BIOCHEMICAL OXYGEN DEMAND AND DEGRADATION OF LIGNIN IN NATURAL WATERS

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The purpose of this preliminary study was to determine the biochemical oxygen demand of lignin in natural waters and the extent of its degradation under these conditions, and to explore the interrelationships of BOD and degradation.

The project was initiated because of the need to determine the extent to which lignin, a chief constituent in pulp and paper mill wastes, is a contributor to the long-term BOD exerted on the stream. Lignin presents a water pollution problem and the many attempts to solve the problem have been only partially successful.

Although various methods of measurement of this type of waste have been used, including short- and long-term BOD's, none has been found that continued observations over the extended time used in this study.

The plan followed for this work was to obtain a river sample which contained wastes discharged from a paper mill and to determine the oxygen demand at regular intervals for a period of 100 days. Concurrently, a portion of the river sample was set up under the same conditions, and lignin determinations were made at the same time intervals as the BOD analyses.

Previous Work

Disposal of liquid wastes has long been a problem in the pulp and paper industry. The Senate Select Committee on National Water Resources (1) reported that in 1954 the water usage for the pulp and paper mills of the United States was 1,607 bil gal (6.1 bil cu m). From Besselivre's data (2) it may be estimated that over two million tons of lignin products are included in the five million tons of solids discharged annually to the nation's waterways; this amount constitutes a heavy pollutional burden on the streams.

Woodard and Etzel (3) stated that in the production of paper the primary objective of the pulping process is to separate cellulose from other constituents of the wood. The cellulose is used to make paper while the other constituents, along with the spent pulping chemical, are discharged as waste. The main constituents of the waste are wood sugars and lignin, the lignin representing about 30 percent of the original weight of the raw material.

Lignin discharged to a stream has a high coloring effect and introduces a large organic load. The waste is objectionable because it reduces the value of the stream for domestic and industrial use and contributes to the BOD exerted on the stream.

Nemerow (4) states that spent waste sulfite liquor from digesters contains sulfur compounds which possess an immediate oxygen demand that accounts for about 11 percent of the 5-day BOD. The sugars (hexoses and pentoses) represent about 65 percent of the BOD. Although lignins account for over half the solids in this waste, they contribute little to the BOD, according to Nemerow.

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Lawrance, in a study for the National Council for Stream Improvement (5), found that the discharge of paper-mill wastes into a stream places a rapid oxygen demand on the water, chiefly because of the carbohydrate content of the waste. Data compiled by the Council from different sources show very definitely that the lignin has only a small biochemical oxygen demand. Whether this demand is a result of the conversion of lignin into organic compounds available to microorganisms as nutrients or is due to other organic substances which were not removed in the purification process has yet to be determined.

Considerable speculation concerning the decomposition of lignins in surface waters is contained in the literature. Lawrance (5) concluded that calcium lignosulfonate contributes little, if at all, to the oxygen demand of spent pulp liquor. He found no evidence to indicate that calcium lignosulfonate in the presence of pure carbohydrate is converted into compounds having a high BOD.

The hypothesis of Woodard *et al.* (6) proposed to explain the mechanisms involved in the biological oxidation of lignin as: (*a*) lignin is removed from solution by an immediate adsorption onto the bacterial cells, and (*b*) the adsorbed fraction is utilized by the bacteria at a slow, constant rate.

In general, Zobell and Stadler (7) found that the literature indicates the following on the decomposition of lignin: (*a*) the species of microorganisms that utilize lignin are relatively limited; (*b*) biological decomposition is very slow and incomplete; (*c*) the susceptibility of lignin to biological attack varies with its source, age, treatment, chemical nature, etc.; and (*d*) higher organisms and fungi are more capable of utilizing lignin than are bacteria and simple microorganisms.

The extent of biological degradation of lignin has been measured by Woodard *et al.* (6) by the oxygen uptake and reduction in lignin concentration.

Lawrance and Sakamoto (8) found the microbial oxidation of lignin alone and in the presence of certain pure carbohydrates to be very small. The 5-day BOD's of the sugars, with the exception of xylose, were usually unaffected by the presence of lignin. The bacterial oxidation of lignin is unaffected by the cellulose degradation products, but the BOD of butyric and lactic acids and their calcium salts was lower when lignin was present. The BOD may be due to impurities in the sample or to the oxidation of a portion of the lignin.

Pearl (9) noted that, when lignin is subjected to oxidation, even under mild conditions, complete disruption of the molecule takes place and simple degradation products are obtained.

Eldridge (10) found that the BOD curves for sulfate liquor and white water from paper mills show quite different rates, with an apparent transitional stage for the former in 5 days and a slow transition for the latter in 12 days.

Holderby and Moggio (11) found that the BOD of spent sulfite liquor is variable and is controlled largely by the sugar content. West (12) states that the *k* rate for rapidly oxidized wastes like sugars is greater than 0.20.

Apparently little is known about the decomposition of lignin in natural biological processes. Even less is known about the biological decomposition of industrial forms of lignin in streams receiving these wastes.

Laboratory Work

Experimental Procedure

A river sample was collected approximately 6 miles (9.6 km) below the waste discharge point of a paper mill using the Kraft pulping process. The sample was received in the laboratory two days after collection and a portion was diluted to five percent with formula "C" dilution water for BOD determinations. The standard BOD procedure (13) was modified as follows: (*a*) dissolved oxygen (DO) determinations were made by means of the dropping mercury electrode; (*b*) in place of the conventional BOD bottle, 125-ml reagent bottles with glass stoppers were used, since the polarograph uses small amounts of sample; (*c*) when the DO approached depletion a reaeration technique was used; and (*d*) duplicate DO determinations were made up to and including 100 days. Results were plotted as BOD in milligrams per liter vs. time in days.

Simultaneously with the BOD determinations, the river sample for lignin determinations was set up under exactly the same conditions (5-percent dilution, 125-ml glass stoppered bottles stored under water in a 20°C incubator). When a DO determination for the BOD was made, a simultaneous lignin determination was made using the "Tannin and Lignin" method described in "Standard Methods" (13). Lignin standards were prepared from superior-grade lignosulfonic acids—sodium salts. Standards were run as a part of the routine lignin determination. The results were plotted as lignin concentration in milligrams per liter vs. time in days.

The BOD reaction rates were calculated by the "daily difference method" suggested by Tsivoglou (14). The ultimate demands were calculated according to the formula $Y = L(1 - 10^{-kt})$.

Results

The fast component of the first stage of the BOD progressed at a fast rate ($k = 0.455$) and the ultimate demand was 33 mg/l. A slower decomposition rate ($k = 0.072$) was calculated for the slow component of the first stage, with an ultimate demand of 65 mg/l. A second stage beginning after about 23 days incubation had a reaction rate of $k = 0.080$ and an ultimate demand of 103 mg/l.

The experimentally observed demand for the 100-day BOD was 210

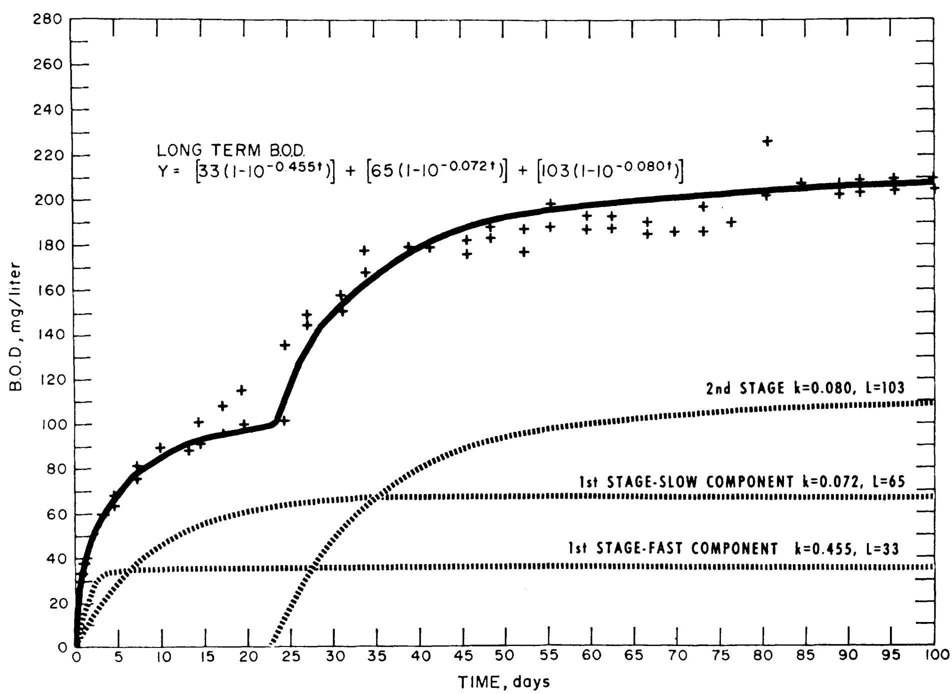

FIGURE 1.—Long-term BOD over 100-day period.

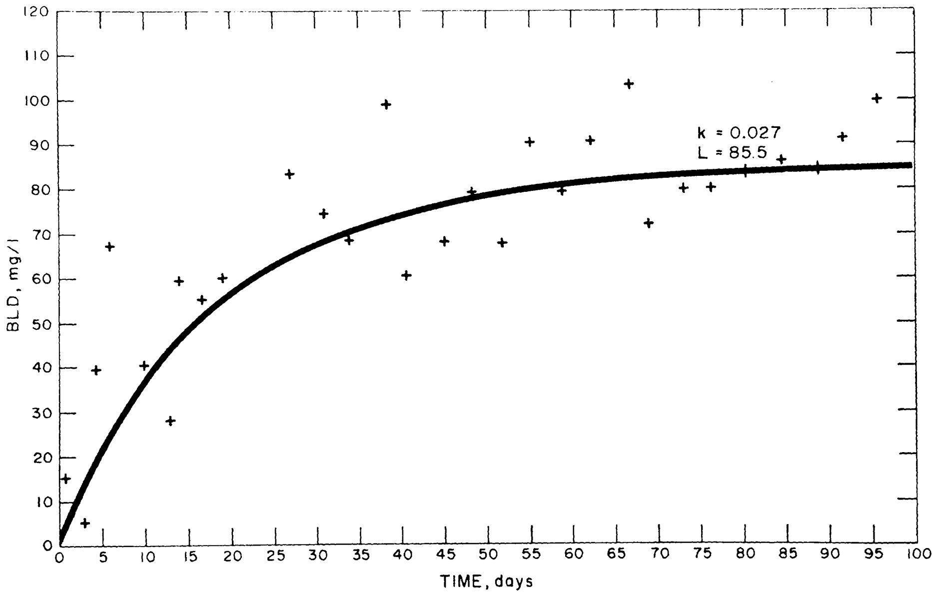

FIGURE 2.—Long-term BLD over 100-day period.

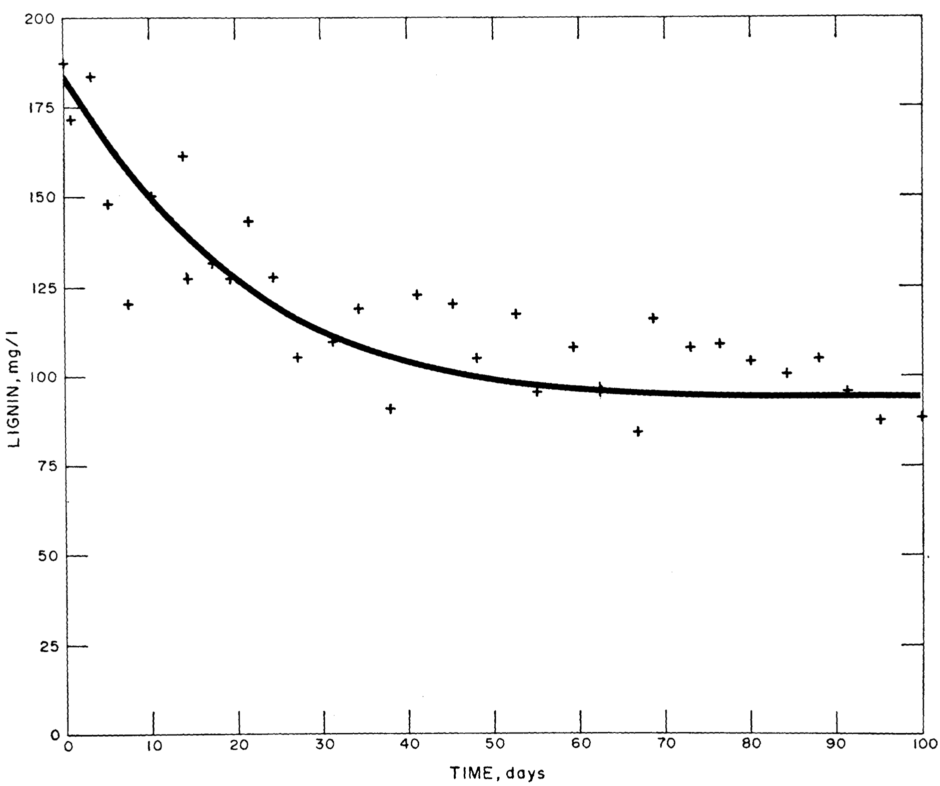

FIGURE 3.—Degradation of lignin over 100-day period.

mg/l compared to the calculated ultimate demand of 201 mg/l. The 100-day BOD is illustrated in Figure 1.

The lignin decomposition was calculated from the lignin data as a biochemical lignin demand (BLD), using the same method as for the BOD, so that a comparison between the k and L values of the BOD and BLD could be determined.

The overall reaction rate for the lignin decomposition was calculated to be $k = 0.027$. The ultimate demand for the BLD was calculated to be 85.5 mg/l compared to the experimentally observed 100-day BLD of 84 mg/l. The 100-day BLD is illustrated in Figure 2. The lignin decomposition is shown in Figure 3.

The data show that 51.6 percent of the lignin compounds present originally were not oxidized after 100 days of incubation at 20°C.

Discussion

Previous investigators have suggested that the first stage of the BOD of paper mill wastes represents the oxidation of wood sugars and other readily decomposable organics. Nemerow (4) found that in the 5-day BOD of spent sulfite liquor from digesters, the sugars (hexoses and pentoses) represented about 65 percent of the BOD, corresponding to the fast component of the first stage ($k = 0.455$) in this study. This fast component (presumably sugars) represented about 47 percent of the 5-day BOD. In this study a sulfate waste from the Kraft pulping process was used, whereas Nemerow reported on spent sulfite waste liquor.

The curve (Figure 1) suggests that the 5-day BOD is related to the carbohydrate concentration of the waste while the long-term BOD is indicative of an oxidizable fraction of lignin compounds, as well as any other slowly decomposable organics present in the sample. In 50 days, 93 percent of the oxidizable fractions had been destroyed, while 88 percent of the total BOD had been satisfied.

The BOD curves for sulfate liquor and white water from paper mills described by Eldridge (10) showed an apparent transition stage at 5 and 12 days, respectively. In this study the transition stages occurred in about 7 and 23 days, respectively.

This study confirms the results of Kroner and Moore (15), which showed that 41 to 46 percent of the lignin remained after about 20 wk, whereas in this experiment about 51 percent of lignin remained after about 15 wk. The slight difference in results may be due to the difference in the type of lignin compounds or to difference in the incubation temperatures, since Kroner's samples were held at room temperature while 20°C was maintained in this study.

Additional Work

This study has shown that paper-mill waste decomposition has a multi-component BOD curve and that the lignin decomposes less than 50 percent in 100 days. Since the fast component of the first stage is related primarily to the concentration of the carbohydrates, a long-term BOD on pure lignin compounds should help to identify the portions of the curve due to lignin.

Further investigation might include: (*a*) determining long-term BOD of pure lignin compounds, (*b*) determining BOD and lignin degradation in a stream polluted with raw Kraft waste, (*c*) studying the effect of time of passage on BOD characteristics of a polluted stream, and (*d*) studying BOD and lignin degradation of waste after biological treatment.

Conclusions

1. This study shows that the BOD of a stream polluted with Kraft mill wastes apparently is related to the concentration of carbohydrates, the fraction of lignin compounds oxidizable in 100 days, and possibly to other slowly decomposable organics.

2. The fast component of the first stage of the BOD is indicative of the

oxidation of wood sugars and other readily decomposable materials. The reaction rate is very rapid ($k = 0.455$).

3. The slow component of the first stage, and the second stage of the BOD ($k = 0.072$ and 0.080, respectively) are suggestive of the decomposition of the lignin and its by-products and other decomposable organics.

4. Over a period of 100 days, less than 50 percent of the lignin compounds present were oxidizable under test conditions.

5. The lignin decomposed at an overall slow rate of $k = 0.027$.

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