carbonate	[471-34-1]
cellobiose	[528-50-7]
glucose	[492-62-6]
hydrochloric acid	[7647-01-0]
sulfuric acid	[7664-93-9]
water	[7732-18-5]
xylose	[58-86-6]

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