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The Influence of Cellulose, Lignins, and Organic Compounds on Biochemical Oxygen Demand

Dale Phenicie
Western Michigan University

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Biochemical Oxygen Demand (B.O.D. as it is commonly called) is the oxygen, in parts per million, required during stabilization of the decomposable organic matter by aerobic bacterial action.

This thesis represents work done to find out what, if any, B.O.D. is exerted by cellulose, lignins, and other organic materials found in paper and pulp mill effluents.

I became interested in this topic during the past summer while working for a local mill which produces pulp from the neutral sulfite semi-chemical pulping process. It came to mind that cellulose, and particularly the lignins, hemi-celluloses and pentosans, might exert a portion of the high B.O.D. recorded in the mill effluent. Observations throughout the summer indicated that the B.O.D. had a tendency to rise as the suspended solids of the effluent increased. No direct relationship between suspended solids and B.O.D. could be found however.

Theoretically, these materials should exert a B.O.D.. Let us first take a look at some of the nutritional requirements of microorganisms. The majority of all bacteria obtain their supply of energy from their food. The food which is consumed is used for two purposes: one, as a source of energy; and two,

as the actual material or building blocks which make up the cell. We will be concerned with the food only as a source of energy for the microb. The food of the microb is made up of several inorganic elements which are necessary to sustain life. These elements include (listed in order of decreasing amounts which are required): carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfar, with much smaller amounts of iron, calcium, magnesium, potassium, sodium, and chloride. Trace elements of such things as manganese, zinc, copper, boron, molybdenum, iodine, and strontium are also required.

The carbon requirement and the energy requirements of the cell is all that this paper will be concerned with.

The majority of all bacteria is a type of bacteria known as the heterotrophic bacteria. These organisms must obtain their supply of carbon and energy from the breakdown of organic materials. What better supply of organic materials could bacteria find in a pulp and paper mill than the cellulose, lignins, and other organic compounds which are the direct resultant of the pulp and paper making process. However, most people are of the opinion that cellulose by itself does not exert B.O.D. The problem seems to lie in the fact that in order for the bacteria to obtain their supply of carbon from cellulose they must first depolymerize the cellulose molecule. This belief is contrary to the fact that a certain species of bacteria will attack and break down the cellulose molecule. In fact it will even go back one step further and

break down the cellulose complex found in the cellulose fiber. The decomposition of cellulose by this micorb goes stepwise in the following manner.

Cellulose complex → Cellulose molecule → Linear glucose compound → Cellobiose → Glucose → CO_2, Organic acids, CH_4, H_2

If this cellulose decomposition process can and does take place, it would seem that the hemicelluloses, which have a much lower D.P. (degree of polymerization) than does the alpha cellulose, would be even more readily decomposed. If all this were true, cellulose would certainly exhibit a substantial B.O.D.

Lignin is a complex organic substance which certainly should supply a good deal of carbon to the microbs. Like cellulose, the molecule would have to be broken up or depolymerized before it could yield carbon to the bacteria. This fact might suggest that the lignin would not exert B.O.D. because it would not be readily broken down. However, there is also a bacteria species which devotes its carbon seeking time to the decomposition of lignins. In view of this fact, it would be expected that lignins would exert B.O.D.

The other organic materials such as the pentosans and gamma celluloses of very low D.P. would not be present in a highly purified pulp. However, a pulp sample such as unbleached semi-chemical, unbleached kraft, or groundwood would contain a small amount of these materials. These materials are very close in structure to simple sugars. These materials would show a high B.O.D. because the simple sugars are a very accessible

source of energy for microbs. However, the relative amounts of these materials in a pulp mill effluent would be small and might not affect B.O.D. of the system.

A survey of literature was made to determine what work had been done on this subject and what results were recorded. Upon completion of the literature survey, it was found that very little, if any, work has been done in the area of the B.O.D. exerted by pulp fractions. However, evidence which could be made to apply to the problem was found. This material seemed contradictory as there were facts to support both sides of the issue of whether or not these materials had any influence on B.O.D. An article in Sewage and Industrial Wastes 29, No. 11:1267072 titled "Biochemical Oxygen Demand of Organic Chemicals", the author established the following things:

> "The biological oxidation of organic chemicals does not proceed always with ease and certainty. An inflexible analytical procedure, such as that for a 5-day B.O.D., may not express the true nature of biological oxidation of a given waste."

From TAPPI 32, No. 12:559-62 comes the following statement:

> "Formaldehyde will show B.O.D. while oxalic acid which exerts oxygen demand on the stream shows no B.O.D."

Harry Gehm in an article in the Sewage Works Journal 19, No. 5:865-71 titled "Problems in the Application of Biochemical Oxygen Demand Test to Pulp and Paper Waste" says this:

> "One of the major questions regarding Pulp and Paper wastes which is as yet unsolved is the rate of oxidation of the fiber. Experimental work

> at Rutgers University dealing with the B.O.D. rates of paperboard mill waste before and after fiber removal yielded variable results, the most puzzling being the variation between the various types of pulp since the oxygen demand in no way follows the cellulose content."

The latest edition (11th) of Standard Methods for the Examination of Water and Sewage, has a standard solution which is a check on B.O.D. procedure. This solution is made up of glucose of a certain concentration.

In another article by Dr. Gehm, Technical Association Papers, 30:64-65, he says:

> "The presence in others of chemical oxygen absorbers such as bisulphites and of wood sugars capable under some conditions as pointed out by Martin of promoting the decomposition of even resistant lignins further complicates the picture."

The articles listed above are just a few of the many surveyed for this thesis. This is the only information that could be found dealing with this subject.

All of the literature which was found to deal with this subject is quite old. Also no statement of any quantity of B.O.D. was made.

Before starting the investigatory work on this project, a method of measuring B.O.D. had to be found. It was decided to use the standard Winkler Method for determining B.O.D. This procedure consists of three samples to be tested which are diluted to a standard volume of 300 milliliters in a special B.O.D. bottle. The water which is used for dilution is a specially prepared dilution water containing an inoculum of bacteria. The dilution water is prepared by saturating

deionized water with air to a dissolved oxygen content greater than 8.0. To each liter of dilution water is added the following nutrient solutions:

0.5 ml. $FeCl_3$
2.5 ml. $CaCl_2$
2.5 ml. $MgSO_4$
1.25 ml. Phosphate buffer solution

These nutrients make up the necessary inorganic elements mentioned earlier which constitutes the microbs' food supply. The inoculum of microbs is introduced into the dilution water containing the nutrients. I used influent to the local sewage disposal plant. One milliliter of sewage per one liter of dilution water proved to be sufficient.

Along with the three samples, three blanks are also set up. The blanks consist of only dilution water to which no sample has been added. Both samples and blanks are incubated for five days at 20 degrees C. Upon completion of the incubation period, the samples and blanks are analyzed for dissolved oxygen content.

The analysis for oxygen content is made by reacting the samples and blanks with 2.0 ml. of the following three reagents:

Manganous Sulfate solution
Alkaline Potassium Iodide
Concentrated Sulfuric Acid

After the addition of the sulfuric acid and the precipitate has dissolved, 200 ml. of the 300 ml. are titrated with standard .0250 normal sodium thiosulfate solution, using a starch indicator. The result of the titration is equal to the dissolved oxygen content in parts per million. The B.O.D. is obtained by subtracting the dissolved oxygen (D.O.) of the sample from the

D.O. of the blank.

As samples the following pulps were used:

Cotton
Bleached kraft
Bleached sulfite
Unbleached kraft
Unbleached sulfite
Neutral sulfite semi-chemical
Groundwood

To prepare the samples for B.O.D. trials, the pulp was first ground up in a Mead disk type refiner. The purpose of this was to destroy the fiber and eliminate the necessary mechanical decomposition of the fiber which must take place before the bacteria can attack the cellulose complex. This action on the fiber was used as an attempt to get more reproducable results on B.O.D. tests.

The dilution of the sample had to be determined by a series of trials. The series of dilutions found to give the most consistant results was 1, 3, 5, 7, 10, 15, and 16 per cent with most tests run in the 5 per cent range. The term dilution means the per cent sample added to the entire volume of the B.O.D. bottle. For a 5 per cent dilution, 5 per cent of 300 ml. or 15 ml. of pulp sample would be added to the bottle. The bottle is then filled with 285 ml. of dilution water.

The data obtained from the five day B.O.D. test on the pulp samples is shown in Table 1.

The data of this table is taken from the average of fifteen tests on all pulp samples. The pulps are listed in the order of increasing lignin content. This table shows a tendency toward an increase in B.O.D. as the amount of lignin present in the pulp sample is increased. To arrive at a more specific indication

of the effect of lignins on B.O.D., let us first consider the amount of lignin which was present in each pulp sample. Table 2 shows the amount of lignin of each pulp sample tested.

TABLE 1

THE B.O.D. OF PULP SAMPLES

Pulp	Observed B.O.D.
Cotton	8.37 p.p.m.
Bleached sulfite	11.10 p.p.m.
Bleached kraft	10.06 p.p.m.
Unbleached sulfite	14.55 p.p.m.
Unbleached kraft	21.3 p.p.m.
Groundwood	26.5 p.p.m.
Neutral sulfite semi-chemical	52.7 p.p.m.

TABLE 2

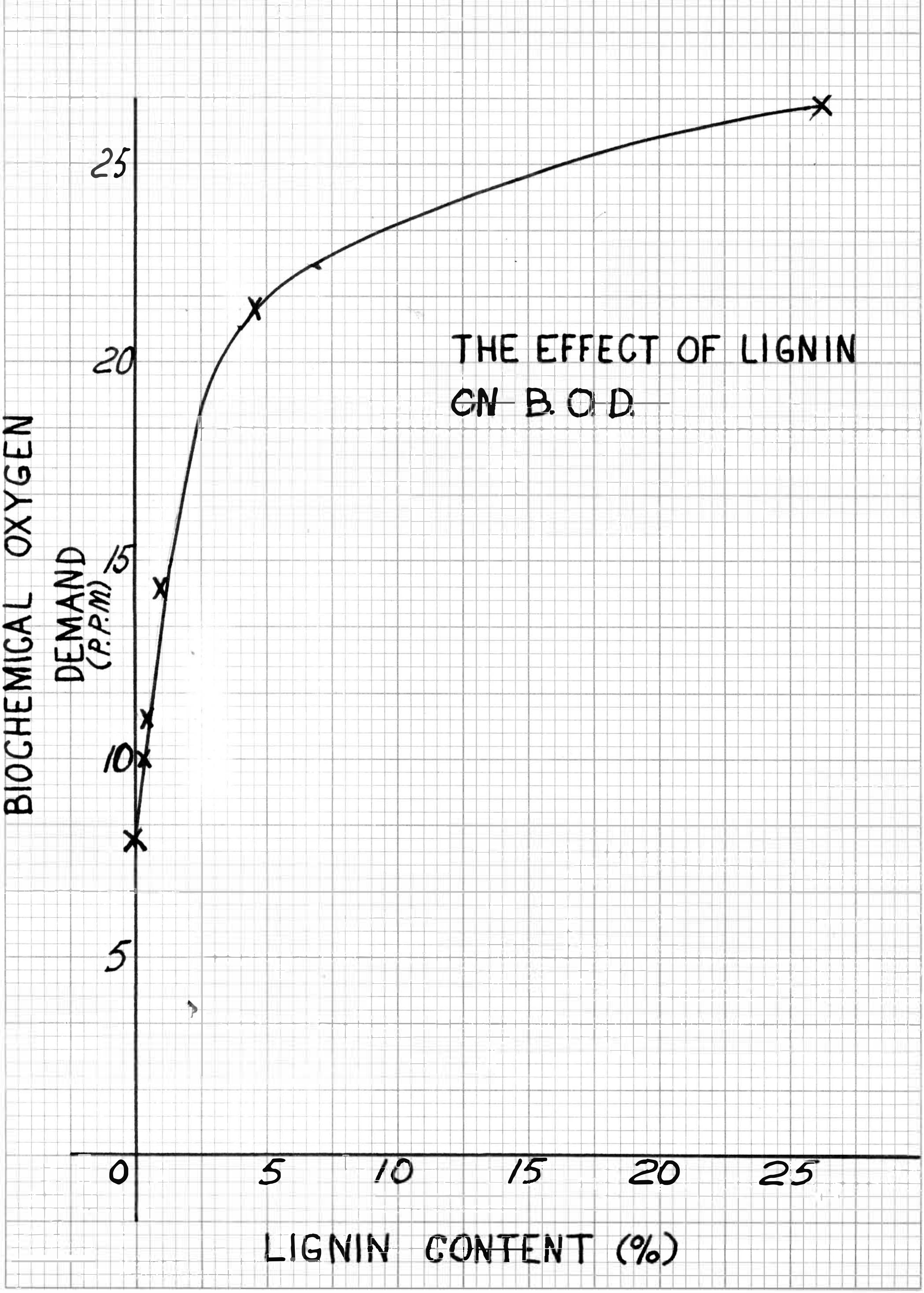
LIGNIN CONTENT OF PULP SAMPLES

Pulp Sample	Lignin Content
Cotton	0 %
Bleached sulfite	.47%
Bleached kraft	.58%
Unbleached sulfite	.8 %
Unbleached kraft	4.6 %
Groundwood	26.3 %
Neutral sulfite semi-chemical	1.93%

The values in Table 2 are the values which most often appear in literature.

Using the data from Tables 1 and 2, a plot of lignin content versus B.O.D. can be made. Figure 1 shows just such a plot. We can see from this graph that with the exception of the neutral sulfite semi-chemical as the amount of lignin of the pulp increases the B.O.D. also tends to rise. In fact, it appears as if there is a sharp increase in B.O.D. with increase of lignins.

FIG. 1


THE EFFECT OF LIGNIN
ON B.O.D.
BIOCHEMICAL OXYGEN DEMAND (P.P.M.)
25
20
15
10
5
0
5
10
15
20
25
LIGNIN CONTENT (%)

The neutral sulfite semi-chemical does not comform with the other pulps, as it shows the highest B.O.D. There may be two reasons for this. (1) The pulp sample may have been partially spoiled before the test meaning that bacteria would have already started to decompose it, or (2) the chemical action performed on the pulp may have made the lignin more accessable to the bacteria. I suspect that the first reason is more nearly the case.

What about the B.O.D. exerted by the pentosans of the pulp? Table 3 shows the pentosans content of the pulps which were tested.

TABLE 3

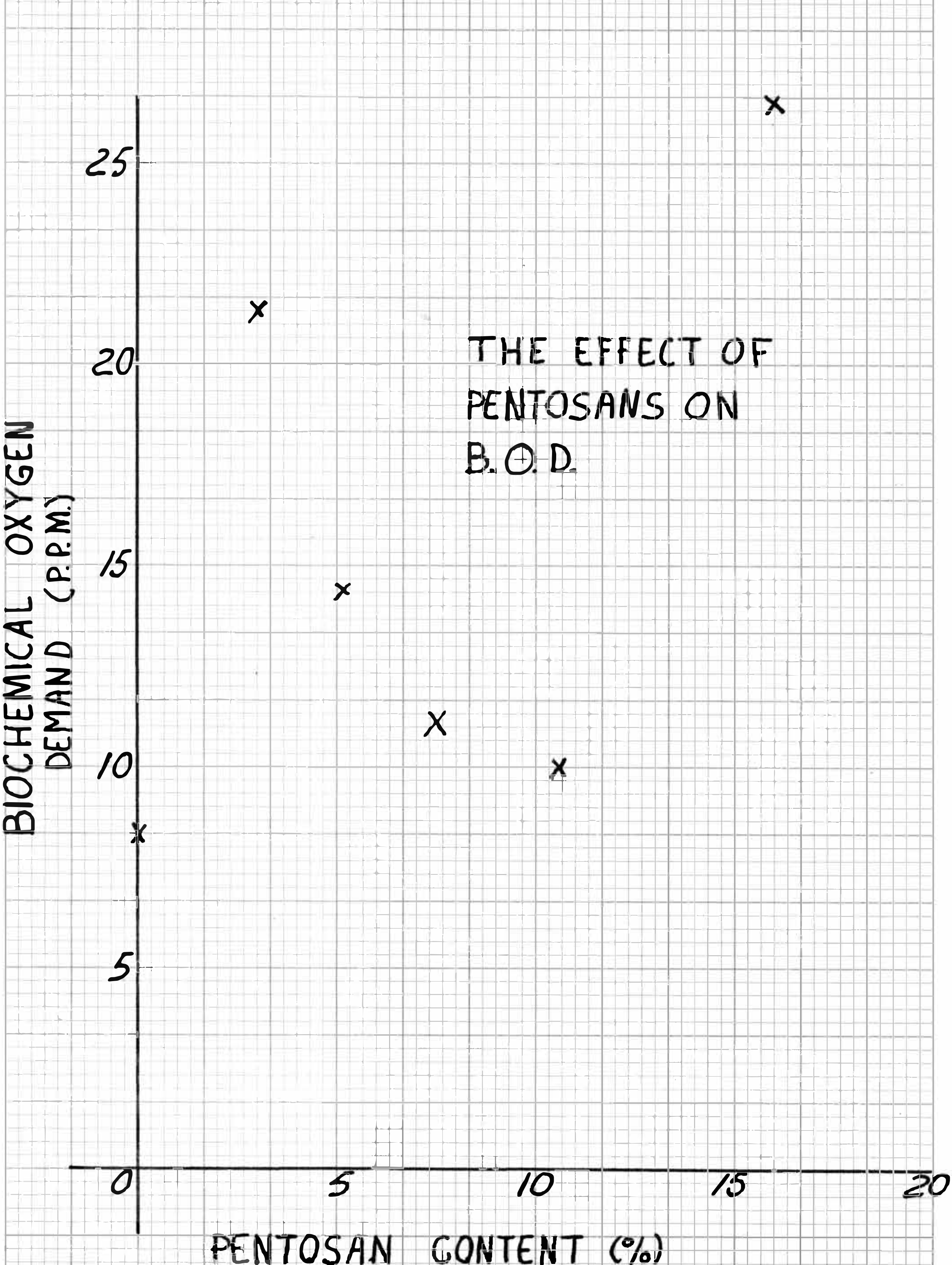
THE PENTOSAN CONTENT OF PULP SAMPLES

Pulp	Pentosans (%)
Cotton	Less than 0.9%
Bleached sulfite	8.5
Bleached kraft	10.6
Unbleached sulfite	5.1
Unbleached kraft	3.0
Groundwood	16.0
Neutral sulfite semi-chemical	19.3

These values we obtained from the same source as was the lignin composition of the pulps. A plot of the data from Tables 1 and 3 is shown in Figure 2. As can be seen there is no easily discernable curve as there is in the case of the lignin versus B.O.D. curve in Figure 1. For this reason not too much of a conclusion can be drawn about the B.O.D. of pentosans.

As far as the B.O.D. of cellulose goes, this work has shown that cellulose does exert some B.O.D. on a five day basis. However, to be able to find out the total B.O.D. exerted by

FIG. 2


THE EFFECT OF PENTOSANS ON B.O.D.
BIOCHEMICAL OXYGEN DEMAND (P.P.M.)
PENTOSAN CONTENT (%)
0
5
10
15
20
25

cellulose alone, extended time B.O.D.'s of more than five days would have to be run.

SUMMARY:

Through this work the following things have been investigated and established.

(1) The B.O.D. of a pulp sample which has been made to simulate the conditions of paper mill white water tends to show an increase as the lignin content of the pulp is increased.

(2) The total B.O.D. of cellulose cannot be effectively measured by the standard five day B.O.D. procedure.

(3) The effect of the pentosans on B.O.D. cannot be effectively measured by this procedure.

BIBLIOGRAPHY

Association of British Chemical Manufacturers and Society for Analytical Chemistry, "Determination of Oxygen Demand", Analyst 82, No. 979;683-708 (Oct., 1957)

Berger, Herbert F., "B.O.D. From Oxygen Consumed Determination", TAPPI 38, No. 9:534-538

Borden, George C., Jr., and Woodcock, Irene, "Reseeding B.O.D. Bottles; A Means of Determining Toxic Interference in the B.O.D. Text", TAPPI 32, No. 11:506-14

Busch, Arthur W., and Sawyer, Clair N., "Determination of the B.O.D. of Sewage and Industrial Wastes with the Polarograph", Analytical Chemistry 24, No. 12:1887-91

Chakravarti & Som, Anal. Chem. 24, No. 2:295-200

Gehm, Harry W., "Problems in the Application of the Biochemical Oxygen Demand Test to Pulp and Paper Wastes", Sewage Works J. 19, No. 5:865-71

Hood, Norman R., "Paper and Board Research Progress" World's Paper Trade Rev. 145, No. 3:178-180, 187-88; No. 4:256,261-2

Kittrell, F. W., "Evaluation of the Biochemical Oxygen Demand in Streams", TAPPI 32, No. 12:540-2

Lardier, Nicholas J., "The Aerobic and Benthal Oxygen Demands of Cellulosic Materials", Proc 11th Ind. Waste Conf., Purdue Univ. Eng. Bull., Extension Serv. No. 91:1-13

Love, S.K. and Thatcher, L.L., "Water Analysis", Anal. Chem. 22 No. 2:264-8
23 No. 2:253-7
24 No. 2:295-300
25 No. 1:65-74
27 No. 4:680-90

Meinck, Fritz, "Investigations of the B.O.D. of Spett Sulfite Liquors", Das Papier 9. No. 13/14:327-32

Oberton, Arthur C.E., and Stack, Vernon T., Jr., "Biochemical Oxygen Demand of Organic Chemicals", Sewage and Ind. Wastes 29, No. 11:1277-72

Paessler, A. H., and Hedgepeth, L. L., "Stream Polution from Pulp Mills in Virginia; Applicability of the B.O.D., TAPPI 32, No. 12:559-62

Rogers, D. Paul, "Technique of the Biochemical Oxygen Demand Determination", TAPPI 32, 11:481-6

"Water and Sewage Works" 97, No. 122 (1950)

Wesley, W. Jr. and Moore, Theodore L., "Biooxidation", Chem. End. 62 No. 9:189-202

Weston, Roy F. "Problems in Application of the Biochemical Oxygen Demand Test in Pulp and Paper Wastes", Sewage Works J. 19, No. 5:865-71