Investigation of Methods for Alleviating the Pollutinal Effects of Douglas-Fir Ethanol Stillage

By

W. B. Bollen

OREGON FOREST PRODUCTS LABORATORY
State Board of Forestry and School of Forestry,
Oregon State College Cooperating
Corvallis
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A cooperative research project of the Oregon State Engineering Experiment Station, the Bacteriology Department, School of Science, Oregon State College, and the Oregon Forest Products Laboratory
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Investigation of Methods for Alleviating the Pollutional Effects of Douglas-Fir Ethanol Stillage

SUMMARY

FULL scale production of ethanol by the hydrolysis of Douglas-fir wood at the government-sponsored plant at Springfield, Oregon, is beset with the problem of disposing of approximately 500,000 gallons of stillage daily. Because of its high biochemical oxygen demand (B.O.D.), this stillage, if discharged into the near-by Willamette River, would seriously pollute the stream, removing dissolved oxygen, destroying fish and green plant life, and producing other undesirable effects. Use of stillage to produce fodder yeast and for recovery of xylose, if commercially feasible, would reduce but not eliminate the pollution problem. This paper presents the results of investigations of several proposed direct methods for reducing the B.O.D. of ethanol stillage.

Samples of ethanol stillage for the purposes of these investigations were obtained from the U. S. Forest Products Laboratory, the Vulcan Copper and Supply Company, and the ethanol plant at Springfield.

Treatment of stillage with chemicals, such as lime, alum, copperas, and ozone resulted in little or no reduction of the B.O.D. Aeration of untreated stillage and aeration of stillage treated with 0.1 per cent ammonium sulphate produced increases in the 10- and 20-day B.O.D. values. Aeration, following inoculation with certain microorganisms, caused a B.O.D. reduction as high as 60 per cent in stillage that had been treated with 0.1 per cent ammonium sulphate, but the pollutional value of the waste was still great.

Laboratory tests of stillage percolation through surface soil from the Springfield plant site indicated that nearly 90 per cent of the B.O.D. could be removed. Field percolation tests, made at the plant site, indicated that the top layers of dry soil were temporarily permeable and that percolation through saturated top soil was too slow
to be of practical significance. The subsoil was slightly pervious, but the underlying stratum of plastic yellow clay was essentially non-porous, producing lateral drainage that might contaminate remote wells and fields.

Laboratory models of barrel, wood-stave tank, and glass-tube gravel-filled bacterial trickling filters were employed in attempts to reduce the B.O.D. of ethanol stillage. Of the various disposal possibilities investigated, only the bacterial trickling filter shows promise. With supplemental aeration and liquor recirculation under favorable experimental conditions, approximately 50 per cent of the 5-day B.O.D. was removed with a stillage throughput equivalent to one gallon per cubic foot of gravel per day. With less rapid throughput, B.O.D. was reduced nearly 70 per cent; 90 per cent of the sugar and 70 per cent of the total carbon were removed. Dilution of stillage with water and filtration in series may be advantageous, but it was impossible to include such studies in the foregoing investigations.

The mold *Fusarium lini* Bolley as an agent of decomposition was studied. This mold is effective in consuming stillage sugar, but it has only a slight action on non-sugars. Roughly, it can reduce sugars 85 per cent, total carbon 35 per cent, and B.O.D. 20 per cent while acting in a trickling filter.

Bacteria isolated in these investigations were tabulated and characterized. Bacteria of the genera *Pseudomonas* and *Achromobacter* occurred most frequently.

**THE ETHANOL STILLAGE DISPOSAL PROBLEM**

Ethanol (ethyl alcohol) can be made from wood waste by hydrolyzing the wood carbohydrates to sugar and fermenting this to alcohol. A multiple-cycle dilute-acid-hydrolysis method, known as the Scholler Process, was developed in Germany about 1928. Because of the large war-time requirements for alcohol in the United States, the German process was intensively studied by the U. S. Forest Products Laboratory at Madison, Wisconsin; it was first duplicated and then improved (7). The process consists briefly of pumping continuously a cooking liquor containing 0.5 to 0.6 per cent sulphuric acid through a column of wood chips and sawdust for 2½ to 3 hours at 150° to 180° C., followed by neutralization of the hydrolyzate, filtration, and continuous fermentation of the neutralized sugar solution by yeast. One ton of wood waste yields 60 to 65 gallons of 95 per cent ethanol (8).

The government-sponsored plant at Springfield, Oregon, strategically located in the Douglas-fir lumber region of the Willamette

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* Numerals in parentheses indicate references listed at the end of this article.
Valley, was designed to produce 5 to 6 million gallons of alcohol per year from a daily consumption of about 600 tons of sawmill wood waste, equivalent to 260 tons of dry bark-free wood. This would yield each day, in addition to 16,500 gallons of 95 per cent ethanol, the following by-products and waste: 100 tons lignin residue, 33 tons gypsum, 3 tons furfural, 2.5 tons methanol, 11.8 tons unfermented sugars (chiefly xylose), 500,000 gallons stillage, and 3,000,000 gallons of condenser water.

The stillage (still bottoms) requires special consideration because of the disposal problem. It contains unfermented sugar, some furfural, miscellaneous organic acids, and lesser amounts of substances as yet unidentified. Although the concentration is low, the volume of stillage is enormous, and present economic factors require its disposal as waste. Dumping in the near-by Willamette River is now prohibited by the State Sanitary Authority. Because of the high biochemical oxygen demand (B.O.D.) of the liquor, the river would be grossly polluted, and the stream's dissolved oxygen reserve would be depleted for many miles below the point of stillage discharge. This would result in the appearance and odors of putrefaction, discoloration of the water, destruction of fish and green plant life, and many miscellaneous undesirable effects; furthermore, the river's ability to destroy the many inevitable minor contaminations and pollutions would be lessened.

The B.O.D. of the stillage liquor results from the presence of sugars and other substances subject to oxidation, chiefly by microbial action. Oxygen required for this transformation must come, directly or indirectly, from dissolved oxygen in the river. Since Springfield stillage (Table 1) may have, on the average, a B.O.D. of about 6,000* parts per million (p.p.m.) and the river may average 8 p.p.m. dissolved oxygen, the enormous pollutional potential becomes apparent. A half million gallons of average stillage would consume approximately 25,000† pounds of dissolved oxygen in the river, an amount that would completely deoxygenate more than 50,000,000 cubic feet of normally aerated river water. The mean annual flow‡ of the Willamette at Springfield is 4,860 cubic feet per second (c.f.s.); approximately 10 miles downstream, the McKenzie River adds 4,760 c.f.s. The average flow of each river in August is 850 c.f.s. and 1,710 c.f.s., respectively. While it is apparent that the stillage alone would not completely deoxygenate the Willamette, the combination with sewage from Springfield and Eugene would result in a critical

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* 5-day B.O.D.
† Equivalent to a sewered population of approximately 150,000.
‡ Data on flow of Willamette and McKenzie Rivers secured from War Department, Office of the District Engineer, Portland District, Portland, Oregon.
Table 1. **Analysis of Stillage**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Source</th>
<th>Specific gravity 20°/4°</th>
<th>pH</th>
<th>Reducing sugars (P.p.m.)</th>
<th>Kjeldahl nitrogen (P.p.m.)</th>
<th>Total carbon (P.p.m.)</th>
<th>Total solids (P.p.m.)</th>
<th>5-day B.O.D. (P.p.m.)</th>
<th>Plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vulcan, 1945</td>
<td>1.015</td>
<td>5.0</td>
<td>8,000</td>
<td>110</td>
<td>14,800</td>
<td>34,300</td>
<td>16,400</td>
<td></td>
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<tr>
<td>2</td>
<td>Madison, 1945</td>
<td>1.014</td>
<td>5.6</td>
<td>8,300</td>
<td>50</td>
<td>16,000</td>
<td>23,750</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Madison, 1946</td>
<td>1.013</td>
<td>5.0</td>
<td>4,300</td>
<td>370</td>
<td>11,880</td>
<td>27,800</td>
<td>15,300</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Springfield, 5/14/47, beer still No. 1</td>
<td>1.058</td>
<td>4.7</td>
<td>4,700</td>
<td>50</td>
<td>4,500</td>
<td>11,750</td>
<td>4,200</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Springfield, 5/14/47, beer still No. 2</td>
<td>1.070</td>
<td>4.5</td>
<td>3,200</td>
<td>40</td>
<td>5,680</td>
<td>14,080</td>
<td>5,600</td>
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<td>6</td>
<td>Springfield, 6/16/47 composite</td>
<td>1.097</td>
<td>4.8</td>
<td>4,200</td>
<td>70</td>
<td>7,390</td>
<td>18,160</td>
<td>6,800</td>
<td></td>
</tr>
</tbody>
</table>

* Parts per million.
† Yeasts predominating.
condition, particularly down to the mouth of the McKenzie. The condition would be alleviated during seasons of high water and low temperature; with low water and warm weather it would be aggravated, and the stream would become dead for many miles. Successful operation of the Springfield plant is thus dependent upon some process to remove most of the B.O.D. from stillage prior to stream disposal. If the process could recover or produce by-products of value, it would reduce the net cost of ethanol, and it might be a deciding factor in the economics of operation.

It has been shown that yeast can be grown on the stillage to produce about 200 pounds of dry fodder-yeast per ton of dry wood processed for ethanol (11), but this removes only 40 per cent of the stillage B.O.D.; moreover, the cost of a fodder-yeast plant at present is prohibitive. A major pollution problem for the yeast slopes would remain. Recovery of the xylose may eventually be feasible; this would lower the stillage B.O.D. by about 25 per cent. It is apparent, therefore, that some direct treatment aimed primarily at B.O.D. reduction is essential. The following investigation was conducted with this objective in mind.

**STILLAGE SAMPLES**

Previous to preliminary operation of the Springfield plant late in the spring of 1947, stillage samples in barrel lots were obtained from pilot plants as follows:

- **Sample No. 1.** Vulcan Copper and Supply Co., Cincinnati, Ohio, received in 1945.
- **Sample No. 2.** Forest Products Laboratory, Madison, Wisconsin, received in 1945.
- **Sample No. 3.** Forest Products Laboratory, Madison, Wisconsin, received as a concentrate in August 1946 for analysis and use. It was diluted to 6.9 volumes to obtain a concentration equivalent to that of the original stillage.

Three samples were obtained from the Springfield plant, as follows:

- **Sample No. 4.** One gallon from beer still No. 1, on May 14, 1947.
- **Sample No. 5.** One gallon from beer still No. 2, on May 14, 1947.
- **Sample No. 6.** Ten gallons of composite, on June 16, 1947.

Analytical data are presented in Table 1. The relatively low B.O.D. values of the Springfield samples are noteworthy. It should be recognized, however, that samples from trial runs are not necessarily representative of what may be expected from the process cycle of typical operation yet to be established.
ANALYTICAL METHODS

Specific gravity was determined by the Westphal balance. The glass electrode was used for pH determinations. Reducing sugars were determined by a modification of the Shaffer-Somogyi method (10); a 30-minute boiling period was allowed to insure reduction of pentoses. Gunning's Kjeldahl procedure (2) with Hibbard's mixture and Hengar granules was used for total nitrogen determination; the ammonia was distilled into boric acid solution and titrated directly with N/14 H₂SO₄. Total carbon was determined by combustion with oxygen at 950° C.; the evolved CO₂ was absorbed in Ascarite towers and weighed. Samples were measured into porcelain boats, dried at 80° C., and covered with 10-30 mesh Alfrax before introduction into the combustion tube. Total solids were determined by evaporating 100 ml. samples to constant weight at 105° C.

Standard procedure was followed for B.O.D. (1). Since the low nitrogen content of stillage liquor would not meet microbial requirements and would thus retard decomposition, the ammonium supplemented phosphate-bicarbonate buffered dilution water of Lea and Nichols (12) was used.

Bacteria were plated on nutrient agar. Peptone-glucose agar adjusted to a pH of 4.0 was used for molds. Adequate dilutions were made to insure plates with a reasonable number of colonies.

EXPERIMENTAL WORK

Methods of reducing ethanol stillage B.O.D. included treatment with chemicals, aeration of untreated and treated stillage, laboratory and field ponding, filtration through lignin columns, and passage through laboratory scale bacterial trickling filters of several types. *Fusarium lini* Bolley, as an agent of decomposition, was studied. Bacteria isolated in the foregoing investigations were tabulated and characterized.

Treatment with chemicals

Treatment of stillage with various chemicals, including lime, alum, copperas, and ozone resulted in little or no reduction in B.O.D. These experiments indicated that ordinary chemical treatments were impracticable.

Aeration studies

Aeration of stillage from sample 1, plus 0.1 per cent (NH₄)₂SO₄, for 48 hours had no influence on the 5-day B.O.D. The 10-day B.O.D. of this was 9 per cent greater than the 10-day B.O.D. of un-aerated stillage; similarly, the 20-day B.O.D. showed a 34 per cent increase. Similar increases had been obtained previously by aeration
of untreated liquor. The reason for this is not clear, but it might be the result of partial oxidation of substances not oxidizable in the B.O.D. procedure, the partial oxidation products then being susceptible to slow oxidation by dissolved oxygen under quiescent conditions. Some support for this was found when comparing the 20-day B.O.D. value of the liquor with its total carbon. Since the total carbon content is approximately 15,000 p.p.m., complete oxidation according to the reaction C + 2(1/2O2) = CO2 would require 40,000 p.p.m. oxygen.

\[
15,000 : x = 12 : 32
\]

\[
x = 40,000 \text{ p.p.m. O}_2 \text{ required}
\]

Oxygen necessary for complete oxidation of carbonaceous matter in stillage or other wastes is determined by the nature of the organic compounds present. Since these compounds contain oxygen and hydrogen in varying amounts, the actual quantity of additional oxygen required for ultimate decomposition may be greater or less than the theoretical amount based on carbon alone. In the case of sugars and other carbohydrates, where hydrogen and oxygen occur in the same proportions as in water, the oxygen demand for complete oxidation is directly proportional to the amount of total carbon. The same is true of furfural and some organic acids; in other organic acids the hydrogen-oxygen ratio may depart from 2:1 either way. It is, thus, a fair assumption that the 20-day B.O.D. value of 22,000 indicated that slightly more than one-half of the carbonaceous material in the stillage was oxidized in 20 days. This is in contrast to ordinary sewage, which is 99 per cent oxidized in 20 days. Although Springfield stillage was much lower in B.O.D. than the pilot plant samples, the implications of down-river pollutional effects are obvious.

Following inoculation with various microorganisms, aeration of stillage containing 0.1 per cent (NH4)2SO4 gave the results shown in Table 2. The data for Oidium lactis* are of interest because this organism offers a potential source of fodder protein, and it grows readily in acid media; hence, the pH of waste liquor needs no adjustment. The bacteria, on the other hand, grew well only when the reaction was adjusted to near neutrality. The Pseudomonas was most effective in lowering B.O.D., but even the approximate 60 per cent reduction left a considerable pollutional load in the waste.

**Laboratory ponding**

Ponding was proposed as a possible method of disposal, particularly as a temporary expedient to permit trial runs of the plant. A sample of Wapato clay loam soil from the plant site was brought to

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* Revised nomenclature designates this yeast-like fungus by the earlier term Geotrichum candidum.
the laboratory and lightly packed in a tub to form a shallow pond with a diameter of 12 inches, with about 0.5 gallon capacity, and with an average depth of 6 inches to the collecting drain. The pond was filled with sample No. 2. Although the rate of percolation was only 0.01/gal./ft.²/day, the percolate became almost colorless, and nearly 90 per cent of the B.O.D. was removed.

Ponding without percolation, carried on by allowing a gallon of stillage to stand on a 6-inch layer of Wapato surface soil in a glass jar, was of no apparent value. Although the surface of the liquor became moldy, the B.O.D. was unchanged after seven days.

In view of these results, it seemed desirable to carry on percolation studies on a larger scale and with better control. Six pieces of 6-inch steel pipe, each 4 feet long, were driven into the soil of the proposed pond site in pairs near cluster well locations. One of each pair was removed with the included soil column and brought to the laboratory. Apparatus was devised to maintain a 6-inch layer of stillage in the head-room above one of the soil columns. A small volume of liquor was absorbed, but percolation did not extend through the column in 41 days.

It was evident that some compaction of the soil column had occurred during driving of the pipe. To alleviate this condition, one of the original soil columns was removed from the pipe and replaced layer by layer in original order with a constant tamping pressure of

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Table 3. Change in B.O.D. of Stillage No. 2, Following Percolation Through Wapato Surface Soil in a Laboratory Pond

<table>
<thead>
<tr>
<th>Days after filling pond</th>
<th>5-day B.O.D.</th>
<th>Reduction in B.O.D.</th>
<th>Volume of percolate</th>
<th>Appearance of percolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.p.m.</td>
<td>Per cent</td>
<td>ml.</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23,750</td>
<td>87</td>
<td>250</td>
<td>Black liquor</td>
</tr>
<tr>
<td>10*</td>
<td>2,500</td>
<td>89</td>
<td>350</td>
<td>Pale straw color</td>
</tr>
<tr>
<td>16</td>
<td>4,000</td>
<td>83</td>
<td>480</td>
<td>Brown straw color</td>
</tr>
</tbody>
</table>

* Pond was nearly dry. At 13 days shrinkage cracks appeared; more liquor was then added, but leakage occurred, as indicated by the increased B.O.D. and the darker color of the percolate at 16 days.
Liquid from the bottom of this column appeared after 14 days, indicating a rate of percolation of 0.08 gal./ft.²/day. Because of the very slow percolation, the experiment was abandoned before sufficient percolate was available for B.O.D. determinations.

To approach the natural soil structure, a column 6 by 6 inches by 4 feet deep was carved out in a pit in the field and transferred intact to a confining box. Attempts to use this column in the laboratory were unsuccessful because liquor by-passed much of the column along the sides and through unavoidable cracks in the lower soil.

Field ponding

Since the percolate from ponding showed a marked reduction in B.O.D., disposal by ponding showed promise. Field studies were therefore warranted, and a survey of the proposed ponding area and its water table was made; at the same time percolation studies were conducted in the field.

Type of soil. The proposed ponding area west of the Spring-field plant is Wapato silty clay loam soil. The topsoil, having depth of 8 to 12 inches, cracks extensively when dry, shows a coarse crumb structure, and is grayish-brown in color. The saturation capacity is 59 per cent (water-free basis). A first subsoil layer about 6 inches thick consists of grayish-brown, more or less mottled, silty clay to clay loam; rodent tunnels filled with loose crumbly infiltrations of surface soil are numerous. Below this is a second subsoil layer 14 to 18 inches thick, consisting of compact, grayish-yellow, vesicled clay containing occasional root channels. The underlying stratum, of undetermined depth, is a plastic yellow clay, mottled with gray and brown. A sandy streak about 2 inches thick was found in the clay at a depth of 7 feet; water was struck at 88 inches. Because of the poor drainage of this soil type, the following field studies were undertaken to determine the rate and extent of percolation as well as the general drainage and water table characteristics, each a factor influencing a ponding operation.

Water table. Ten depth wells 6 inches in diameter were bored in the field at locations indicated in Figure 1. These were set up with bench marks, and the water levels were determined by electrometric contact to 0.001 foot.

On June 18, 1946, when the first measurements were made, the water table encountered varied from 461.866 to 463.278 feet mean sea level; the level changed from well to well, and the maximum difference was 1.412 feet. On August 9, 1946, this difference was 1.479 feet; the average drop in water level since June 18 was 0.155 ft. per week. The data are presented in Table 4 and Figure 2. The
Figure 1. Proposed field ponding site showing location of the 2-inch cluster-wells and the ten 6-inch depth-wells. (Based on Oregon State Engineering Experiment Station data.)
evident slope of the water table forms a trough bearing from the east center to the northwest corner of the field; this is shown graphically in Figure 3.

**Drainage characteristics.** Three sets of cluster wells, located as shown in Figure 1, were sunk to a depth of 6 feet. Each cluster consisted of six 2-inch holes disposed at 60° and 2 feet distant from a central hole of the same diameter. A heavy charge of fluorescein was placed in the central well of each cluster, and water samples were drawn from each peripheral well at daily intervals for 10 days. Additional samples were taken at weekly intervals for 10 weeks. During this time, there was no lateral transfer of the dye through the distance of two feet separating the central and outer wells. It was evident that lateral drainage in the yellow clay layer was highly

![Figure 2. Water level chart for 6-inch depth-wells at proposed ponding site. (Based on Oregon State Engineering Experiment Station data.)](image-url)
Figure 3. Isometric view of underground water levels in proposed field ponding area. Indicated vertical distances from datum plane show relative water levels in the ten 6-inch depth-wells. (Based on Oregon State Engineering Experiment Station data.)

restricted; also, it is probable that water in this layer did not represent drainage from the overlying soil.

Percolation studies. A well 18 by 18 inches by 2 feet deep, cased with water-tight wooden walls, revealed that the subsoil at 2 feet was relatively pervious. Water passed through at 0.623 gal./ft.²/min. Test borings in the soil around the well revealed lateral as well as some downward drainage. When another well, an 8-inch steel pipe, was sunk to a depth of 36 inches, drainage was still rapid. With a similar well extended to a depth of 49 inches, percolation became virtually negligible, amounting to approximately 0.005 gal./ft.²/hr.

Evaluation of results. Perculation through Wapato surface soil is effective in lowering the B.O.D. of stillage. While the dry soil under field conditions is temporarily permeable because of granulation and because of channeling by roots, worms, and rodents, the rate of percolation through the saturated soil is far too low for prac-
Table 4. Elevation and Drop of Water Surface in 6-inch Depth Wells for Various Dates in 1946*
(Values in feet)

Data by Oregon State Engineering Experiment Station.

<table>
<thead>
<tr>
<th>Well number</th>
<th>June 18</th>
<th>July 1</th>
<th>July 12</th>
<th>July 18</th>
<th>July 26</th>
<th>August 2</th>
<th>August 9</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Elevation</td>
<td>Drop</td>
<td>Elevation</td>
<td>Drop</td>
<td>Elevation</td>
<td>Drop</td>
<td>Elevation</td>
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<td>463.040</td>
<td>0.029</td>
<td>463.001</td>
<td>0.054</td>
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<td>0.028†</td>
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<td>463.229</td>
<td>0.109</td>
<td>463.129</td>
<td>0.052</td>
<td>463.068</td>
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<td>462.647</td>
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<td>462.507</td>
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<td>462.292</td>
<td>0.190</td>
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<td>462.193</td>
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<td>461.950</td>
<td>0.127</td>
<td>461.823</td>
<td>0.058</td>
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<td>462.083</td>
<td>0.147</td>
<td>461.936</td>
<td>0.122</td>
<td>461.814</td>
</tr>
</tbody>
</table>

* Data by Oregon State Engineering Experiment Station.
† A plus quantity; five gallons of water poured into the well required about two weeks to drain off.
tical consideration. Percolation in saturated soil is less than 400 gal./acre/day. The subsoil is slightly permeable by virtue of a specific vesicular porosity. The underlying yellow clay is plastic and essentially non-porous; at this horizon, therefore, drainage becomes lateral.

Percolation and drainage obviously depend on soil porosity, but porosity is a complex function. Primary soil porosity is determined by soil texture and consistency. Since Wapato soil is high in clay content and is of a plastic consistency when moist, the porosity of the unmodified soil is extremely low. Ordinary soil porosity is a function of soil structure and mechanical cleavages. Wapato soil is compact in structure, but when dry it becomes coarsely granular and ramified with cracks. Specific soil porosity is partly a biological porosity represented by channels produced by plant roots, worms, and small animals, combined with specific cavities, such as vesicular porosity, produced by physical forces. All forms of soil porosity are subject to modification by the liquid ingredient of the soil mass.

It is thus apparent (a) that any percolation and drainage to be expected from a pond on the proposed site will be the result of cracks, fissures, and channels, and (b) that infiltration of the liquor would probably result in some restrictive modification. Consideration must also be given to lateral drainage imposed by the underlying stratum of impervious clay; this could be far-reaching from the proposed ponding area, since surrounding soils overlie similar impervious layers. Remote fields and wells thus could be subject to contamination.

**Filtration through lignin column**

Highly sorptive by-product lignin residue will be available at the plant in quantity. The effect of passing stillage liquor through a column of wood-sugar lignin residue obtained from the Forest Products Laboratory at Madison was, therefore, investigated. Stillage liquor No. 2 was used. The first percolate decolorized to a straw color, and the B.O.D. was reduced approximately 10 per cent. By the time three volumes, each equivalent to the column volume, had passed through, the liquor emerged unchanged.

**Trickling filter studies**

Preliminary work with gravel columns in glass tubes gave indications that a film, biologically active on stillage, could be established. Using soil, raw sewage, and various pure cultures as inocula, including *Aerobacter aerogenes*, *Pseudomonas fluorescens*, and zoogloal...
isolates from sewage filters,* growth was established in mixtures of synthetic sewage and stillage under aerobic conditions. These mixed cultures were then passed repeatedly over the gravel columns until films were established. Channeling was extensive because of the small diameter (1½ to 2½ inches) of columns; consequently, film development was unsatisfactory. Nevertheless, some B.O.D. reduction was obtained with diluted (1 to 4) stillage. Straight stillages were only slightly altered by the filters. The results seemed promising, however, so the study was extended on a larger scale.

_Barrel filter Run No. 1._ A filter of 2- to 3-inch gravel was set up in a 50-gallon barrel provided with a rotating-arm distributor and a pump for recirculation. Five gallons of stillage (Sample No. 2) were added to the barrel filter after inoculation with 0.5 gallon liquid and film from the glass tube columns. After 23 days of operation, the 5-day B.O.D. dropped from 23,750 p.p.m. to 4,750 p.p.m. Additions of ammonium hydroxide to supply available nitrogen increased the rate of action. Decrease in specific gravity and increase in pH show correlation with decrease in B.O.D. Odor entirely disappeared and viscosity decreased, but the dark color remained. After 14 days additional operation, the B.O.D. was further lowered to 1,250 p.p.m. During this latter period, results were erratic because of high evaporation losses which, with restoration, resulted in concentration changes. Data for the complete run are given in Table 5.

_Barrel filter Run No. 2._ The barrel was emptied, and a fresh charge of stillage, fortified with 500 p.p.m. nitrogen as urea, was added. The bacterial flora was maintained at a higher average in this run, and the B.O.D. reduction was more rapid, falling to 12,000 p.p.m. in 8 days (Table 6).

_Barrel filter Run No. 3._ The charge for this run was made up from a new lot of stillage from the concentrated Madison Sample No. 3 (this was diluted 1 to 6.9 to obtain the concentration of the original material). B.O.D. of this sample was considerably less than that of previous lots. As shown in Table 7, the B.O.D. was lowered to 750 p.p.m. in 19 days, a reduction of 95 per cent. Although the other data are incomplete, the higher values for bacterial counts are noteworthy.

_Wood-stave tank filters._ While results with the barrel filter showed that an active flora could be built up to decompose stillage and greatly lower the B.O.D., the rate of action was too slow for practical application. Believing that limited aeration was an important factor in this slow action, provision was made for forced aeration in two 400-gallon wood-stave tanks assembled for use as larger filters.
Table 5. Change in B.O.D. and Other Properties of Stillage No. 2 on Trickling Filter (Barrel)  
Run No. 1

<table>
<thead>
<tr>
<th>Operation time</th>
<th>5-day B.O.D.</th>
<th>Kjeldahl nitrogen</th>
<th>pH</th>
<th>Specific gravity 20°C/4°C</th>
<th>Bacterial count</th>
</tr>
</thead>
<tbody>
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<td>Days</td>
<td>P.p.m.</td>
<td>P.p.m.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.014</td>
<td>250</td>
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<td>50</td>
<td>7.0</td>
<td>1.011</td>
<td>3,000</td>
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<td>50</td>
<td>7.2</td>
<td>1.010</td>
<td>24,000,000</td>
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<tr>
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<td>8,770</td>
<td>60</td>
<td>7.1</td>
<td>1.010</td>
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<tr>
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<td>9,250</td>
<td>50</td>
<td>7.6</td>
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<tr>
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<td>50</td>
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<td>1.009</td>
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<tr>
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<td>45</td>
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<td>110</td>
<td>7.7</td>
<td>1.003</td>
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<td>1,250</td>
<td>75</td>
<td>7.6</td>
<td>1.005</td>
<td></td>
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</tbody>
</table>

* More liquor, sufficient to double the volume in the system, and 40 p.p.m. N, as NH₄OH, were added after taking the 18-day sample.

† 300 p.p.m. N, as NH₄OH, added and evaporation loss restored 24 hours previous to sampling.

‡ Evaporation loss, which was excessive because of hot weather, and its replacement were factors contributing to irregularity of results.

Table 6. Change in B.O.D. and Other Properties of Stillage No. 3 on Trickling Filter (Barrel)  
Run No. 2

| Operation time | 5-day B.O.D. | Kjeldahl nitrogen | pH | Specific gravity 20°C/4°C | Bacterial count |
|----------------|--------------|-------------------|----|--------------------------|                |
| Days           | P.p.m.       | P.p.m.            |    |                          |                |
| 0              | 23,750       | 105               | 5.6| 1.014                    | 250            |
| 2              | 11,000       | 50                | 7.0| 1.011                    | 3,000          |
| 18*            | 9,500        | 50                | 7.2| 1.010                    | 24,000,000     |
| 19             | 8,770        | 60                | 7.1| 1.010                    |                |
| 21             | 9,250        | 50                | 7.6| 1.010                    |                |
| 23             | 4,750        | 50                | 7.2| 1.009                    |                |
| 25             | 6,500        | 45                | 7.2| 1.008                    |                |
| 27*            | 9,750        | 30                | 7.1| 1.007                    |                |
| 28             | 6,250        | 30                | 7.7| 1.006                    |                |
| 32*            | 7,000        | 65                | 7.5| 1.004                    |                |
| 37             | 6,500        | 170               | 7.5| 1.001                    |                |
| 36             | 3,750        | 110               | 7.7| 1.003                    |                |
| 27             | 1,250        | 75                | 7.6| 1.005                    |                |

* More liquor, sufficient to double the volume in the system, and 40 p.p.m. N, as NH₄OH, were added after taking the 18-day sample.

† 300 p.p.m. N, as NH₄OH, added and evaporation loss restored 24 hours previous to sampling.

‡ Evaporation loss, which was excessive because of hot weather, and its replacement were factors contributing to irregularity of results.

Table 7. Change in B.O.D. and Other Properties of Stillage No. 3 on Trickling Filter (Barrel)  
Run No. 3

| Operation time | 5-day B.O.D. | Kjeldahl nitrogen | pH | Specific gravity 20°C/4°C | Bacterial count |
|----------------|--------------|-------------------|----|--------------------------|                |
| Days           | P.p.m.       | P.p.m.            |    |                          |                |
| 0              | 17,750       | 650               | 6.5| 1.010                    | 130,000,000    |
| 1              | 17,500       | 550               | 6.9| 1.012                    | 145,000,000    |
| 10             | 8,250        | 230               | 7.3| 1.005                    | 440,000,000    |
| 12             | 6,000        | 230               | 7.3| 1.010                    | 400,000,000    |

* 16 liters water added to restore volume to 38 liters, and 150 p.p.m. nitrogen, as urea, added after sampling.
Many mechanical and operational difficulties were encountered with the wood-tank setup. Forced aeration produced vigorous foaming. Although the air pressure was less than 1 p.s.i., the foam was especially difficult to confine and direct to a foam breaker. Continuous operation of the filters was not possible without danger of overflow and consequent damage to the room and adjacent equipment. One discontinuous run totaling 26 hours of aeration lowered the B.O.D. from 10,000 p.p.m. to 3,400 p.p.m., a reduction of 66 percent. It is evident that vigorous aeration and foaming accelerated the stillage decomposition.*

Glass tube filter with air lift recirculation. The operational difficulties encountered with the wood-stave tank filters rendered a small scale laboratory experiment under controlled conditions desirable. After some experimentation, satisfactory apparatus and procedures were devised to combine aeration, foaming, and trickling filter action.

The apparatus consisted of two gravel towers and one foam receiving tower, made with glass tubes 2\(\frac{1}{2}\) inches in diameter by 4 feet high. This was placed in an incubator at 30\(^\circ\) C. The gravel used ranged from \(\frac{1}{2}\) to 1\(\frac{1}{2}\) inches in diameter. All towers were connected in multiple at the bottom. A 2.5-liter charge of stillage was added via the first gravel tower. This soon became distributed in approximately equal volumes in the three towers. By means of a side-arm air lift, liquid was continually transferred from the bottom of the system into the top of the first gravel column, where it trickled down and was exposed to action of the biologically active film as it kept the surface moist. Returning liquid entered the bottom of the second gravel tower and was vigorously aerated through a fritted glass sparger; an abundant foam developed in this tower. Most of the foam was broken by a motor-driven stirrer inserted in the top of the column. Excess foam not mechanically broken was collected in the third tower where the liquid resulting from spontaneous breakdown returned to the system from below.

The first gravel tower provided trickling filter action on the liquid more or less aerated by the air lift. The second tower, while providing similar action, served primarily to maintain about one-third of the total charge in foam. The foam was believed to be desirable because of its relatively enormous surface exposure to air and bacterial action.

Sugar values instead of B.O.D. were chosen as an index of decomposition because of the promptness with which sugars may be

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*Two hundred gallons of processed liquor and 3 cubic yards of gravel from these tank filters were sent to the Springfield plant May 15, 1947, for inoculating a 1-acre gravel-filled pit to be used with spray distribution for disposing of stillage from trial runs. No information is available on the dosage or operation of this pit.
determined. The B.O.D. is partly attributable to sugar; therefore, lowering the sugar should also lower the B.O.D. The exact relationship existing between these two criteria is not known; but, as shown later, about 50 per cent of the B.O.D. of stillage remains after most of the sugar has been decomposed.

Madison No. 3 stillage was used in this series of runs. Urea was added to give 0.14 per cent nitrogen, or a carbon-nitrogen ratio of 10:1 in the liquid, thus insuring an adequate supply of available nitrogen for rapid microbial action. The first run was inoculated with several predominating isolates from the barrel filters, suspended in 25 ml. of previously processed stillage plus an equal volume of an active flax-retting liquor. For subsequent runs, urea without inoculum was added to the stillage.

Analytical data are given in Table 8. The first three runs are of interest in showing the gradual development of an active flora. On

<table>
<thead>
<tr>
<th>Operation time</th>
<th>pH</th>
<th>Kjeldahl nitrogen</th>
<th>Reducing sugar</th>
<th>Bacteria count</th>
</tr>
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<td>Run number 1</td>
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<td>860</td>
<td>4,300</td>
</tr>
<tr>
<td></td>
<td>16 hours</td>
<td>5.0</td>
<td>1,060</td>
<td>4,300</td>
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<td></td>
<td>3 days</td>
<td>5.7</td>
<td>750</td>
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<td>4 days</td>
<td>5.3</td>
<td>880</td>
<td>3,200</td>
</tr>
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<td>7 days</td>
<td>7.9</td>
<td>680</td>
<td>1,500</td>
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<td>8 days</td>
<td>7.5</td>
<td>460</td>
<td>1,100</td>
</tr>
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<td>1,100</td>
<td>4,200</td>
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<td></td>
<td>1 hour</td>
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<td>2,800</td>
</tr>
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<td></td>
<td>24 hours</td>
<td>7.1</td>
<td>860</td>
<td>2,000</td>
</tr>
<tr>
<td></td>
<td>40 hours</td>
<td>7.8</td>
<td>760</td>
<td>1,200</td>
</tr>
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<td></td>
<td>64 hours</td>
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<td>660</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>88 hours</td>
<td>7.7</td>
<td>650</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>112 hours</td>
<td>7.7</td>
<td>720</td>
<td>900</td>
</tr>
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<td>1,240</td>
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<td>5 minutes</td>
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<td>1,710</td>
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<td>2,700</td>
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<td>40 hours</td>
<td>7.3</td>
<td>1,500</td>
<td>2,400</td>
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<td></td>
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<td></td>
<td>136 hours</td>
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<td>700</td>
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<td></td>
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<td>990</td>
<td>800</td>
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<td>9 hours</td>
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<td>810</td>
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<td>32 hours</td>
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<td>750</td>
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<td>98 hours</td>
<td>7.5</td>
<td>810</td>
<td>1,300</td>
</tr>
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</table>

* A fresh charge of liquor was added after taking the 88-hour sample.
† The foaming tower was dry at 93 hours; a partially processed charge was added and aerated 5 hours.
the fourth run, more than 90 per cent of the sugar was decomposed in 3 days. During this rapid decomposition, the number of bacteria attained the maximum characteristic of liquid cultures. Determination of numbers developing simultaneously on the foam would have been of interest.

As decomposition proceeded, the tendency to foam decreased so that, after 48 hours, a full blast of air could be used. This was accompanied by rapid evaporation and change in appearance; the liquid became turbid and light brown. During the 19-hour period, after the 74-hour sample was taken, a 500 ml. residual charge confined to the foaming tower evaporated to dryness. A 500 ml. charge was then admitted from the foam-collecting tower and aerated for five hours; the 98-hour sample was then taken, completing the experiment. Data for this last sample are of little significance, since the foam-collecting tower previously had been disconnected from the bottom of the system because of mechanical difficulties.

Glass tube filters with pump recirculation. Excessive foaming was avoided in this run by replacing the air lift with a small centrifugal pump and reducing the air flow through the sparger; consequently, the foam-collecting tower and foam breaker could be omitted. Two-liter charges were used. About 1 per cent of the charge was recirculated through each tower per minute. Air was admitted by sparger to one tower only, at a rate of approximately 20 liters per hour. Results of three runs are presented in Table 9. It was apparent that sugar decomposition was as extensive as, or even more rapid than, the decomposition associated with excessive foaming. This may have resulted from better adaptation of the bacterial flora, particularly since the plate counts were much lower. The data show that, although the filter removed most of the sugar from the liquor, it was less effective in lowering the B.O.D., since only 50 to 60 per cent was removed in 2 to 4 days.

The low efficiency of the filter in reducing B.O.D. of the Springfield stillage may have resulted from lack of additional available nitrogen or from some major difference in organic content other than sugars.

Single column bacterial filter. This experiment was similar to the glass tube filter with pump recirculation, except that only one tower was used and the rate of aeration was decreased while recirculation was increased. The study was made more extensive by including carbon determinations so that carbon balances could be established for comparison with changes in B.O.D. A taxonomic study of representative bacteria from this and previous filters was also included.
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<td>1,200,000</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>6.8</td>
<td>300</td>
<td>91</td>
<td>91</td>
<td>8,100</td>
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<td>1,200,000</td>
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<tr>
<td></td>
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<td>350</td>
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<td>92</td>
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<td>47</td>
<td>1,200,000</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>6.7</td>
<td>400</td>
<td>91</td>
<td>91</td>
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</tr>
<tr>
<td></td>
<td>65</td>
<td>6.8</td>
<td>400</td>
<td>91</td>
<td>91</td>
<td>8,100</td>
<td>47</td>
<td>1,200,000</td>
</tr>
</tbody>
</table>

* 500 p.p.m. N, as urea, added.
† No N added.
‡ Tower liquor diluted by residual from previous run.
§ With continuous flow of added stillage through tower at 30 ml./hr.
¶ Sugar in drainage liquor.
‖ Data on this line are for the sample as received.
A mixture containing one part \(\frac{1}{2}\)-inch and five parts 1- to 1\(\frac{1}{2}\)-inch dry gravel previously used in other filters was placed in a 3- by 48-inch Pyrex tube to give a 40-inch column. Suitable connections for a recirculation pump, air sparger, and inlet and outlet tubes for continuous feed and discharge were provided. As in previous experiments, the apparatus was placed in the incubator at 30° C.

Before making the run with the stillage sample, a preliminary zoogloeal film was built up by operating the tower for 12 days on a charge of old processed liquor, which was fortified with 0.5 per cent xylose and inoculated with a mixture of sewage and isolates No. 233 and No. 235.

The only stillage available for this experiment was about 4 liters of Springfield No. 6 that had been stored in a stoppered bottle for 4 months. It was apparent that the stillage had undergone some change, as a finely granular dark precipitate had formed. This was filtered off to avoid clogging of the capillary feed required to regulate the slow rate of continuous flow into the tower. One-tenth per cent urea was added to the filtered stillage to support active bacterial development. The analysis as given in Table 10 for this “original lot” in the experiment compares favorably with the original analysis in Table 1. Significant differences occurred only in the plate count of bacteria, which increased from 900 to 38,000 per ml., and in reducing sugars, which decreased from 4,200 to 3,200 p.p.m.

An initial volume of 650 ml. was introduced; about 150 ml. were required to fill a screen tower installed to protect the recirculation pump, while 500 ml. remained in the bottom of the gravel tower, rising to a height of 7 inches. This left 33 inches or slightly more than three-fourths of the gravel column exposed to aeration and trickling. The surface exposure of the gravel was measured roughly; including the inside surface of the tube, the total area available for development of an active zoogloeal film was estimated to be 10 square feet. The filter was placed in operation with recirculation at 500 ml. an hour and aeration through the sparger at 2 liters an hour. These rates were maintained at approximate constancy for the duration of the experiment. The rate of input from a reservoir of "fresh" stillage to the top of the column was regulated to match as nearly as possible the rate of discharge from the bottom. It was planned to maintain a throughput of about 10 ml. per hour for the first 48 hours, a slow rate being desirable while the zoogloeal film was being built up further. The rate was then increased to about 25 ml. per hour, and finally, to approximately 50 ml. per hour. The last rate corresponds to about 0.03 gallon per square foot of gravel surface per day, or to approximately 1 gallon per cubic foot of \(\frac{1}{2}\)- to 1\(\frac{1}{2}\)-inch gravel per day.
Table 10. Decomposition of Springfield Stillage, No. 6, in Single Column Gravel Tower, with Continuous Flow, Recirculation by Pump, Aeration by Sparger
Analysis of Throughput

<table>
<thead>
<tr>
<th>Lot number</th>
<th>Throughput period</th>
<th>Rate of throughput</th>
<th>Equivalent/M cu. ft. gravel per day</th>
<th>Bacteria count</th>
<th>Specific gravity 20°C</th>
<th>pH</th>
<th>Reducing sugars</th>
<th>Total carbon</th>
<th>Kjeldahl nitrogen</th>
<th>C/N ratio</th>
<th>5-day B.O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original §</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>ml./hour</td>
<td>Gallons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>7</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>7</td>
<td>150</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>26</td>
<td>550</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>15</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
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<td>550</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>28</td>
<td>600</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>48</td>
<td>1,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Filtered.
† Carbon-nitrogen ratio.
§ 0.1 per cent urea added; equivalent to 470 p.p.m. nitrogen and 200 p.p.m. carbon.

Table 11. Decomposition of Springfield Stillage, No. 6, in Single Column Gravel Tower with Continuous Flow, Recirculation by Pump, Aeration by Sparger
Carbon Balance and B.O.D.

<table>
<thead>
<tr>
<th>Lot number</th>
<th>Carbon in sugars</th>
<th>Non-sugar carbon*</th>
<th>Carbon losses</th>
<th>Total</th>
<th>Reduction in 5-day B.O.D.</th>
<th>Calculated 20-day B.O.D.</th>
<th>Ultimate B.O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1280</td>
<td>6420</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>240</td>
<td>2860</td>
<td>1,040</td>
<td>81</td>
<td>3,560</td>
<td>4,600</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>2210</td>
<td>1,140</td>
<td>89</td>
<td>4,210</td>
<td>5,350</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>480</td>
<td>3720</td>
<td>800</td>
<td>63</td>
<td>2,700</td>
<td>3,550</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>480</td>
<td>3820</td>
<td>800</td>
<td>63</td>
<td>2,600</td>
<td>3,400</td>
<td>44</td>
</tr>
<tr>
<td>6</td>
<td>520</td>
<td>4580</td>
<td>760</td>
<td>59</td>
<td>1,840</td>
<td>2,600</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>540</td>
<td>4660</td>
<td>740</td>
<td>58</td>
<td>1,760</td>
<td>2,500</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>480</td>
<td>4170</td>
<td>500</td>
<td>63</td>
<td>2,350</td>
<td>3,550</td>
<td>39</td>
</tr>
</tbody>
</table>

* Difference between total carbon and sugar carbon.
† 5-day B.O.D. X 1.46.
‡ Total C X 32/12. Based on C + O₂ = CO₂; C:O₂ = 12:32.
The output from the filter was collected at the intervals indicated in Table 10. Each lot was analyzed by the methods previously described; in addition, colonies of the more numerous types of bacteria were fished from the plates and cultured for further study to determine their natures and possible significance in filter action. Examination of the zoogloeal film at the close of the run revealed the predominance of small Gram negative rods.

It is of interest that during the first 48 hours with slow throughput, the B.O.D. reduction was maximum, being nearly 63 per cent. Although the plate count on the effluent reached 100,000,000, the zoogloeal film on the gravel was barely visible. Subsequent counts varied considerably and do not necessarily indicate a corresponding development or activity of surface film; they were not proportional to the decrease in B.O.D. The last two counts were significantly higher and coincided with a readily observed increase in development of zoogloea. Although B.O.D. reduction for the last lot of processed stillage was not so high as that initially obtained, the effectiveness of the filter under the considerably increased operating rate was increasing rapidly. Had more stillage been available for longer operation, a greater efficiency in removal of B.O.D. might have been attained.

As in previous experiments, the stillage became less acid; therefore, it can reasonably be expected that a properly operating filter will give an approximately neutral effluent. The data for Kjeldahl nitrogen show that at first all added urea nitrogen was utilized; as the bacterial film became better developed, slightly less was consumed. Lower carbon-nitrogen ratios during the latter part of the run indicate the presence of more nitrogen than the microorganisms require; smaller additions may be effective to obtain the desired activity of a well established film. The specific gravity of the effluent was significantly lower than that of the raw stillage; with few exceptions, the decrease paralleled the decrease in B.O.D. From the carbon balance, as shown in Table 11, it is apparent that in five of the eight lots the percentage decrease in B.O.D. is fairly well paralleled by reduction in sugar; but the latter is more extensive. These relationships are shown graphically in Figure 4. Similar indications were noticed in the less extensive decompositions obtained with Fusarium lini (Table 13).

When the rate of throughput was first increased, carbon loss and B.O.D. reduction decreased significantly. This was doubtless the result of insufficient development of the zoogloea. More nearly adequate development occurred toward the close of the run, since further increase in throughput was accompanied by a definitely increasing efficiency on the last two lots. Expressing the final data on a
practical basis, it appears that under operating conditions similar to those of the experiment, a trickling filter with continuous effluent may process 1,000 gallons of stillage per 1,000 cubic feet of gravel per day to effect a 50 per cent reduction in B.O.D. Removal of 50 per cent of the B.O.D. is far from sufficient to permit discharge of the effluent into a stream for final disposal. Further treatment on one or more additional filters would be required. The possible value of diluting stillage before treatment and of diluting the effluent should also be considered, particularly since 3,000,000 gallons of condenser water would be available each day at the Springfield plant. Studies of the effects of dilution and of filtration in series were not made because of limitations imposed by the preliminary nature of this investigation. No prediction of the efficiency of successive filters can be made, but
a more dilute stillage should be more rapidly oxidized. In this connection, the calculated 20-day and ultimate B.O.D. values of the stillage are of interest.

For ordinary domestic sewage, the 20-day B.O.D. value represents the amount of oxygen required for 99 per cent decomposition, which is essentially the ultimate B.O.D. The calculated 20-day B.O.D. of stillage is less than one-half of the theoretical ultimate value; therefore, it is high in substances unusually resistant to decomposition. Whether or not these may be significant in downstream pollution, if left in discharged effluent, would be determined by characteristics of the stream.

**Fusarium lini Bolley as an agent of decomposition**

This mold was shown by Heines and Nord (9) to be highly effective in fermenting the residual pentoses in Douglas-fir hydrolysates, following fermentation by yeast. From a culture of the organism (kindly furnished by Dr. F. F. Nord, Department of Organic Chemistry, Fordham University), a heavy mat was developed on the surface of 1/2- to 1 1/2-inch gravel in a single tower. The culture was first grown in flasks on Nord's medium (9) and then mixed with an equal volume of stillage on this medium. The tower was charged with a liter of culture growing on this mixture; aeration and recirculation were provided by a side-arm air lift. Each day for a period of one week, about one-half the charge was withdrawn and replaced with fresh stillage containing 0.1 per cent added urea; after one week, a heavy growth of *Fusarium* was observed on the gravel and the sides of the tower.

Results of three runs are given in Table 12. In all cases, 0.1 per cent urea was added to give available nitrogen. With side-arm lift aeration and recirculation, the mold in 6 days decomposed about 90 per cent of the sugar in Springfield stillage No. 4. Because of the exhaustion of Springfield stillage No. 4, Springfield stillage No. 5 was used on subsequent runs. Sample No. 5 contained about 20 per cent less sugar; this may or may not have been a factor in the more rapid sugar decomposition indicated in the second run. It is more likely that the additional air supplied by the sparger was responsible. In view of the rapid action obtained, the tower was next operated with continuous flow; fresh stillage was added at the top, while processed liquor was withdrawn from the bottom. Side-arm aeration and recirculation were employed as before. In most instances, sugar consumption was about as high as with batch operation. Discontinuance of aeration and recirculation by a power failure caused a marked drop in efficiency. Best results, in general, were obtained
with a moderate rate of throughput and a relatively low liquid level, exposing more active surface in the towers. Limitations of accessories for the apparatus prevented adjustment to constant rates of flow and aeration.

A fourth run was made, using continuous flow also, under better controlled conditions and with more extensive analysis. The air lift was operated by 370 ml./min. of air at an average pressure of 30 mm. mercury, and it circulated 15 ml. of tower liquor per minute. The continuous-flow throughput was maintained reasonably close to 20 ml. per hour. Analytical data are given in part (A) of Table 13; efficiency of the tower is shown on the basis of carbon transformation and B.O.D. in part (B) of the table.

<table>
<thead>
<tr>
<th>Operation time</th>
<th>Sugar in tower liquor</th>
<th>Sugar decomposed</th>
<th>Flow through tower</th>
<th>Average height of liquor in tower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P.p.m.</td>
<td>Per cent</td>
<td>Total ml.</td>
</tr>
<tr>
<td>Run No. 1 (1,000 ml. Springfield stillage, No. 4, with side-arm aeration and recirculation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0*</td>
<td>4,100</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>24</td>
<td>3,450</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>1,250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>144</td>
<td>500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Run No. 2 (500 ml. Springfield stillage, No. 5, with 250 ml. fresh culture; with side-arm aeration and recirculation; additional air from sparger from 24 to 48 hours)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0†</td>
<td>2,100</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>24</td>
<td>500</td>
<td></td>
<td>76.3</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>300</td>
<td></td>
<td>55.7</td>
<td></td>
</tr>
<tr>
<td>Run No. 3 (Springfield stillage, No. 5; continuous flow of added stillage through tower, plus side arm aeration and recirculation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original stillage</td>
<td>3,200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0‡</td>
<td>250†</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>400</td>
<td>87.5</td>
<td>810</td>
<td>34</td>
</tr>
<tr>
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<td>67.3</td>
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<td>61.0</td>
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<td>39</td>
</tr>
<tr>
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<td>650</td>
<td>27</td>
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<td>61.4</td>
<td>830</td>
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</tr>
<tr>
<td>334</td>
<td>950</td>
<td>70.5</td>
<td>1,305</td>
<td>54</td>
</tr>
</tbody>
</table>

* Original stillage.
† Low sugar because of dilution with culture and residual liquor from previous run.
‡ Residual liquor from previous run.
§ No aeration for 4 hours because of power failure.
¶ Sugar in drainage liquor.
After the third day, sugar decomposition was maintained at well over 80 per cent. The B.O.D. values varied but showed a reduction of nearly 20 per cent after the second day. With one erratic exception, the B.O.D. reduction closely parallels the loss in total carbon. The plate counts are of interest because they show that bacterial contaminants developed in moderate numbers. The mold counts show only that numerous *Fusarium* spores were liberated from the mycelial mat; no free mycelium occurred in the throughput liquor. It is doubtful if the bacterial contaminants produced much action on the liquor, since their active mass could be only relatively minute in comparison with the mycelial mat.

While *Fusarium lini* Bolley is effective in consuming the sugar from stillage, it has only slight action on the non-sugars. Stated in round numbers, it can reduce stillage sugars 85 per cent, total carbon 35 per cent, and B.O.D. 20 per cent while acting in a trickling filter.

**Study of isolates**

Colonies of predominating types were fished from plates used for counts during the course of this project and cultured for study and future reference. Most of the 40 isolates obtained in this project were taken from the various samples of processed stillage, although a few were taken from untreated samples. The transfers were maintained on nutrient agar slants. At the end of the experimental work, all isolates that appeared to be replicates were discarded, except the most recent one; this was necessary to stay within reasonable limits on the time allowed for systematic investigation.

Each isolate reserved for intensive study was plated out three successive times to insure purity. Except where reference is made to other procedures, standard methods as outlined in *The Manual of Methods for Pure Culture Study of Bacteria* (6) were used to determine morphology, staining, cultural characters, and biochemical activity. An incubation temperature of 30° C. was used in all cases.

Cell dimensions given are average measurements made on 50 cells in Congo red preparations from 24-hour old nutrient agar slant cultures. For detection of spores, Schaeffer and Fulton’s modification of the Wirtz method was followed. Kopeloff and Beerman’s procedure was used for the Gram stain. Motility was checked on wet mounts in the dark field, and Leifson’s modified stain (13) was employed to determine flagellation. Nutrient agar slant cultures 10 to 14 hours old gave the best flagella stains except with isolates No. 286, No. 295, and No. Z100, for which 5-, 36-, and 20-hour old cultures respectively were most satisfactory. The Leifson and Anthony methods were used to demonstrate capsules.
Table 13. Decomposition of Springfield Stillage No. 6 by Fusarium lini with Continuous Flow Through Gravel Tower
Run No. 4

(A) Analysis of throughput

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Time from beginning of run (Hours)</th>
<th>Volume of throughput (ml.)</th>
<th>Plate count (Bacteria No./ml., Molds No./ml.)</th>
<th>pH</th>
<th>Reducing sugars (P.p.m.)</th>
<th>Total carbon (P.p.m.)</th>
<th>5-day B.O.D. (P.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>0</td>
<td>0</td>
<td>900, 0</td>
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<td>4,200</td>
<td>7,290</td>
<td>6,800</td>
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<td>18</td>
<td>276</td>
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<td>2,700</td>
<td>5,220</td>
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<td>4</td>
<td>72</td>
<td>600</td>
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<td>1,100</td>
<td>5,020</td>
<td>5,650</td>
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<td>96</td>
<td>500</td>
<td>100,000,000, 200,000</td>
<td>7.00</td>
<td>700</td>
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<td>7.70</td>
<td>600</td>
<td>4,270</td>
<td>5,800</td>
</tr>
</tbody>
</table>

(B) Carbon transformation and B.O.D.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Carbon in sugars (P.p.m.)</th>
<th>Non-sugar carbon* (P.p.m.)</th>
<th>Carbon loss (P.p.m., %)</th>
<th>Reduction in B.O.D. (Per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>1,680</td>
<td>5,710</td>
<td>1,250, 21.4</td>
<td>21.8</td>
</tr>
<tr>
<td>1</td>
<td>1,320</td>
<td>4,460</td>
<td>600, 35.7</td>
<td>21.0</td>
</tr>
<tr>
<td>2</td>
<td>1,080</td>
<td>4,140</td>
<td>1,400, 85.7</td>
<td>19.8</td>
</tr>
<tr>
<td>3</td>
<td>440</td>
<td>4,140</td>
<td>1,100, 85.7</td>
<td>19.8</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>4,550</td>
<td>600, 35.7</td>
<td>20.3</td>
</tr>
<tr>
<td>5</td>
<td>240</td>
<td>4,110</td>
<td>1,440, 85.7</td>
<td>19.8</td>
</tr>
<tr>
<td>6</td>
<td>240</td>
<td>4,100</td>
<td>1,440, 85.7</td>
<td>19.8</td>
</tr>
<tr>
<td>7</td>
<td>240</td>
<td>4,510</td>
<td>1,440, 85.7</td>
<td>19.8</td>
</tr>
<tr>
<td>8</td>
<td>240</td>
<td>4,340</td>
<td>1,440, 85.7</td>
<td>19.8</td>
</tr>
</tbody>
</table>

* Calculated.
Tests for acetymethylcarbinol in the Voges-Proskauer reaction were made with O'Meara's reagent, using the medium and procedure recommended by Smith (14). The lead acetate paper method as recommended by ZoBell and Feltham (15) was employed to detect hydrogen sulphide production.

Because acid production from fermentation of carbohydrates added to nutrient broth is often masked by the production of ammonia from peptone, additional fermentation studies were made with filter-sterilized carbon sources added to the synthetic inorganic basal medium of Ayers, Rupp, and Johnson (3).

Table 14. ISOLATE SOURCE AND FREQUENCY OF OCCURRENCE

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Source</th>
<th>Time in filter</th>
<th>Occurrence</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>223</td>
<td>Springfield stillage No. 6, 5 days after collection</td>
<td></td>
<td>850</td>
<td>95</td>
</tr>
<tr>
<td>225</td>
<td>Madison stillage No. 3, 15 months after collection</td>
<td></td>
<td>2,000</td>
<td>60</td>
</tr>
<tr>
<td>228</td>
<td>Foaming trickling filter, Run No. 4</td>
<td>9 hours</td>
<td>37,000,000</td>
<td>55</td>
</tr>
<tr>
<td>224</td>
<td>do</td>
<td>74 hours</td>
<td>1,490,000,000</td>
<td>65</td>
</tr>
<tr>
<td>225</td>
<td>do</td>
<td>47 hours</td>
<td>525,000,000</td>
<td>25</td>
</tr>
<tr>
<td>226</td>
<td>do</td>
<td></td>
<td>775,000,000</td>
<td>50</td>
</tr>
<tr>
<td>228</td>
<td>do</td>
<td></td>
<td>71,000,000</td>
<td>20</td>
</tr>
<tr>
<td>281</td>
<td>Single column tower build-up run</td>
<td>12 days</td>
<td>27,000,000</td>
<td>45</td>
</tr>
<tr>
<td>282</td>
<td>do</td>
<td></td>
<td>6,000,000</td>
<td>10</td>
</tr>
<tr>
<td>283</td>
<td>do</td>
<td></td>
<td>12,000,000</td>
<td>20</td>
</tr>
<tr>
<td>286</td>
<td>Single column tower run</td>
<td>24 hours</td>
<td>12,000,000</td>
<td>50</td>
</tr>
<tr>
<td>289</td>
<td>do</td>
<td>48 hours</td>
<td>1,000,000</td>
<td>1</td>
</tr>
<tr>
<td>295</td>
<td>do</td>
<td>119 hours</td>
<td>87,000,000</td>
<td>50</td>
</tr>
<tr>
<td>297</td>
<td>do</td>
<td>111 hours</td>
<td>112,000,000</td>
<td>40</td>
</tr>
<tr>
<td>298</td>
<td>do</td>
<td>119 hours</td>
<td>70,000,000</td>
<td>40</td>
</tr>
<tr>
<td>305</td>
<td>do</td>
<td>0 hours</td>
<td>34,000</td>
<td>90</td>
</tr>
<tr>
<td>286</td>
<td>Single column tower run</td>
<td></td>
<td>12,000,000</td>
<td>50</td>
</tr>
<tr>
<td>289</td>
<td>do</td>
<td></td>
<td>1,000,000</td>
<td>1</td>
</tr>
<tr>
<td>295</td>
<td>do</td>
<td></td>
<td>87,000,000</td>
<td>50</td>
</tr>
<tr>
<td>297</td>
<td>do</td>
<td></td>
<td>112,000,000</td>
<td>40</td>
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<tr>
<td>298</td>
<td>do</td>
<td></td>
<td>70,000,000</td>
<td>40</td>
</tr>
<tr>
<td>305</td>
<td>do</td>
<td></td>
<td>34,000</td>
<td>90</td>
</tr>
<tr>
<td>281</td>
<td>Single column tower build-up run</td>
<td>12 days</td>
<td>27,000,000</td>
<td>45</td>
</tr>
<tr>
<td>282</td>
<td>do</td>
<td></td>
<td>6,000,000</td>
<td>10</td>
</tr>
<tr>
<td>283</td>
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<td></td>
<td>12,000,000</td>
<td>20</td>
</tr>
<tr>
<td>286</td>
<td>Single column tower run</td>
<td>24 hours</td>
<td>12,000,000</td>
<td>50</td>
</tr>
<tr>
<td>289</td>
<td>do</td>
<td>48 hours</td>
<td>1,000,000</td>
<td>1</td>
</tr>
<tr>
<td>295</td>
<td>do</td>
<td>119 hours</td>
<td>87,000,000</td>
<td>50</td>
</tr>
<tr>
<td>297</td>
<td>do</td>
<td>111 hours</td>
<td>112,000,000</td>
<td>40</td>
</tr>
<tr>
<td>298</td>
<td>do</td>
<td>119 hours</td>
<td>70,000,000</td>
<td>40</td>
</tr>
<tr>
<td>305</td>
<td>do</td>
<td>0 hours</td>
<td>34,000</td>
<td>90</td>
</tr>
<tr>
<td>286</td>
<td>Single column tower run</td>
<td></td>
<td>12,000,000</td>
<td>50</td>
</tr>
<tr>
<td>289</td>
<td>do</td>
<td></td>
<td>1,000,000</td>
<td>1</td>
</tr>
<tr>
<td>295</td>
<td>do</td>
<td></td>
<td>87,000,000</td>
<td>50</td>
</tr>
<tr>
<td>297</td>
<td>do</td>
<td></td>
<td>112,000,000</td>
<td>40</td>
</tr>
<tr>
<td>298</td>
<td>do</td>
<td></td>
<td>70,000,000</td>
<td>40</td>
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<tr>
<td>305</td>
<td>do</td>
<td></td>
<td>34,000</td>
<td>90</td>
</tr>
</tbody>
</table>

* Isolates from other processed stillage judged to be similar on the basis of morphology, staining, and colony and streak characters.
† C. T. Butterfield, U. S. Public Health Service, Cincinnati, Ohio.

Properties of the isolates are shown in Tables 15, 16, 17, and 18. Most of the isolates were Gram negative, nonspore-forming rods of medium size similar to predominating types found in trickling filters for sewage purification. All produced mucoid growth on solid media, and all but one of the rod-shaped forms were more or less heavily capsulated, a property imparting ability to produce zoogloeal growth and flocs having high absorptive capacity for organic substrate. The two types most commonly encountered and found in high proportion on plates are represented by isolates No. 233 and No. 235. Both are active xylose fermenters, a character of particular interest because
of the wood sugar in stillage; No. 233, however, was unable to form acid from this sugar in the synthetic medium. The same limitation was shown by 5 of the 10 isolates fermenting xylose in nutrient broth. This suggests a stimulating factor in peptone or beef extract and invites speculation as to possible effects of such a factor, which could possibly be introduced with sewage to facilitate operation of a stillage filter. It should be noted, incidentally, that the sewage filter isolates, Z60 and Z100, are nonxylose fermenters. Isolate No. 225 with synthetic medium revealed xylose fermentation that was masked in the broth. In several instances with the other sugars, particularly dextrose, masked fermentations were similarly revealed.

Other isolates of interest are No. 223, a xylose-fermenting yeast predominating in the Springfield composite sample, and No. 225, a slow xylose-fermenting bacterium found to predominate in an old stored sample of Madison stillage. The sarcina, No. 305, was the only coccus encountered, and it is apparently unique in the genus for fermenting xylose (4). Isolate No. 289, a nonxylose-fermenting Bacillus, low in order of occurrence, was probably a contaminant. Lack of xylose-fermenting ability, however, does not necessarily preclude significance of an organism in filter action. A nonxylose fermenter may function efficiently in decomposing other constituents of stillage, or it may transform by-products of xylose fermentation and thus hasten or complete the oxidation. It is nonetheless significant that, of the 16 stillage isolates, 11 fermented xylose.

The relatively slow rates of fermentation indicated in Table 18 for most of the isolates may be attributed to the more or less strongly aerobic nature of the organisms. With more adequate aeration, as on a trickling filter, the action would be more rapid and also probably more complete.

Other characteristics of the various isolates are not discussed, since they are of interest primarily to the systematist and are recorded for reference in possible future investigations. The data have been used in an attempt to classify and identify the bacteria according to Bergey's Manual (4). While it is possible to give each a generic designation (Table 19), none has the combination of characters necessary to agree with any described species. Representatives of Pseudomonas and Achromobacter were of most frequent occurrence.
### Table 15. Morphology and Staining Reactions of Stillage Isolates

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>General morphology</th>
<th>Size (microns)</th>
<th>Capsule thickness (microns)</th>
<th>Spores*</th>
<th>Motility and flagellation</th>
<th>Gram reaction†</th>
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</thead>
<tbody>
<tr>
<td>223</td>
<td>Apiculate yeast</td>
<td>3.2 x 4.4</td>
<td>Absent</td>
<td>+</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>225</td>
<td>Rod, single and short chains</td>
<td>1.0 x 2.3</td>
<td>0.5</td>
<td>-</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
<tr>
<td>228</td>
<td>Pleomorphic rod, single and in short chains</td>
<td>1.0 x 2.7</td>
<td>0.3</td>
<td>-</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>233</td>
<td>Rod, single</td>
<td>0.5 x 1.8</td>
<td>0.5</td>
<td>-</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
<tr>
<td>234</td>
<td>Rod, single</td>
<td>0.8 x 2.4</td>
<td>0.3</td>
<td>-</td>
<td>Monotrichous</td>
<td>+</td>
</tr>
<tr>
<td>235</td>
<td>Rod, single, pairs</td>
<td>0.9 x 2.5</td>
<td>0.5</td>
<td>-</td>
<td>Monotrichous</td>
<td>+</td>
</tr>
<tr>
<td>236</td>
<td>Rod, single</td>
<td>0.5 x 1.7</td>
<td>0.5</td>
<td>-</td>
<td>Monotrichous</td>
<td>+</td>
</tr>
<tr>
<td>237</td>
<td>Rod, single</td>
<td>0.6 x 2.6</td>
<td>0.4</td>
<td>-</td>
<td>Monotrichous</td>
<td>+</td>
</tr>
<tr>
<td>238</td>
<td>Rod, oval to long, pleomorphic, yeast-like</td>
<td>1.0 x 3.3</td>
<td>Absent</td>
<td>+</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>239</td>
<td>Rod, coccoid to long</td>
<td>0.8 x 1.9</td>
<td>0.3</td>
<td>-</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
<tr>
<td>240</td>
<td>Rod, single, pairs, filaments, irregular</td>
<td>0.9 x 1.8</td>
<td>0.9</td>
<td>-</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
<tr>
<td>241</td>
<td>Rod, single, pleomorphic, tapering, curved, clubbed</td>
<td>1.1 x 4.3</td>
<td>1.0</td>
<td>+</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
<tr>
<td>242</td>
<td>Rod, single</td>
<td>1.0 x 2.5</td>
<td>0.5</td>
<td>-</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
<tr>
<td>243</td>
<td>Rod, single</td>
<td>0.7 x 2.0</td>
<td>0.5</td>
<td>-</td>
<td>Lophotrichous</td>
<td>+</td>
</tr>
<tr>
<td>244</td>
<td>Rod, plump to oval</td>
<td>1.0 x 1.6</td>
<td>0.7</td>
<td>-</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
<tr>
<td>245</td>
<td>Sarcina</td>
<td>0.8</td>
<td>Absent</td>
<td>-</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>246</td>
<td>Rod, single</td>
<td>0.6 x 1.7</td>
<td>0.5</td>
<td>-</td>
<td>Monotrichous</td>
<td>+</td>
</tr>
<tr>
<td>247</td>
<td>Rod, single</td>
<td>0.5 x 2.6</td>
<td>0.3</td>
<td>-</td>
<td>Peritrichous</td>
<td>+</td>
</tr>
</tbody>
</table>

* = --- absent; + = present.  
† = — negative; + = positive; +— = variable.  
‡ Ability to form spores not determined
<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Form</th>
<th>Diameter (mm.)</th>
<th>Surface</th>
<th>Elevation</th>
<th>Margin</th>
<th>Internal structure</th>
<th>Optical properties</th>
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<tbody>
<tr>
<td>223</td>
<td>Circular</td>
<td>3</td>
<td>Smooth</td>
<td>Pulvinate</td>
<td>Entire</td>
<td>Granular</td>
<td>White, opaque, matte</td>
</tr>
<tr>
<td>225</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>Curled</td>
<td>Filamentous</td>
<td>White, opaque, glistening</td>
</tr>
<tr>
<td>227</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>Finely erose</td>
<td>Granular</td>
<td>Yellowish-white, opaque, translucent</td>
</tr>
<tr>
<td>228</td>
<td>...do...</td>
<td>2</td>
<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Finely granular</td>
<td>...do...</td>
</tr>
<tr>
<td>229</td>
<td>...do...</td>
<td>2</td>
<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Granular</td>
<td>Yellowish-white, opaque, glistening</td>
</tr>
<tr>
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<td>1</td>
<td>...do...</td>
<td>Pulvinate</td>
<td>do</td>
<td>Finely granular</td>
<td>...do...</td>
</tr>
<tr>
<td>231</td>
<td>...do...</td>
<td>2</td>
<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Granular</td>
<td>...do...</td>
</tr>
<tr>
<td>232</td>
<td>...do...</td>
<td>2</td>
<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Finely granular</td>
<td>...do...</td>
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<tr>
<td>233</td>
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<td>2</td>
<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Coarsely granular</td>
<td>Coral pink, opaque, glistening</td>
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<tr>
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<td>Convex</td>
<td>Entire</td>
<td>Granular</td>
<td>...do...</td>
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<tr>
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<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Granular</td>
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<tr>
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<td>2</td>
<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Granular</td>
<td>...do...</td>
</tr>
<tr>
<td>237</td>
<td>...do...</td>
<td>2</td>
<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Granular</td>
<td>...do...</td>
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<tr>
<td>238</td>
<td>...do...</td>
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<td>...do...</td>
<td>Convex</td>
<td>Entire</td>
<td>Granular</td>
<td>...do...</td>
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<tr>
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<td>2</td>
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<td>Entire</td>
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<table>
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<th>Isolate number</th>
<th>Form</th>
<th>Lustre</th>
<th>Consistency</th>
<th>Optical character</th>
<th>Chromogenesis</th>
<th>Odor</th>
<th>Surface growth</th>
<th>Turbidity</th>
<th>Sediment</th>
<th>Odor</th>
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<td>223</td>
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<td>Butyrous</td>
<td>Opake</td>
<td>White</td>
<td>Yeasty</td>
<td>None</td>
<td>Slight</td>
<td>Granular</td>
<td>None</td>
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<td>...do...</td>
<td>...do...</td>
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<td>...do...</td>
<td>...do...</td>
<td>Viscid</td>
<td>...do...</td>
</tr>
<tr>
<td>227</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>None</td>
<td>...do...</td>
<td>...do...</td>
<td>Ring</td>
<td>Moderate</td>
</tr>
<tr>
<td>229</td>
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<td>...do...</td>
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<td>...do...</td>
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<td>Moderate</td>
</tr>
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<td>...do...</td>
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<td>...do...</td>
<td>...do...</td>
<td>Viscid</td>
<td>Granular</td>
</tr>
<tr>
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<td>...do...</td>
<td>...do...</td>
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<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>...do...</td>
<td>None</td>
<td>...do...</td>
<td>...do...</td>
<td>Viscid</td>
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* Irregular margin.
† Strong odor.
‡ Slight.
§ Moderate.
Table 17. Physiological Reactions of Stillage Isolates

<table>
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<tr>
<th>Isolate number</th>
<th>Dextrose shake*</th>
<th>Gelatin liquefaction†</th>
<th>Starch hydrolysis on plates‡</th>
<th>Nitrate reduction§</th>
<th>Indol†</th>
<th>MR‡</th>
<th>VP§</th>
<th>H₂S¶</th>
<th>Fermentations in nutrient broth¶</th>
<th>Litmus milk</th>
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* f = facultative, a = aerobic.
† + = reaction or product produced, - = no reaction or product produced.
‡ + = methyl red positive, - = methyl red negative.
§ - = Voges-Proskauer reaction negative.
¶ + = hydrogen sulphide produced, - = hydrogen sulphide not produced.
Il Indicates acid, = indicates neutral, = indicates alkaline, 0 indicates no change, ± indicates slight acidity.

No gas was formed in any of the fermentations.
Table 18. Fermentation Reactions of Stillage Isolates in Synthetic Media*†

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<tr>
<th>Isolate number</th>
<th>Dextrose</th>
<th>Levulose</th>
<th>Lactose</th>
<th>Sucrose</th>
<th>Xylose</th>
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* Synthetic medium of Ayers, Rupp and Johnson plus filter-sterilized solution of sugar to give 1 per cent concentration of the carbohydrate.
† Acid formation indicated by plus sign; figure following plus sign indicates number of days for reaction to appear. Minus sign indicates alkaline; 0 indicates no growth.

CONCLUSIONS

Of the various disposal possibilities investigated, only the bacterial trickling filter shows promise. With supplemental aeration and recirculation under favorable experimental conditions, approximately 50 per cent of the 5-day B.O.D. was removed with a throughput equivalent to one gallon of stillage per cubic foot of gravel per day. With less rapid throughput, B.O.D. was reduced nearly 70 per cent, while 90 per cent of the sugar and 70 per cent of the total carbon were removed. Bacteria of Pseudomonas and Achromobacter types were most abundant on the filters. If further investigation is desired, it is suggested that it be made on a larger scale with adequate apparatus and controls and only when a large supply of typical stillage, completely analyzed, is available.

Table 19. Generic Designations of Bacteria from Stillage

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Table 18. Fermentation Reactions of Stillage Isolates in Synthetic Media*†

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* Synthetic medium of Ayers, Rupp and Johnson plus filter-sterilized solution of sugar to give 1 per cent concentration of the carbohydrate.
† Acid formation indicated by plus sign; figure following plus sign indicates number of days for reaction to appear. Minus sign indicates alkaline; 0 indicates no growth.
LITERATURE CITED


