THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

STUDY OF THE CARBOHYDRATE PEELING AND STOPPING REACTIONS UNDER
THE CONDITIONS OF ALKALI-OXYGEN PULPING

Project 3265

Interim Report

to

THE PROGRAM COMMITTEE

February 18, 1977

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SUMMARY

Reactions between dissolved oxygen and a carbohydrate substrate have been carried out in solutions of varying pH and at different temperatures. These reactions were carried out in the absence of a supporting oxygen atmosphere, so that mass transfer from a gaseous phase was not a factor.

The extent of oxidation (yield of disaccharide acids formed from the disaccharide cellobiose) ranged from 6 to 23%. The bulk of the cellobiose was destroyed by the peeling reaction. The oxidation was favored, relative to peeling, by high pH, low temperatures, and a slow heat-up period at the beginning of the reaction. Measurement of oxygen consumed during the reaction showed that about 3 to 4 moles of oxygen reacted with one mole of cellobiose. No disaccharide acids were formed when nitrogen was used in place of oxygen.

Reactions in the flow reactor were normally carried out by the rapid mixing of alkali-oxygen and carbohydrate solutions that were already at the reaction temperature. Reactions run at 100°C showed two effects. First, the rate of reaction tended to level out due to the formation of disaccharide acids. The yield of these acids was about 15% for a high pH system (sodium hydroxide) and about 5% for a lower pH (sodium carbonate). Secondly, the rate of reaction in the initial phases was much slower for an oxygen system than for a nitrogen system, due again to the formation of the alkali-stable disaccharide acids.

When the reaction temperature was increased to 130°C, the yields of disaccharide acids in a sodium hydroxide-oxygen system were about 6-8%. When

the two solutions were mixed at room temperature and then brought to temperature within 30 seconds, this heat-up period increased the yield of disaccharide acids to about 23%. This increase was attributed primarily to a faster rate of oxidation relative to peeling, using the lower temperatures encountered in the heat-up period. The possibility of an induction period has been considered.

When sodium carbonate was used instead of sodium hydroxide, the reaction system with a 30-second heat-up period gave about half the yield of disaccharide acids (10.5%) showing the effect of lower pH. This same pH effect was shown for reactions carried out in a digester with either sodium hydroxide or sodium carbonate for a heat-up period of 90 minutes. However, the use of a heat-up period with sodium carbonate at a higher temperature seems to be more beneficial (10.5% at 130°C) than the use of a lower temperature with no heat-up period (5% at 100°C).

INTRODUCTION

The purpose of this project is to study the relative rates of peeling
of glucose end groups present in oligosaccharides in comparison with the stopping
(oxidation) of the same groups in a system composed of oxygen and alkali or buffer
solutions of lower pH. Experimentally the problems to be solved were (a) to pre-
pare solutions of dissolved oxygen under pressure in various alkaline or buffer
solutions, (b) to analyze these solutions for oxygen content, (c) to maintain the
oxygen in solution while these solutions were mixed with carbohydrate solutions
at various temperatures, and (d) to analyze the resulting systems for the extent of
reaction by either oxidation or peeling. Most of these problems have been solved.

In this report details of various reactions run in the flow reactor are
given which show that dissolved oxygen will react when mixed with solutions
of carbohydrates, when the resulting reaction system is isolated from any external
contact with an oxygen supply, such as an oxygen atmosphere which is used in a
normal digester reaction. Thus, there is no problem of mass transfer, i.e., the rate
of solution of gaseous oxygen into the aqueous system. In the flow reactor there
is no gaseous phase, just the mixing of two aqueous solutions to ensure a reaction.
In this reactor system the concentration of dissolved oxygen is about ten times
(on a molar basis) that of the cellobiose, so that an excess of oxygen is always
present during the reaction. Control experiments have shown that the dissolved
oxygen will remain in solution during the course of the reaction. To our knowledge,
this is the first time that such an isolated system, containing dissolved oxygen,
has been operated successfully.

Because the reaction in the flow reactor is started by mixing rapidly
two solutions already maintained at the reaction temperature, there is no period

of "heat-up" of the reaction solution, in contrast to heat-up periods of 20 to 90

minutes usually required to bring the contents of a conventional digester to re-

action temperature. Thus, there is no problem of what will happen to competing

reactions at the lower temperature in the first part of the heat-up period. Also,

the reaction is stopped rapidly and there is no loss of products due to continued

reaction during the cooling period.

In some of our reactor operations a certain heat-up period was desired,

and for this purpose the reaction was carried out by mixing the cold solutions

first and then forcing them into a heated coil in an oil bath. With the light

mass and thin walls of the coil allowing rapid heat transfer, a heat-up period

of a few seconds could be achieved. This coil technique is called an "isothermal

digester" in this report.

Since the solubility of oxygen in aqueous solutions is rather low, of

the order of 300 milligrams per liter, reactions were carried out with equally

low concentrations of carbohydrate, to ensure an excess of oxygen in the reactions

studied. The molar ratio of oxygen to cellobiose was about 10 to 1, and the mass

ratio about 1 to 1. The alkali present, either sodium hydroxide or sodium carbo-

nate, of course, is in much higher concentration, and so the oxygen has to compete

with this greater amount of alkaline reagent for the cellobiose.

The quenched reaction solutions were analyzed by gas chromatography,

either as total disaccharides (a combination of unreacted disaccharide and disac-

charide acids formed by oxidation) or as the acids remaining at the end of the

reaction, when all original disaccharide has been removed, either by peeling or

by oxidation.

Finally, it should be pointed out at present the parameters of the

alkali-oxygen system have been explored rather widely but not very much in depth.

Precision of data is needed by running duplicate experiments and showing the

importance of the various parameters.

BACKGROUND

In the oxidation of a carbohydrate end group with oxygen to form an

alkali-stable acid end unit, alkali is needed to promote the reaction. Oxygen

is relatively inert, except in the presence of alkali or free radicals. In the

alkaline system the carbonyl end unit is converted to a 1,2-enediol primarily

and the resulting double bond is attacked, with loss of one carbon atom (Fig. 1).

Thus, cellobionic acid will be converted from the C-12 disaccharide to a C-11 acid,

glucosyl-arabinonic acid.

```
        CHO                        CHOH                    HCOOH
         |                                                 Formic Acid
       H-COH                       --||--                      +
         |                          |
       HOC-H    ---  OH⁻     --    C-OH-     ---  O₂  --    COOH
         |      ----->             |         -------->       |
       H-C-O-GL                  HO-C-H                     HO-C-H
         |                          |                         |
       H-COH                     H-C-O-GL                   H-C-O-GL
         |                          |                         |
       CH₂OH                      H-C-OH                     HC-OH
                                    |                         |
                                  CH₂OH                     CH₂OH

     Cellobiose                1,2-Enediol                 C-11 Acid
```
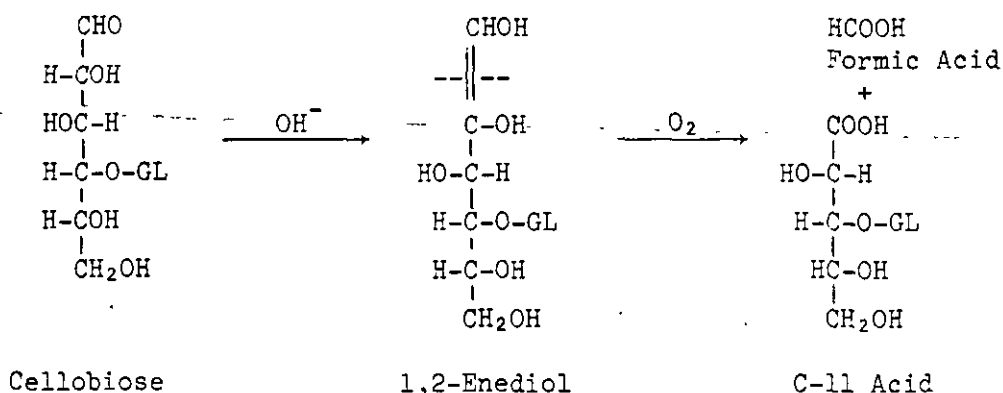
Figure 1. Oxidation of Cellobiose with Loss of One Carbon Atom

Other alkaline rearrangements can also occur, such as rearrangement of

cellobiose to the ketose isomer cellobiulose, and subsequent formation of a

2,3-enediol. The double bond is then attacked, with loss of two carbon atoms,

and the formation of a C-10 acid, glucosyl-erythronic acid (Fig. 2). Further loss
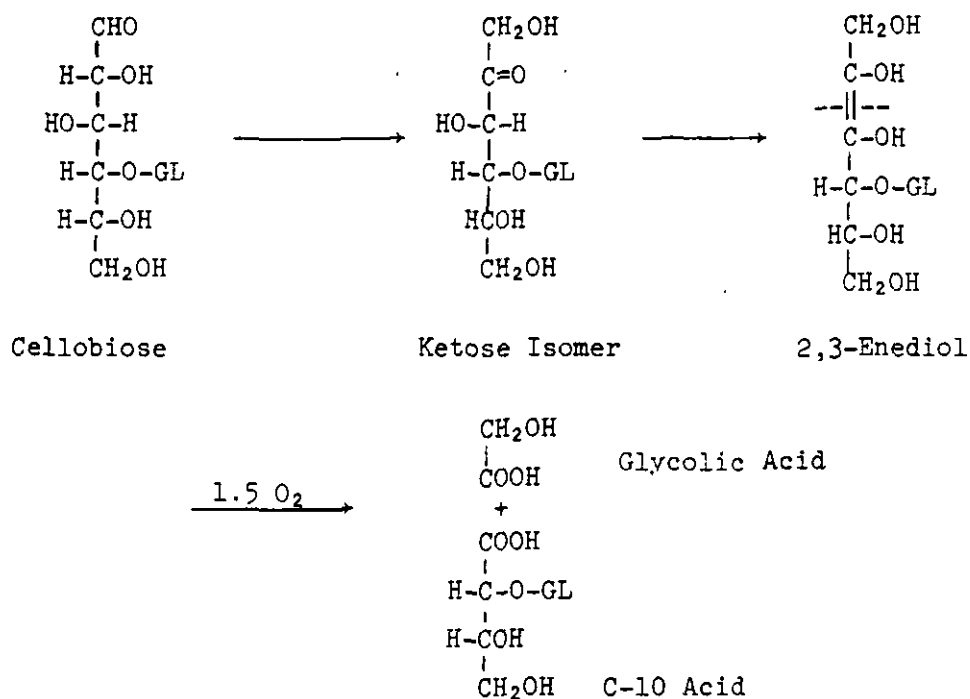
of carbon can lead to a C-9 acid.

```
      CHO                  CH2OH                 CH2OH
       |                    |                     |
    H-C-OH                 C=O                   C-OH
       |                    |                    --||--
   HO-C-H      ------>    HO-C-H      ------>     C-OH
       |                    |                     |
    H-C-O-GL             H-C-O-GL              H-C-O-GL
       |                    |                     |
    H-C-OH                HCOH                   HC-OH
       |                    |                     |
     CH2OH                CH2OH                  CH2OH

   Cellobiose           Ketose Isomer         2,3-Enediol
```

```
                        CH2OH
                         |
                        COOH        Glycolic Acid
          1.5 O2          +
        ------->
                        COOH
                         |
                      H-C-O-GL
                         |
                      H-COH
                         |
                        CH2OH    C-10 Acid
```

Figure 2. Oxidation of Cellobiose with Loss of Two Carbon Atoms

Several studies of the oxidation of cellobiose have been reported in the literature. These reactions employ relatively long reaction times, involving a heating up period, and the minimum reaction time at the desired temperature is at least 5 minutes. Usually the reactions involving the peeling or oxidation of cellobiose are complete during the heat-up period, long before maximum temperature is reached.

Malinen and Sjostrom (1) reported data for reaction times from 5 to 120 minutes with a heat-up time of 20 minutes in addition. They found that the yields of disaccharide acids (C-12 to C-9) were proportional to (a) the pressure of oxygen, and (b) the pH of the system, and inversely proportional to (c) the temperature and (d) the length of reaction time. They attributed the effect of reaction time to the fact that the disaccharide acids were unstable when exposed for periods of 1 to 2 hours to either alkali or alkali-oxygen at elevated temperatures (2).

Rowell, et al. (3,4) compared the rate of peeling and oxidation for cellobiose by calculating the relative yields of isosaccharinic acid (as the main product of peeling) and of the glucosyl aldonic acids at the end of an alkali-oxygen reaction. In our present work we have either carried the reaction to completion and calculated the yield of disaccharide acids (based on starting disaccharide) or have used very short reaction times (1 to 100 seconds) and determined total disaccharides (unconverted starting material plus disaccharide acids). The latter technique has not been perfected yet, as we lack response factors for the isomers of cellobiose (glucosyl-mannose and cellobiulose) and for the several disaccharide acids.

Kolmodin and Samuelson (5) have studied the oxidation of cellulose and found that the aldonic acid end groups, liberated upon hydrolysis, were primarily the C-5 acid, arabinonic acid, and C-4 acid, erythronic acid, and the C-6 acid, mannonic acid. The first two would correspond to the C-11 and C-10 acids in the case of cellobiose. They found very little gluconic or ribonic (C-5) acids. The absence of gluconic acid agrees with our work where we get no cellobionic acid (glucosyl-gluconic acid). (See Table I.)

In the fragmentation of cellobiose to C-11 and C-10 acids, the carbon atoms are lost as formic acid and glycolic acid, respectively. Malinen and Sjostrom (1) reported appreciable amounts of these acids. However, they did not differentiate between the several disaccharide acids, as to the extent of C-9, C-10 or C-11 acids. They also reported appreciable amounts of glyceric acid, a C-3 acid which would be formed in the fragmentation of cellobiose to a C-9 acid.

The minor fragments formed are interesting to investigate, from the viewpoint of terminating and fragmentation mechanisms. Our primary interest is

in the total yield of disaccharide acids, since the glucosidic bond, so important

to pulp yield, is preserved in these alkali-stable products.

TABLE I

YIELDS OF ALDONIC ACIDS FORMED FROM END UNITS OF
HYDROCELLULOSE AND CELLULOSE AFTER
ALKALI-OXYGEN REACTION (5)

(Starting material — aldonic acid)

	Cellulose[a], %	Hydrocellulose,[b] %
Gluconic acid	11	3
Mannonic acid	27	27
Arabinonic acid	15	50
Ribonic acid	2	2
Erythronic acid	45	18

[a]Cotton was impregnated with aqueous solution containing
1% NaOH and 1% lignin, pressed to dry content of 27%,
and heated under oxygen pressure of 30 psig at 100°C
for 22 hours.

[b]Hydrocellulose, treated with 0.5% NaOH 2 hours at 100°C.

DISCUSSION OF EXPERIMENTAL RESULTS

INTRODUCTION

If we confine ourselves to a simplified scheme of an alkali-oxygen
system for disaccharides, then there are two primary reactions, the peeling of
the disaccharide and the oxidation of the disaccharide to one or more alkali-
stable disaccharide acids.* Such a system is shown in Fig. 3, where the overall
reaction (Curve A) levels off after a certain time due to the formation of these
alkali-stable products. Also, it can be seen that this rate initially is much
slower than that observed in an alkali system where only peeling occurs (C),
and no disaccharide acids are left.

This scheme ignores any oxidation of fragments containing six carbons
or less, and for the purpose of our chromatographic analyses it simplifies things
greatly. In the work cited below we have analyzed for (a) total disaccharides in
kinetic runs (Curve A), and (b) for disaccharide acids only after Curve A has
leveled off (Region A'). So far we have not analyzed for the rates of formation
of disaccharide acids (Curve B). The separation of these acids from the unreacted
disaccharides is a bit involved and we have not perfected this technique yet. So
our efforts have been concentrated on Curve A. In earlier work (6) the peeling
reaction with alkali alone was studied and first-order kinetic plots obtained, as
shown in the straight line C.

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*The disaccharide acids are defined as a group of peaks obtained in a gas chromato-
graphic analysis. These peaks have retention times in the "disaccharide region,"
i.e., retention times greater than that of the internal standard inositol. Also
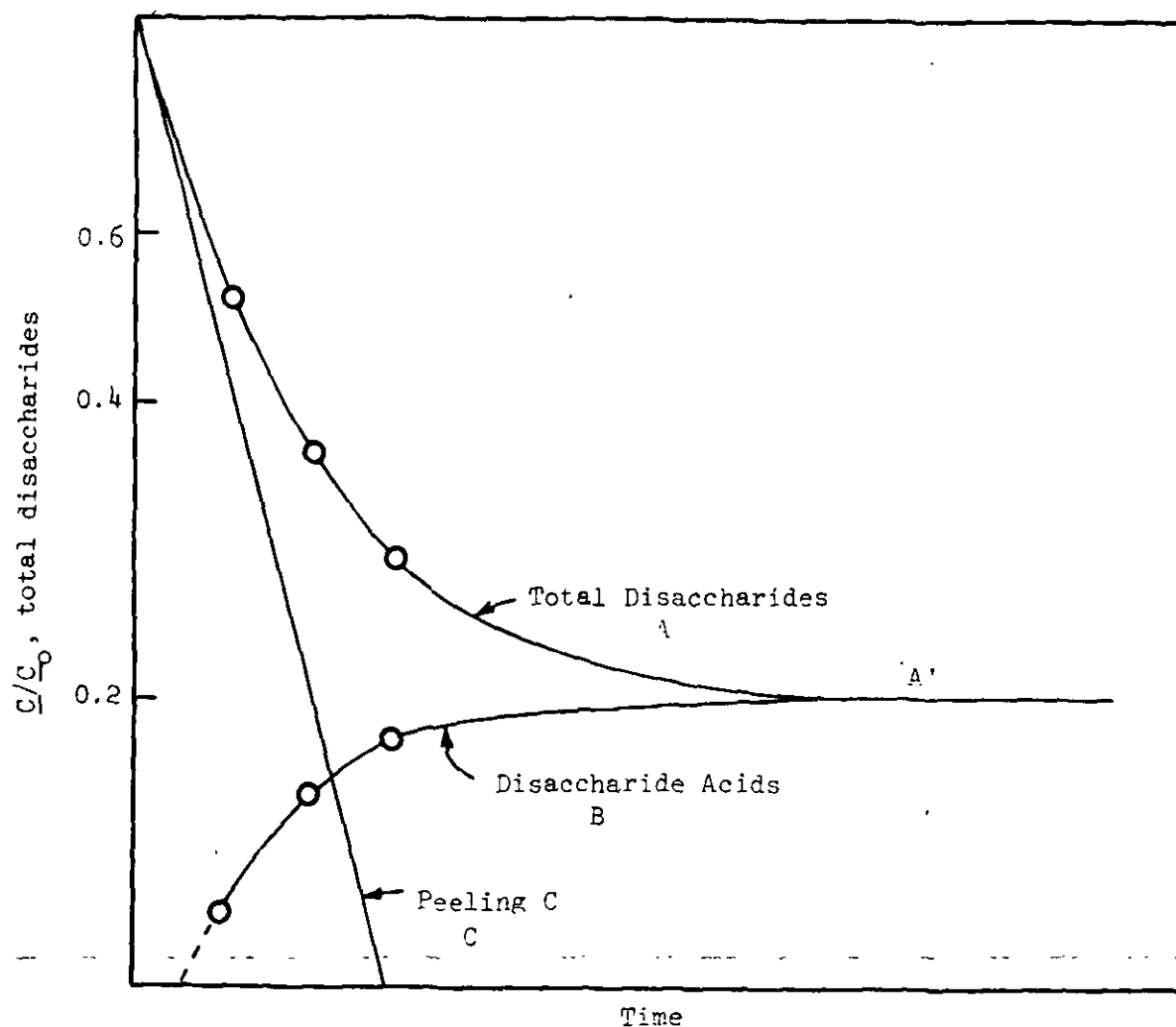these peaks are not formed in an alkaline system containing nitrogen in place of
oxygen.

Figure 3.   Idealized Plot of Rate of Oxidation, Compared to Peeling

KINETIC RUNS

A series of three kinetic runs were made at 100°C with solutions of
varying alkalinity, for orientation purposes.   The first-order plots are shown
in Fig. 4, and it can be seen that the rate of disappearance of total disaccharides
increases with increasing alkalinity.   The reaction is very slow with sodium bi-
carbonate,. and in contrast with sodium hydroxide it is almost complete in one
minute and the leveling off of the curve is due to the presence of alkali-stable
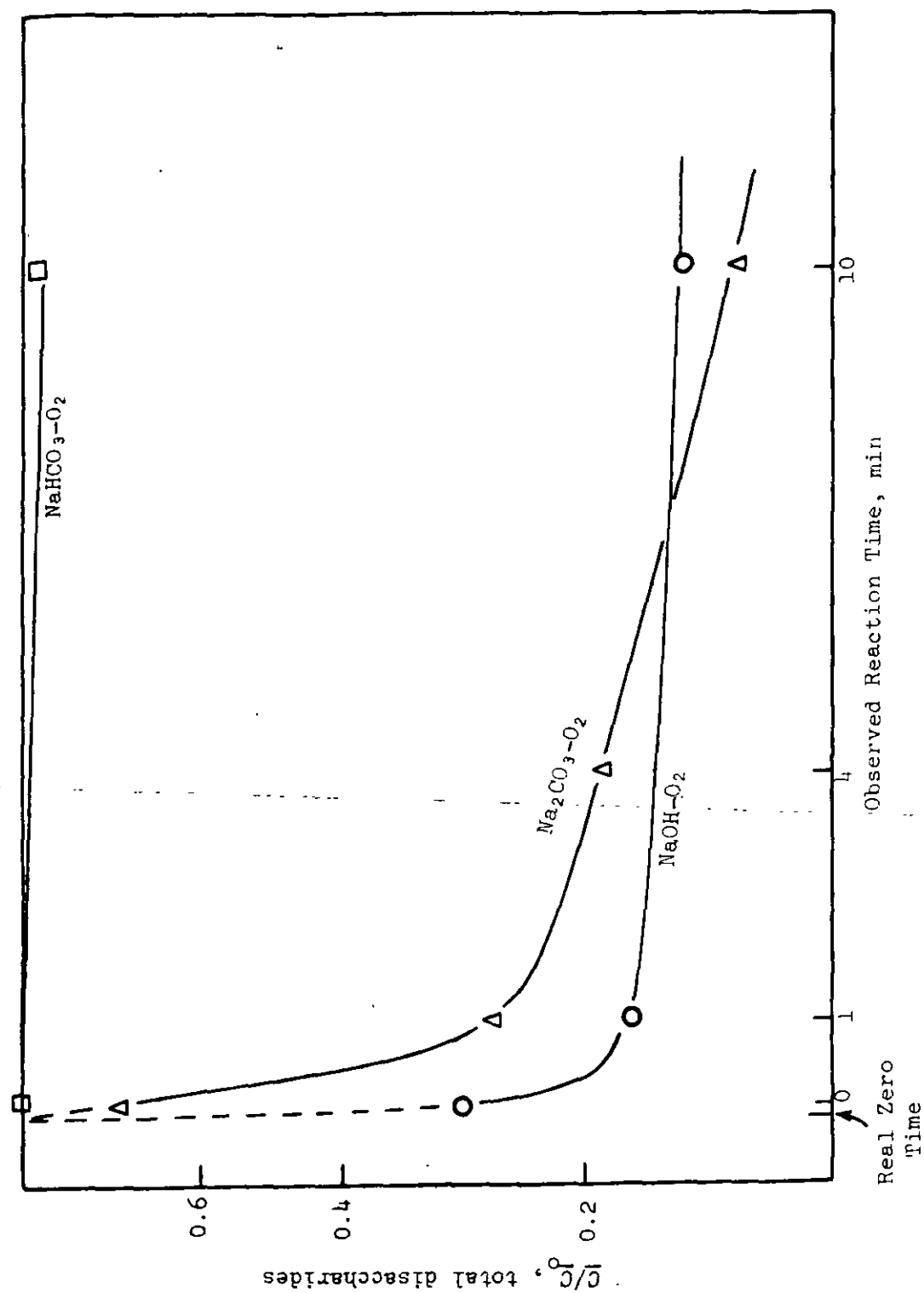
Figure 4. Intermittent Flow Reactor Study of Oxygen Reactions with Cellobiose at 100°C

disaccharide acids. The sodium carbonate reaction is intermediate in rate, and has not leveled off in 10 minutes.

It can also be noted that the yield of disaccharide acids is greater for the sodium hydroxide system, in contrast to that for sodium carbonate. This confirms the data obtained by Malinen and Sjostrom (5) who worked only with varying concentrations of sodium hydroxide (see Table II).

TABLE II

YIELDS OF TOTAL DISACCHARIDE ACIDS IN ALKALI-OXYGEN RUNS

Alkali Concn.	Oxygen, ppm	Sugar Concn., molar	Temp., °C	Heat-up Period, min	Yield, %
0.375$\underline{N}$ NaOH	175	0.00044	100	0	15
0.375$\underline{M}$ Na$_2$CO$_3$	175	0.00044	100	0	<5
0.375$\underline{N}$ NaOH	175	0.00044	130	0	6-8
0.75$\underline{N}$ NaOH	350	0.00044	130	0.5	23
0.75$\underline{M}$ Na$_2$CO$_3$	350	0.00044	130	0.5	10.5
0.25$\underline{N}$ NaOH	[a]	0.03	120	90	19
0.25$\underline{M}$ Na$_2$CO$_3$	[a]	0.03	120	90	--

[a]These were digester runs, with a supporting oxygen atmosphere.

To confirm the effect of oxygen in the reactions, two short kinetic runs were made with sodium carbonate at 100°C (Fig. 5). One run was made with dissolved oxygen and the other with only nitrogen present. In the short reaction times the plots are first order and have not started to level off as shown in Fig. 4. It can be seen that the rate of reaction with oxygen is slower than that with nitrogen, due to the formation of alkali-stable disaccharides in the former system. In the nitrogen system only peeling occurs.
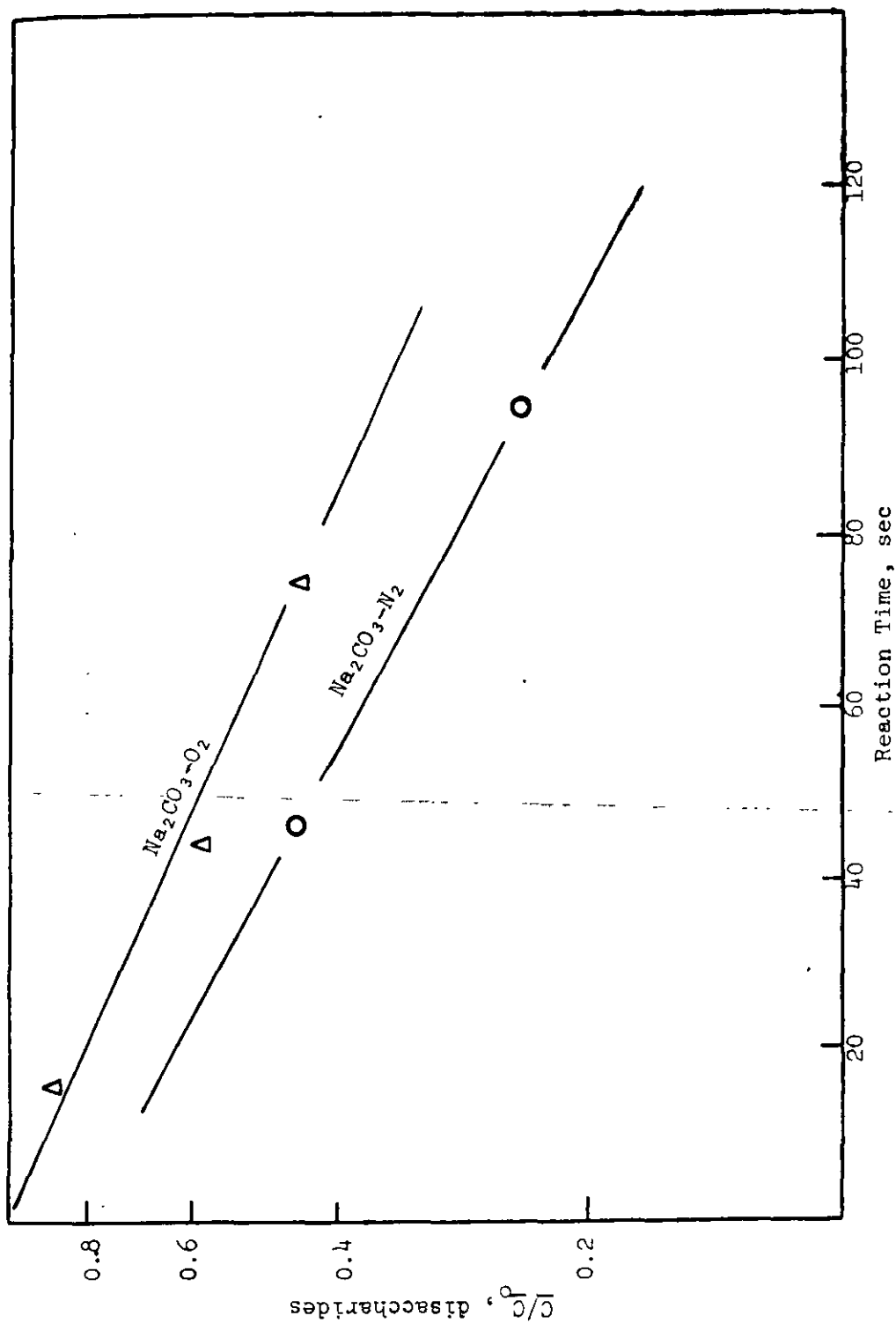
Figure 5. First-order Kinetic Plot of Disappearance of Disaccharides in the Reaction of 0.0004M Cellobiose with 0.005M Dissolved Oxygen in 0.15M Sodium Carbonate at 100°C.

REACTIONS CARRIED TO COMPLETION

We were interested in the extent of oxidation at higher temperatures, more in the realm of pulping conditions, so a series of experiments were carried out at 130°C. These experiments were carried out for relatively long reaction times (6 seconds with 0.36$\underline{N}$ NaOH) in an effort to determine the total amount of acids formed, and to obtain a correlation with digester cooks where the time involved is a matter of minutes and hours rather than seconds.

To our surprise, the yield of disaccharide acids was of the order of 6 to 8%, based on the original cellobiose. This yield seemed quite low to us, compared to digester cooks (see below and in Table II). At first we thought perhaps our oxygen had leaked out of the flow reactor but control experiments assured us that this was not so. Finally, it dawned on us that we were determining the "true" yield of acids, the true extent of oxidation relative to peeling, at a given temperature, without going through a preliminary heat-up period such as that encountered in laboratory digesters. With our flow reactor, the two solutions containing alkali-oxygen and carbohydrate are brought to temperature separately and then mixed rapidly to start the reaction immediately. So at this temperature we are dealing with the true relative rates of peeling and of oxidation. In a heat-up period we go through a low-temperature stage first, which may be more favorable to oxidation than to peeling. This temperature effect is shown by the data for flow reactor runs at 100°C and at 130°C; the yields of disaccharide acids are much greater at the lower temperature (see Table II).

To check the heat-up period we altered our flow reactor so that the reactants were mixed cold and then forced into a heated coil where the heat-up period was about 30 seconds. This period is rather short, compared to that for a laboratory

digester but is very long compared to the "half-life" of the carbohydrate at

elevated temperatures. This relationship is given in Table III for several

temperatures and it can be readily seen that most reactions are complete long

before maximum temperature is reached*.

TABLE III

HEAT-UP TIME RELATIVE TO REACTION TIME

Temp., °C	Half-life of Cellobiose[a] in $\underline{N}$ NaOH	Number of Half-lives in Heat-up Time	
		of 30 sec	of 20 min
65	30 hr	1/3600	1/90
60	500 sec	1/16	3
100	10 sec	3	120
120	2 sec	15	600
130	0.5 sec	60	2400

[a]See Ref. (6).

The yield of disaccharide acids obtained by this modified reactor showed

a great increase to about 23%. The alkali and oxygen concentration were higher in

this experiment also, compared to the flow reactor run with zero heat-up time, but

the main effect seems to be attributed to the appreciable heat-up time.

The increased yields obtained with use of heat-up periods can be attributed

to either a greater rate of oxidation (relating to peeling) at lower temperatures,

or else the presence of an induction period for oxidation. Sinkey and Thompson (7)

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*In a reaction time, equivalent to ten half-lives for the organic substrate, only
0.1% of the substrate should be still present $(1/2^{10})$.

have noted such an induction period in the oxidation of methyl β-D-glucopyranoside in an alkali-oxygen system, and such a delay in the start of a reaction could also occur in the oxidation of the end group on cellobiose. Kinetic runs at low temperatures will be made to evaluate this possible factor.

Once we had determined the significance of the heat-up time, we made several runs in our "isothermal digester" and also in a laboratory digester where the heat-up time was about 90 minutes. In both cases we found that the yield of disaccharide acids was much lower for sodium carbonate systems than for sodium hydroxide systems, and this agrees with the data reported by Malinen and Sjostrom (1), for their digester runs with varying concentrations of sodium hydroxide; the greater yields were found at the higher pH values. The yields of disaccharide acids in our carbonate runs were about half of that for a sodium hydroxide run of the same molarity. This effect can be seen again in Fig. 3, where the carbonate run is starting to level off at a much lower yield than the sodium hydroxide run.

Malinen and Sjostrom (1) did not report their yields of disaccharide acids on the basis of original disaccharide, but only as percentage of total nonvolatile acids formed. However, their lowest pH run, with 0.25N NaOH, gave a very low yield of acids, and must be viewed as suspect, since the molar concentrations of alkali and cellobiose were relatively close (0.06 and 0.03M, respectively) and most of the alkali would be consumed during the cook and lower the pH drastically from the starting value. With our use of sodium carbonate for lower pH values, we have ample buffer capacity to maintain the original pH. Most of our alkali and cellobiose concentrations were in the molar ratio of 300 to 1 (see Fig. 4), so there was always an excess of alkali or buffer.

To insure that the disaccharide acids were really a product of the alkali-oxygen system, we made two digester runs with sodium hydroxide and with sodium carbonate, and with a nitrogen atmosphere instead of an oxygen atmosphere. The gas chromatographic analyses revealed no peaks in the disaccharide region, showing the completeness of peeling.

CONSUMPTION OF OXYGEN DURING RUNS

It would be very desirable to measure the rate of consumption of dissolved oxygen during a reaction with cellobiose. However, as long as there is unreacted cellobiose present, this sugar may interfere with the oxygen determination by the Winkler method*. However, control runs with a mixture of aldonic and saccharinic acids, obtained after a completed run where no cellobiose is present, showed no interference. Therefore, we carried out an "isothermal digester" run, with about 30 mg of cellobiose and 3 mg of dissolved oxygen in 10 ml of 0.75$\underline{N}$ sodium hydroxide, and allowed a 30-second heat-up time to 130°C, and then 10 minutes at temperature. Under these conditions we have found that about 25% of disaccharide acids are formed.

This run was carried out twice, and the liquid at the end of each run pushed directly into a Winkler trap and analyzed for oxygen. Most of the oxygen (350 ppm) had disappeared, and after comparison with control runs with no cellobiose present, it was found that about 0.87 mole of oxygen had reacted per mole of cellobiose present originally. If we assume that only 25% of the original

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*A communication by Dr. Richard B. Barker informs us that manganese, in a higher oxidation state which occurs in the Winkler determination, will oxidize sugars very rapidly.

cellobiose disappeared by reaction with oxygen*, and the rest was destroyed by

the peeling reaction, then this value of 0.87 will be changed to about 3.5 moles

of oxygen per mole of cellobiose oxidized.

In our normal runs we use a much higher ratio of oxygen to cellobiose,

a 10 to 1 molar ratio, in contrast to the 1 to 1 ratio in the present experiment.

This was necessary to insure that we obtained an experimentally valid difference

in oxygen concentration; actually 2.5 of the 3.0 mg of oxygen reaction. In our

normal runs the difference in determination would be much less and closer to

experimental error.

At present it is impossible to calculate the exact stoichiometry of the

reaction. Glucose, an intermediate in the peeling reaction, will also react

readily with oxygen. Also some of the acids present may react more slowly at

the high temperature involved (although they apparently do not react with the

Winkler reagent at room temperature). The reaction time used here, 10 minutes,

is a relatively short time, compared to the stability studies carried out by

Malinen and Sjostrom (2) for 1 to 2 hours.

We can only state at present that one equivalent (16-gram atoms) of

oxygen would be required to oxidize a C-12 sugar to a C-12 acid, and that one

mole (32 g) of oxygen would be required to form a C-11 sugar, and formic acid,

with breaking of the double bond. The formation of a C-10 acid, with the

breaking of a 2,3-double bond and subsequent oxidation of the 2-carbon fragment

to glycolic·acid, will require 1.5 moles of oxygen. The same stoichiometry

would hold for the formation of a C-9 acid and glyceric acid, the C-3 fragment.

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*This assumption is based on the yield of 23% disaccharide acids obtained at 130°C
(see Table II). We have no true way of knowing where all the oxygen consumption
goes, but we can evaluate a part of it, based on the disaccharide acids formed.

All three of these low molecular weight acids have been reported as products in

alkali-oxygen reactions with cellobiose (1).

In all our kinetic studies the oxygen concentration has been kept at

approximately the same value, about 175 ppm, and the ratio of oxygen to carbo-

hydrate maintained at a 10 to 1 molar ratio to insure a strong driving force in

the oxidation. However, it would be interesting to use a lower molar ratio and

note the effect of such a change on the rate of reaction.

EXPERIMENTAL

The flow reactor has been operated in three modes: (a) a continuous operation, where the reaction time is inversely proportional to the flow rate through a reaction coil; (b) an intermittent operation, for times above 30 sec, where the reaction solution is mixed rapidly, but maintained motionless in the reaction coil for a period of several minutes; and (c) an "isothermal digester" operation, where there is an appreciable heat-up time for the reaction system. The first two give the true rates of reaction for a given system, and the third mode is related to the operation of a typical digester of appreciable mass.

A FLOW REACTOR IN CONTINUOUS OPERATION

This is the normal mode for which this reactor was designed and this has been discussed in detail in earlier reports ($\underline{8}$). The two reaction solutions are heated in heating coils for about 2 minutes to bring them to the desired temperature. (The coils are heated empty for fifteen minutes first to insure that they and the nickel mixer housings are equilibrated with the oil bath.) Tests with thermocouples have shown that heat transfer at 100°C is almost complete in 30 sec, and so two minutes have been used as a standard time.

The two solutions are then pushed into a mixer and the resulting reaction solution driven through the reaction coil at a given rate; quench reagent is added as the reaction solution reaches the end of the coil. The quench reagent (usually cold boric acid) lowers the pH and the temperature and the reaction is, thus, brought rapidly to a stop.

The quenched solution is collected in a bottle in a pressure chamber (nitrogen) which serves to keep the dissolved oxygen in solution and also prevents the aqueous solutions from boiling at temperatures above 100°C.

A FLOW REACTOR IN INTERMITTENT OPERATION

Here the two heated solutions are pushed into a reaction coil at a rapid rate to insure good mixing. The reaction solution is left in the coil for a given period of time, then pushed out of the coil by displacing with fresh solution from the heating coils. The movement of the liquid must be adjusted so that only a portion of the reaction solution is removed, and that there is no mixing with fresh unreacted solution entering the reaction coil. The longer the reaction time, the more chance there is of diffusion of liquid from the heating coil through the mixer into the reaction system and, thus, affecting the kinetic history of the reaction solution. So only the first part of the solution in the reaction coil is removed, and is mixed with quenching reagent in the usual manner.

This procedure has been described in detail in a report for an earlier project (9).

THE FLOW REACTOR OPERATED AS AN "ISOTHERMAL DIGESTER"

This is also an intermittent operation. The two solutions are mixed at room temperature and then pushed into a single heating coil connected to a mixer and quench syringe, placed in the oil bath. This is shown in Fig. 6. The solution warms up to temperature rapidly as it enters the heated coil. After a given time in the coil, the solution is pushed out through the mixer and quenched.

OPERATION OF A LABORATORY DIGESTER

This was a small one-liter container, fitted with a Teflon enclosure (7). A run contained about 250 ml of solution, and the amount of cellobiose was 2.5 grams. The contents were stirred with a magnetic stirrer. The digester was brought to

100 psig oxygen at room temperature, and then immersed in an oil bath at 120°C

after temperature was reached, the digester was immediately removed and cooled,

and the contents worked up in the usual manner by stirring with IR-120 resin,
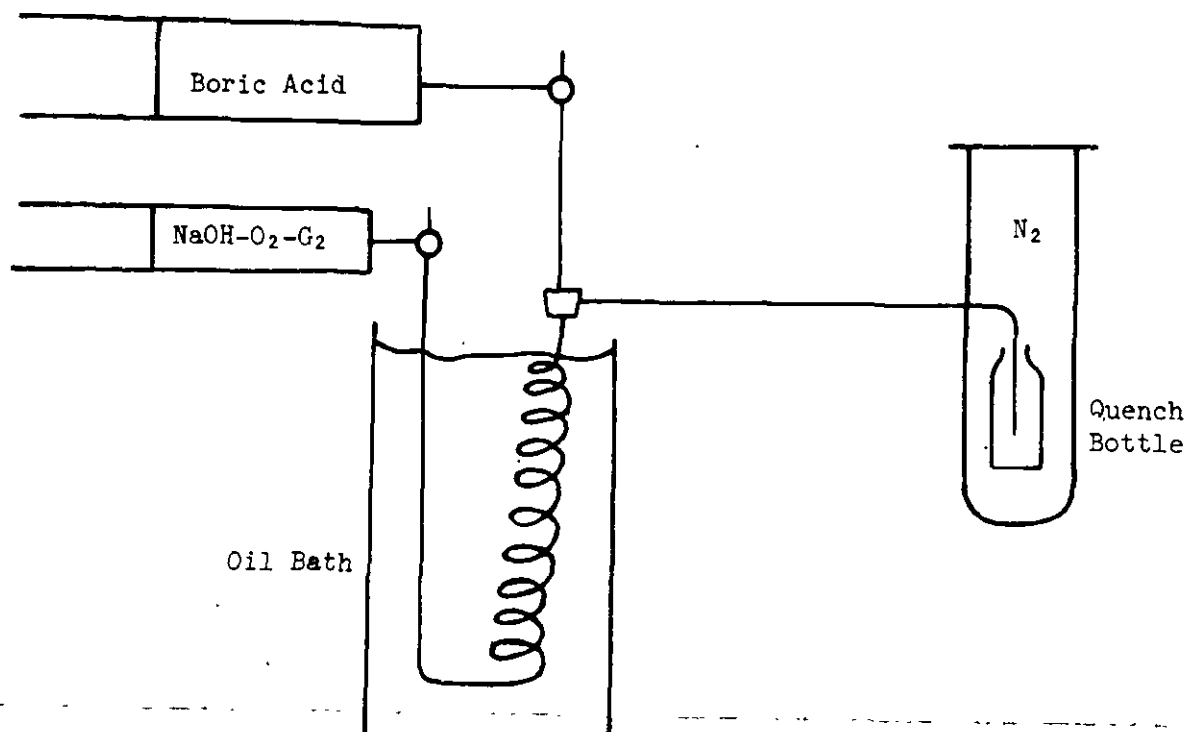
etc.

Figure 6. "Isothermal Digester" Mode of Flow Reactor

PREPARATION OF CARBOHYDRATE SOLUTIONS

In most of the runs an aqueous solution of 300 mg cellobiose per liter

was prepared, with the addition of 50 mg of inositol as an internal standard.

The concentration of cellobiose is about 0.00088 molar. During subsequent gas

chromatographic analyses the relative areas of the inositol and the combined di-

saccharide peaks were compared, to calculate the change in the latter with time.

The solutions were made up fresh each day to avoid any bacterial attack.

In the batch digester runs, with long heat-up times, inositol was not added as an internal standard initially, as it was felt that the long reaction time might affect the standard adversely. The short reaction and heat-up times in the flow reactor are not considered harmful to inositol.

HANDLING OF DISSOLVED OXYGEN IN THE FLOW REACTOR

Dissolved oxygen was prepared for kinetic runs in the usual manner by spraying a given aqueous solution into pressurized oxygen (10) and then analyzing the liquor by the modified Winkler method. A 20.7-ml aliquot of this solution was drawn into one of the mixing syringes and then pushed into a heating coil for the normal operation of the flow reactor.

The oxygen chamber was usually maintained at a pressure of 200 psig, and the resulting dissolved oxygen was normally about 300-350 ppm after the spray process. The pressure of the nitrogen chamber at the quench end of the flow reactor was maintained at 250 psig to keep the oxygen in solution.

For some unknown reason analyses for dissolved oxygen in sodium carbonate solution were very erratic, in contrast to the analyses obtained for sodium hydroxide solutions. This agreed with previous results (10).

WORK-UP OF QUENCHED SAMPLES

The samples, about 120 ml volume, after being quenched with boric acid, had a pH in the region of 8.5 to 9. They were stirred up with IR-120 cation exchange resin that had been regenerated with N HCl and washed with water to a pH of 5.0. The pH of the solutions dropped to about 3.5, due to the presence of both boric acid and saccharinic acids.

The solutions were each filtered through paper, and concentrated _in vacuo_ at 60°C to dryness in a 500-ml flask. The bulky white residue was treated with about 100 ml of methanol and the concentration repeated to remove boric acid as methyl borate. The small amount of organic residue was then dissolved in a little water and transferred to a 50-ml flask and this solution concentrated again to dryness.

The residue in the small flask was carefully dissolved in about 0.5 ml water. This solution was transferred in four equal aliquots to 6-ml vials for concentration before silylation. This transfer was done with the aid of a disposable transfer pipet; this pipet has a fine tip that forms about 40 drops per ml. The 0.5 ml, equivalent to about 20 drops, was divided dropwise into the four vials. Then about 0.25 ml water was added to the flask and the solution again divided into the four vials.

The several vials were then carefully concentrated to dryness over P_2O_5 in a vacuum desiccator. With care this can be done without bumping and takes about an hour. On the assumption that the original quench solution contained about 2 mg of organic material (original cellobiose substrate), each vial will contain about 500 µg of material.

After the residues have dried, they are pumped down hard over P_2O_5 for about an hour to remove the last traces of moisture. Then a given vial is treated with 400 µl each of dimethyl sulfoxide and Tri-Sil Concentrate (11), the vial capped with Teflon disks and crimped aluminum seals, and shaken overnight. The next day the caps are removed and the contents transferred with a transfer pipet to 1-ml Reacti-Vials, and 10 µl of the upper layer injected in the gas chromatograph.

While dividing the quench sample into 1/4 aliquots before silylation

reduces the size of the sample that is injected into the gas chromatograph, this

procedure does provide spare samples that can be analyzed in case one is lost

through a mishap. The silylated samples are usually stable for only a day or so

after preparation, and it is best to inject them on the gas chromatograph as

promptly as possible. They can be stored in a desiccator in the refrigerator

without too much degradation, if a fresh Teflon-lined disk is placed in the cap.

A disk that has already been pierced by a microsyringe should be replaced before

cold storage.

Because of the small size of the sample obtained from the flow reactor,

all of the quench solution must be worked up for subsequent silylation. This is

in contrast to earlier work with the flow reactor for peeling only, where larger

samples of carbohydrate substrate could be used, and only a portion of the quenched

sample used. Here, with the low concentration of dissolved oxygen present, the

concentration of cellobiose must be kept at the same low level to insure an

excess of oxygen as a reactant.

CHROMATOGRAPHIC IDENTIFICATION OF DISACCHARIDE AND DISACCHARIDE ACIDS

In Table IV are listed the retention times for the peaks of the tri-

methylsilyl ethers of the major compounds investigated in this study. It can

be seen that the parent sugars generally give two peaks, for the α- and β-anomers,

and that the acid derived from a given sugar gives a peak that is slightly slower

(a larger retention time). On the other hand, the sugar alcohol, obtained by

borohydride reduction, has a peak that is faster than the acid peak.

TABLE IV

RETENTION TIMES FOR VARIOUS DISACCHARIDES AND DERIVATIVES
(AS THE TRIMETHYL SILYL ETHERS)

	Retention Time on OV-17 Column, min[a]		
Parent Sugar	Parent Sugar[b]	Acid	Alcohol
Cellobiose	17.9 19.0	19.1	18.1
Glucosyl-mannose	17.0 17.8	--	18.0
Glucosyl-fructose	--	--	18.0[c]
Glucosyl-arabinose (C-11 sugar)	16.4 17.0	17.9	--
Glucose	8.0 9.2		
Inositol (internal standard)	9.8		

---

[a] The chromatography was done on a 6-foot x 1/8-inch OV-17 column, programmed from 130°C to 250°C at 6°/min.

[b] The two peaks for the parent sugar represent the α- and β-anomers.

[c] The alcohol derived from the reduction of glucosyl-fructose is a mixture of glucosyl-mannitol and cellobiitol.

At present we have not tried to separate mixtures of disaccharides and the related acids. As can be seen from the table, there is too much overlap of peaks. We have explored briefly the reduction of such a mixture by sodium borohydride, with the hope that we could separate the two C-12 alcohols (retention time at 18.0 to 18.1 min) from the several sugar acids.

From our reaction mixtures we have obtained a mixture of acid peaks, as shown in Fig. 7. These peaks range in retention time from 19.0 to 15.5 min and notably the peak at 19 min, that for cellobionic acid, is very small. The pattern of these peaks varies from run to run and we will have to explore this
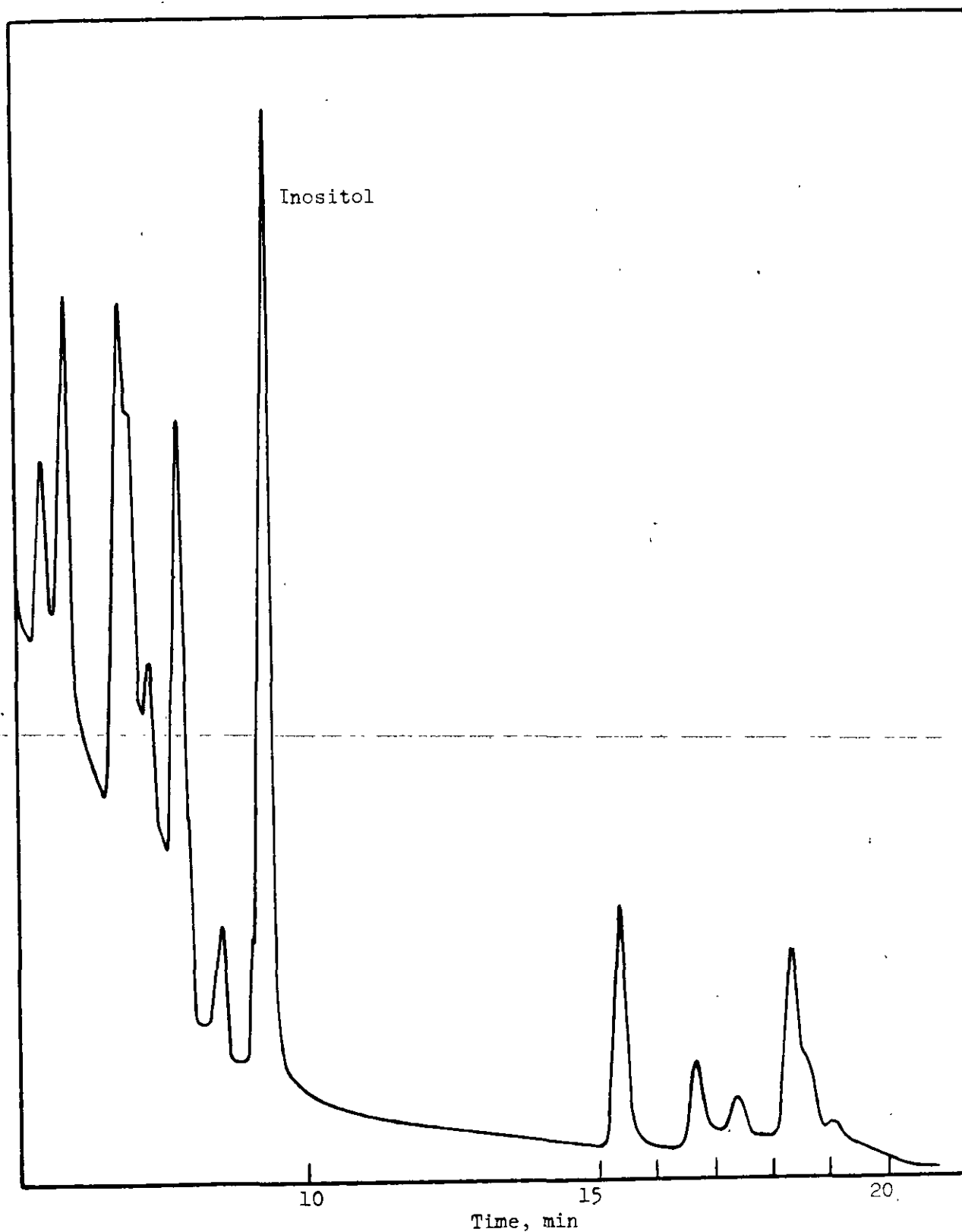
Figure 7. Typical Gas Chromatogram of Trimethylsilyl Derivatives of Disac-
charide Acids (15 to 20 Min Retention Time) and of the Internal
Standard Inositol (Retention Time 9.5 Min).

more in the future. At present we have been determining only the total area of
these peaks, relative to the internal standard inositol (retention time 9.8 min),
and attempting to get the maximum yield of disaccharide acids as such.

Cellobionic acid was prepared by oxidation of cellobiose with iodine
and potassium hydroxide in methanol (12). The potassium salt precipitates from
the alcohol solution and is purified by further precipitation.

The C-11 acid, glucosyl-arabinonic acid, was prepared by degradation
of cellobiose oxime to the C-11 sugar (13) via the nitrile acetate, and then
oxidation as above to the C-11 acid. We have not prepared the glucosyl-mannonic
acid, due to a shortage of the parent sugar, nor the C-10 acid, glucosyl-
erythronic acid.

FUTURE WORK

In the present report the several parameters involved in the alkali-oxygen reaction have been explored rather widely but not in too much detail. It was desired to put the several important variables in perspective before getting too involved in any one aspect. The technique involved, both for the flow reactor and for handling dissolved oxygen, is new to the field of pulping technology and it has been briefly applied over a rather large area.

The following aspects should be studied rather thoroughly:

(a) Variations in the heat-up period, starting with a 30-sec period.

(b) Effect of dissolved oxygen concentration on the rate of oxidation. Does a higher concentration cause a faster reaction and, thus, better competition with peeling? Does the use of higher concentrations of alkali or buffers depress the dissolved oxygen concentration sufficiently to slow down the rate of oxidation?

(c) A study of the distribution of the several disaccharide acids, ranging from C-9 to C-12 and the conditions that affect this distribution. The most efficient use of oxygen would be to form only the C-12 acid, instead of smaller fragments which consume more oxygen.

(d) Improvement in the method of analysis for the neutral carbohydrates remaining, and their separation from the acidic products.

(e) Investigation of the early phases of the oxidations, with special attention to a possible induction period, and formation of peroxides.

(f) Comparison of the end-group oxidation with the oxidative breaking of a glucosidic bond, and effect of various ions as inhibitors and promoters.
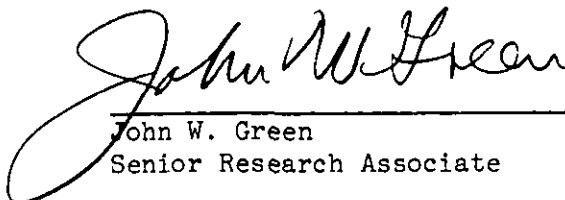
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