RICE UNIVERSITY

MEASUREMENT OF THE
TOTAL BIOLOGICAL OXYGEN DEMAND
BY MASS CULTURE AERATION

by

Leland L. Hiser

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

Thesis Director's Signature:

Houston, Texas
April, 1963
ABSTRACT

Development of the field of environmental engineering has long been impeded by the lack of a quick, accurate method of measuring the biochemically oxidizable organic content of waste waters. Such a test is important to the detection and prevention of pollution of rivers, lakes, and other receiving waters. Proper operation of existing waste water treatment plants and the design of new waste treatment plants also depend on such a parameter. Rapid development of the chemical industry in recent years has further emphasized the need for such a test.

A new test is presented which measures the disappearance of soluble organics from a substrate in contact with an active mass culture of mixed microbial organisms. Soluble organics in the system are measured by the Chemical Oxygen Demand (COD) test and reported in oxygen equivalents. The system substrate COD reaches a minimum when the soluble organics available to the mass culture for metabolism have been depleted. Substrate COD remains relatively constant from this point on. The difference between the initial substrate COD and the minimum substrate COD reached after aeration is then the Total Biological Oxygen Demand (TbOD) of the system.

This TbOD test may be completed in 6 to 8 hours (approximately 4 to 6 hours laboratory time). In addition to measuring the TbOD, the test may be used, with modifications, to define process kinetics for design, to serve as a daily log of plant operation, or be used as a quick method of diagnosing plant operation difficulties. Delineation of plant
cyclic load variations is another use for this test. This $T_b$ OD test will be particularly useful for the control of operating plants since an acclimated culture is available from the plant at all times.
ACKNOWLEDGEMENTS

I wish to extend my sincere thanks and appreciation to:
Professor A. W. Busch for his able support and guidance,
Rice University for an inspiring environment,
Dr. Nugent Myrick and fellow graduate students for their assistance and fellowship,
My wife, Ruth, and children for their patience and understanding,
The U. S. Public Health Service, Division of Water Supply and Pollution Control for their financial support of a portion of this work through Training Grant 1T1-WP12-01 and Research Grant WP-12.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>i</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>iv</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. PREVIOUS WORK</td>
<td>8</td>
</tr>
<tr>
<td>III. THEORETICAL CONSIDERATIONS</td>
<td>10</td>
</tr>
<tr>
<td>IV. EXPERIMENTAL TECHNIQUE</td>
<td>13</td>
</tr>
<tr>
<td>V. EXPERIMENTAL RESULTS</td>
<td>21</td>
</tr>
<tr>
<td>VI. DISCUSSION OF RESULTS</td>
<td>42</td>
</tr>
<tr>
<td>VII. FUTURE WORK</td>
<td>48</td>
</tr>
<tr>
<td>VIII. CONCLUSIONS</td>
<td>49</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>50</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Pure Substrate Systems</td>
<td>23</td>
</tr>
<tr>
<td>II.</td>
<td>Pure Substrates T&lt;sub&gt;b&lt;/sub&gt;OD Data</td>
<td>24</td>
</tr>
<tr>
<td>III.</td>
<td>Pure Substrate-EDTA Mixture Systems</td>
<td>30</td>
</tr>
<tr>
<td>IV.</td>
<td>Pure Substrate-EDTA Mixture T&lt;sub&gt;b&lt;/sub&gt;OD Data</td>
<td>31</td>
</tr>
<tr>
<td>V.</td>
<td>Industrial Waste Systems</td>
<td>37</td>
</tr>
<tr>
<td>VI.</td>
<td>Industrial Wastes T&lt;sub&gt;b&lt;/sub&gt;OD Data</td>
<td>38</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.</td>
<td>$T_b$OD Progression with Mass Culture Aeration</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>Glucose $T_b$OD Progression</td>
<td>27</td>
</tr>
<tr>
<td>3.</td>
<td>Glutamic Acid $T_b$OD Progression</td>
<td>28</td>
</tr>
<tr>
<td>4.</td>
<td>Sodium Acetate $T_b$OD Progression</td>
<td>29</td>
</tr>
<tr>
<td>5.</td>
<td>Glucose-EDTA Mixture $T_b$OD Progression</td>
<td>32</td>
</tr>
<tr>
<td>6.</td>
<td>Glutamic Acid-EDTA Mixture $T_b$OD Progression</td>
<td>33</td>
</tr>
<tr>
<td>7.</td>
<td>Formaldehyde-Methanol Waste $T_b$OD Progression</td>
<td>39</td>
</tr>
<tr>
<td>8.</td>
<td>Phthalic Anhydride Waste $T_b$OD Progression</td>
<td>40</td>
</tr>
<tr>
<td>9.</td>
<td>Ethylene Glycol Waste $T_b$OD Progression</td>
<td>41</td>
</tr>
<tr>
<td>10.</td>
<td>Rate-Concentration Curve</td>
<td>45</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

Currently the 5-day BOD test\(^{(1)}\) is used as the prime measure of the pollutional strength of waste waters. The 5-day BOD determination is a measure of the oxygen utilized in 5 days by a heterogeneous population of microbial organisms mixed with a dilute substrate in a closed environment. The ultimate oxygen demand of the substrate is obtained by extrapolation using the assumption of Streeter and Phelps\(^{(2)}\) that reaction kinetics of this system are "first order".

Busch\(^{(3,4,5)}\) et al have proved the fallacy of the "first order" assumption and have shown the presence of a consistent short term plateau in the BOD progression of pure substrates. Busch has further presented the theory that this "plateau" is coincident with the conversion of all available substrate to bacterial cell material. Although the "short term" BOD test has been shown valid for pure substrates, it is of little use on complex organic wastes in which the relation of the "plateau" value to total oxygen demand is not known. Because of this Busch\(^{(6)}\) proposed a more sophisticated \(T_b\) test with three different methods for its determination.

These methods are:

\[ I. \quad T_b \text{OD} = \text{Plateau BOD (short term)} + 1.414 \times \text{weight of cells synthesized} \]

\[ II. \quad T_b \text{OD} = \text{Plateau BOD (short term)} + \text{COD of cells synthesized} \]

\[ III. \quad T_b \text{OD} = \text{Mass culture} \cdot \Delta \text{COD} \]

The first two methods have been studied by Grady\(^{(7)}\). Method I and II have the disadvantages of requiring rigorous
laboratory technique and having possible indefinite short
term BOD plateaus with complex organic wastes. Determination
of TbOD by these two methods requires 24 to 30 hours for
completion. Although their precision and accuracy are valu¬
able as research tools, this time element limits the use¬
fulness of Methods I and II.

Method III was also studied by Grady(7) using the
micro-terms of the BOD bottle.

Busch(6) proposed the Mass Culture Aeration TbOD test
(Method III) in November, 1961. The purpose of this work
was to determine whether that proposed method holds promise
as a widely applicable analytical procedure.

The TbOD test (Method III) utilizes the COD(1) deter¬
mination for measuring the soluble organic concentration of
substrates. The COD determination consists of refluxing a
known amount of strong oxidizing agent (potassium dichromate)
with an aliquot of the sample in an acid environment. The
amount of oxidizing agent reduced is reported as the Chemical
Oxygen Demand (COD) of the sample. The COD determination
has long been used as a measure of organic pollutional
content of waste waters. However, the relation of the COD
to the BOD of a waste has, for the most part, been confused.
The TbOD test clarifies this relationship by showing that
the net reduction in COD in a particular batch-type biologi¬
cal mass culture-substrate system is the Total Biological
Oxygen Demand of that system.
The following definitions are for the purpose of clarification in this thesis:

substrate--the soluble fraction of the raw or diluted waste which will serve as food for the culture.
culture--the washed, aerobic, microbial organisms suspended in tap water.
mixed liquor--the combination of substrate and culture.
soluble--any material passing through a membrane filter (0.45 micron pore size).
mixed liquor suspended solids or MLSS--the dry weight of solids retained on the membrane filter per liter of mixed liquor filtered.

$\Delta$ COD--the difference between the calculated "O" or initial mixed liquor COD and the minimum mixed liquor COD. The $\Delta$ COD will be specified as either "Calc. $\Delta$ COD" or "Meas. $\Delta$ COD" depending on whether the calculated or measured value of the "O" mixed liquor was used. The term COD will always refer to the chemical oxygen demand of the soluble fraction of an aliquot, as all COD determinations were made on filtered samples.

Further reference in this thesis to the $T_{bOD}$ test refers to its determination by Method III noted above unless otherwise specified.

(a) Manufactured by Millipore Filter Co., Bedford, Mass.
The T<sub>b</sub>OD test involves first, separately measuring the COD of a substrate, and a washed mass culture of aerobic microbial organisms suspended in tap water. Next the mass culture and substrate are combined in known volumes and aerated. Utilization of the substrate by the mass culture may then be traced by determining the COD of incremental time samples of the mixed liquor. Since all mixed liquor samples are homogenous within the system, removal of the samples has no effect on the system kinetics. When the soluble organic content of the mixed liquor available as food to the micro-organisms has been depleted, there will be no further reduction in mixed liquor COD. The concept of mixed liquor soluble COD reduction with time is shown on Figure 1. The T<sub>b</sub>OD of the substrate may be calculated, as shown on Figure 1, to be equal to the \( \Delta \text{COD} \) (net reduction in mixed liquor COD) multiplied by the ratio of initial mixed liquor volume to initial substrate volume.

The T<sub>b</sub>OD test measures the total soluble organic content of a waste or substrate that is amenable to microbiological metabolism. This statement must be qualified to the extent that only the portion of organics measurable by the COD test will be included. With this qualification, the T<sub>b</sub>OD test is a measure of the ultimate oxygen demand of a waste rather than merely the oxygen uptake recorded at some indefinite, incomplete phase of the organic carbon oxidation
Mixed Liquor Soluble COD - mg/l

MLSS - mg/l

\[ T_{b,OD} = \Delta \text{COD} \times \text{DILUTION FACTOR}^* \]

*DILUTION FACTOR = \( \frac{\text{MIXED LIQUOR VOLUME}}{\text{SUBSTRATE VOLUME}} \)

FIG. I - \( T_{b,OD} \) PROGRESSION WITH MASS CULTURE AERATION
to CO₂, typified by the 5 day BOD test. The T_bOD of a waste represents the total oxygen required to convert the organics to carbon dioxide and water. For all practical purposes this oxygen demand will never be realized in treatment plant or stream. Therefore, the T_bOD test contains an inherent safety factor for the oxygen requirement placed on a treatment plant or receiving water by a particular waste.

Cell synthesis during T_bOD progression is also shown on Figure 1. "Mixed liquor suspended solids" (MLSS) concentration increases as mixed liquor COD decreases. In a growth system, the MLSS will reach a maximum as the mixed liquor COD reaches the minimum. It is proposed, in Figure 1, that the T_bOD progression is a first order reaction, with food limitation a significant factor. In this event, endogenous respiration may counterbalance growth sufficiently to produce a MLSS maximum prior to the time of mixed liquor minimum COD as shown.

Test runs were made on 8 different substrates. These included three pure substrates (glucose, glutamic acid, and sodium acetate), two mixtures of pure substrate with EDTA (glucose, and glutamic acid with sodium ethylenediaminetetraacetate) and three industrial waste samples. The industrial waste substrates studied consisted of a formaldehyde-methanol-trioxane waste from plastics manufacture, an organic acid waste from phthalic anhydride manufacture, and a process waste from the manufacture of ethylene glycol.
The pure substrates were selected for their known biological degradability. The mixtures of pure substrates with EDTA were selected in order to provide a synthetic waste of known degradability and a high residual COD. The industrial wastes were used to show the precision of the test when applied to practical situations outside of the research laboratory.
II. PREVIOUS WORK

Symons, McKinney, and Hassis\(^{(8)}\) noted in their work with industrial wastes that "the change in soluble COD through an activated sludge pilot plant tells exactly what portion of the organic matter in the industrial waste is being removed and, discounting special cases, this removal is due to biological action only." They further stated, "If the ultimate BOD of the raw waste were known, this would be a measure of all of the biologically oxidizable material in the waste and would be equal to the change in soluble COD through the pilot plant." However, their work was done on a 24 hour basis and they failed to realize the significance of the COD as a quick test for total biological oxygen demand.

As previously noted, Busch\(^{(3,4,5,6)}\) et al have for some years been working on a faster, more definitive measure of biological oxygen demand than provided by the 5 day BOD test. Their work has led to the concept outlined in this thesis.

Gaudy\(^{(9,17)}\) came close to realizing the usefulness of the T\(_b\)OD test in his work with sequential substrate removals by mixed microbial populations. He used the COD test as a measure of the sequential disappearance of glucose and sorbitol from a mixed culture. T\(_b\)OD calculations made on Gaudy's published curves (on glucose-sorbitol removal in activated sludge systems) gave 96.5% of the theoretical T\(_b\)OD. However, Gaudy failed to point out the use of mass culture
aeration - $\Delta$ COD as a quantitative measure of the $T_b$OD of the system.

Moore et al.$^{(10,11,12)}$ have done considerable work on the COD determination as a definitive test. This work includes the recovery of a wide variety of organic compounds, the study of various oxidizing agents, and the use of silver sulphate for a catalyst as proposed by Muers$^{(13)}$.

Dobbs and Williams$^{(14)}$ have also contributed to the usefulness of the COD test by proposing a mercuric sulphate complexing modification to eliminate the need for a chloride correction and to prevent chloride interference with the silver sulphate catalyst.

Gaudy and Ramanathan$^{(15)}$ have further proposed a colorimetric determination of COD to replace the ferrous ammonium titration step. This procedure will save laboratory time when a considerable number of determinations are to be made.
III. THEORETICAL CONSIDERATIONS

The basic concept of the T\textsubscript{b}OD test (Method III) is that the biochemically available soluble organic material in an acclimated mass culture system will be absorbed and metabolized by the bacteria to the extent that all available substrate will be utilized. From this point on, the remaining concentration of soluble organic material (the COD) will stay relatively constant. This residual COD will consist of material not available to the culture for metabolism, end products from the metabolism of that material which was available, and residuals of lysed organisms of the microbial population.

Proper substrate to culture proportioning or loading of the mass culture should allow this minimum COD to be reached in a reasonably short period of time. Completion of the test within six to eight hours, including the COD determinations should thus be possible.

Biochemical pathways are essentially ignored by this test, other than provision for sufficient nutrients and adjustment of the pH when necessary. It was felt that the T\textsubscript{b}OD of a substrate should be independent of culture age, or previous feeding habits, as long as the culture was developed from a heterogeneous population and acclimated to the substrate involved. Otherwise, the advantages of the test would be lost. It is likely that due to the long acclimation periods of the cultures prior to testing, they tended to consist of fewer species of organisms than originally intro-
duced. This was especially evident in the formaldehyde-
methanol industrial waste culture which had a decided pink
color and may well have been a nearly pure culture of the
same Bacterium methylicum identified by Gellman and Heukel-
ekian\(^{(16)}\) in their work with formaldehyde.

Obviously this \(T_b\)OD test is limited by the accuracy
and precision of the COD determination itself. The work of
Moore et al\(^{(9,10,11)}\) has delineated this accuracy for a
large number of compounds. Based on their work, the
potassium dichromate-silver sulphate method was selected as
providing the most complete oxidation of most organic com-
ounds. Since only a change in COD was being determined
rather than an absolute value, the effect of chlorides
should have cancelled out. Therefore, no correction was made
for chlorides initially. Later the mercuric sulphate\(^{(13)}\)
modification for complexing chlorides was adopted as a use-
ful means of preventing precipitation of silver in the COD
flask.

Air stripping of volatile organics from the mixed
liquor is another factor to be considered. It was felt that,
due to the method of vacuum filtration used on the samples,
volatile fractions present were probably not measured, and
therefore beyond the scope of this work. Certainly the
problem is not pertinent for these pure compounds. It is
suggested that this factor be studied with a different tech-
nique for solids-substrate separation such as centrifugation
of samples.
IV. EXPERIMENTAL TECHNIQUE

The aeration system used for the mass cultures consisted of a 1000 ml graduated cylinder and a porous carborundum diffuser. Use of the graduated cylinder facilitated make up of evaporation losses with distilled water. An important factor in this test is the provision of good mixing. Provision of sufficient air to produce good mixing will normally also provide an excess of oxygen to the system. This may be shown by the conservative assumption of 1% oxygen transfer efficiency for the system used. It was found that approximately 10 scf/hr of air was required to maintain good mixing in the cylinder. Five scf/hr did not provide sufficient mixing to maintain solids in suspension and 15 scf/hr caused excessive surface splashing and carry over. Ten scf/hr at 1% transfer efficiency provides approximately 700 mg. O$_2$ per hour to the system. It is possible that a system could exceed this oxygen requirement. However, the systems studied here did not approach this oxygen utilization rate, especially when consideration is given to cell synthesis. It is also possible that a waste being studied might contain a surface active agent which could adversely affect the oxygen transfer at the film surface. Such a phenomena would probably be characterized by a slow rate of COD progression due to oxygen transfer limitations.

A battery of three filter flasks was set up to accommodate the membrane filters for sample filtering at frequent
intervals. Test tubes (75 ml) with screw caps were used to receive the sample filtrate. These sample tubes were calibrated for 10 ml and 40 ml volumes.

The sample pipette was a standard 25 ml volumetric pipette with the delivery end cut off approximately 1/2" to give a larger opening. This provided free passage of suspended solids into the pipette.

The COD reflux apparatus consisted of a battery of 6 heaters fitted with Allihn condensers with tapered glass necks. Special COD flasks were made by fitting 500 ml Erhrlenmeyer flasks with ground tapered glass necks to fit the Allihn condensers. These flasks were calibrated for the correct dilution volume, so that dilution and titration of the refluxed sample were carried out in the reflux flask. A 25 ml (0.1 ml division) Shellbach Burette was used for all COD titrations.

The general procedure of experiments was as follows:
1. Pretreatment of the acclimated culture consisted of washing in tap water to remove soluble organics remaining from previous feedings and provision for nutrients when required.
2. The membrane filters were tared and the filtering apparatus assembled.
3. The culture and substrate COD samples were taken.
4. Substrate was added to the aerating culture at "0" time, allowed to mix one to three minutes, and
the "0" hour mixed liquor COD samples drawn.

5. The mixed liquor was sampled at intervals to trace the COD reduction with time.

6. Filters were returned to the oven for overnight drying at 103°C.

7. The following morning a final sample was drawn, and with the exception of a few cases, the run ended there.

8. COD's were set up on all samples, using tap water blanks as outlined in the detailed procedure below.

9. Filters were weighed to complete MLSS data.

10. The $T_{b \text{OD}}$ calculation was made using the formula:

$$T_{b \text{OD}} = \frac{\Delta \text{COD} \times \frac{a+b}{b}}{b}$$

where: $\Delta \text{COD} = \text{calculated "0" M. L. COD} - \text{Minimum M. L. COD} = \text{mg/l}$

$a = \text{initial culture volume} = \text{ml}$

$b = \text{initial substrate volume} = \text{ml}$

Several of the above steps require more detailed description.

Sludge pretreatment;

All cultures used in these experiments were initially started from sewage seed and acclimated to the substrate for periods of one week to several months. Immediate pretreatment of the culture then consisted of washing out, by several dilutions, existing soluble organics from the culture liquor
to provide essentially a culture - tap water mixture. This is important in that the test did not differentiate between biodegradation of the existing soluble organics in the culture liquor and the added soluble organics in the substrate. Good washing provided a culture liquor background COD of 10 to 20 mg/l compared to a tap water blank.

Nitrogen and phosphorous nutrients in the form of \((\text{NH}_4\text{)}_2\text{HPO}_4\) were added to the final culture dilution water to give a minimum \(T_b\text{OD}\) to nitrogen ratio of 15:1.

Homogenization of the culture solids was tried on several of the experiments on glucose. The culture was mixed in a Waring Blender for one minute prior to final dilution with tap water. This procedure resulted in better mixed liquor solids data and higher unit substrate utilization rates. However, it also resulted in faster blinding of the filter and a higher culture COD and homogenizing was discontinued. The latter factor was shown in run number 1 in which the culture COD was 27 mg/l. The minimum mixed liquor COD was 20 mg/l. Further dilution of the culture with tap water to the mixed liquor volume would also have resulted in a culture COD of 20 mg/l. The question then arose whether any of the culture COD had been available to the organisms for metabolism. It was decided that when the minimum mixed liquor COD was lower than the culture COD, the culture COD would be used to calculate the \(T_b\text{OD}\) of the substrate. This gave lower results than would have been obtained otherwise.
Preparation of Substrate:

The stock solutions of pure substrates and mixtures were prepared with dried (2 hours at 103°C) analyzed reagent compounds in tap water. Aliquots of these standard solutions were diluted further with tap water for COD samples and use as substrate feed. In some cases aliquots of the standard solutions were fed directly, without further dilution. The theoretical T<sub>b</sub>OD of the pure substrates and mixtures are shown on Tables I and III. These are based on the following ratios of gram substrate per gram T<sub>b</sub>OD for glucose, glutamic acid, and sodium acetate respectively: 0.9345, 1.021, and 1.282.

The industrial wastes were diluted with tap water for COD samples and fed as either concentrated waste or diluted waste to the culture. Since the theoretical T<sub>b</sub>OD and COD of the industrial wastes were not known, the arithmetic averages, T<sub>b</sub>OD and COD, were used for comparative purposes.

Sampling Procedure:

Membrane filters (0.45 micron pore size) were used to filter all samples except tap water blanks and stock solutions. Industrial waste samples were all filtered prior to analysis.

It was found that evaporation during a run was of importance and amounted to 1 to 2 ml/hour. On short aeration periods with large mixed liquor volumes, the effect was negligible. However, the 1000 ml graduated cylinder was adopted for the
aeration vessel to facilitate making up evaporation loss with distilled water prior to sampling.

The 25 ml sample pipette was rinsed onto the filter with tap water, following delivery of the sample, to improve MLSS data. When the sample and pipette rinse water had passed the filter, the filter was rinsed further with tap water to the 40 ml mark on the sample test tube. The sample tube was then capped and stored in the refrigerator at 4°C until COD determinations were made.

In most cases the entire sampling procedure was completed within 2 to 5 minutes. However, in some cases, notably with glucose, the culture was of a character that blinded the filter and as long as 30 minutes were required for filtering. It is felt that this phenomenon would not affect the minimum COD, or the T\textsubscript{bOD}, but might displace the sample point on any graph or function involving time.

COD Procedure:

Following transfer of the 40 ml filtered, diluted sample from its tube to a COD flask, the sample tube was rinsed with 10 ml tap water (10 ml calibration on the tube) and this was added to the COD flask to provide a 50 ml diluted sample. All COD analyses were made according to AWWA Standard Methods\textsuperscript{(1)} with the following modifications:

1. Silver sulphate catalyst was used in all COD determinations. It was dissolved in the concentrated H\textsubscript{2}SO\textsubscript{4} to provide 1 gm Ag\textsubscript{2}SO\textsubscript{4} per COD flask.
2. Ten ml 0.250 N \( \text{K}_2\text{Cr}_2\text{O}_7 \) were used with few exceptions. Fifty ml of total diluted sample, 60 ml of concentrated \( \text{H}_2\text{SO}_4 \) and a final dilution volume of 280 ml for titration were used to maintain identical acid concentrations as called for in Standard Methods.

3. It was felt desirable to rinse all mixed liquor and culture samples through the filter with tap water. This eliminated the possibility of plasmopotomy of the filtered cells (swelling and bursting due to osmotic pressure) with the resultant release of soluble cell components to the sample filtrate. For this reason too, tap water was used for substrate dilutions. Refluxed COD blanks were therefore run on tap water samples (50 ml). Condenser rinses and final dilutions in all COD determinations were made with distilled water. Tap water used for all blanks and rinses of sample pipette, filters, and sample tubes was drawn at one time for each experiment and stored in the dark for use until completion of that experiment.

4. In the last several experiments \( \text{HgSO}_4 \) was used to complex chlorides per Dobbs and Williams. (14)

5. As noted above in the COD apparatus arrangement, special flat bottomed COD flasks were made so that all dilutions and titrations were completed in this
same flask. Allihn reflux condensers were used rather than the Friedrichs type.

**Solids Data:**

As noted above, the Millipore filters were dried overnight in an electric oven at 103°C. Immediately before use they were transferred to a glass dessicator for cooling and tared on a Mettler analytical, single pan, direct reading balance (accuracy ± 0.05 mg). After sample filtration, the filters were again dried overnight at 103°C and weighed the following morning for MLSS measurement.

**Calculations:**

The "0" time or initial mixed liquor COD was measured by sampling after 1 to 3 minutes mixing. It was also calculated by a simple mass balance using the separate COD and volumes of the substrate and culture as follows:

\[ "O" \text{ mixed liquor COD} = \frac{(a \times c) + (b \times d)}{a + b} - \text{mg/l (B)} \]

where:
- \( c \) = culture COD - mg/l
- \( a \) = culture volume - liters
- \( d \) = substrate COD - mg/l
- \( b \) = substrate volume - liters

Both the measured "0" mixed liquor COD (as determined from "0" time samples) and the calculated "0" mixed liquor COD are presented in the data tables.
Experimental results are presented in two tables for each group of substrates. The first table shows the volume and COD of the culture and substrate, the calculated and measured COD of the mixed liquor, and the MLSS. All data in the tables regarding mixed liquor refer to the initial or "O" time condition. The mixed liquor initial volume is the sum of the culture and substrate volumes.

The second table for each group presents the initial loading, time from "O" to minimum COD, unit assimilation rate, COD of the system, calculated $T_b$OD, and $T_b$OD as percent of theoretical $T_b$OD and as percent of substrate COD. In the case of the industrial wastes, the average $T_b$OD or $T_b$OD is used for percentage calculations as the theoretical values were not known.

Loading is shown as grams of $T_b$OD per gram MLSS. No recognition is given to active fraction of the culture in this calculation. This factor is discussed later.

The unit assimilation rate, $\frac{-dc}{dt}$ MLSS, is the maximum slope of the COD regression divided by the average MLSS for the interval involved. Again the active fraction of the MLSS is not considered here. This effect is discussed later.

During the course of experiments it appeared the calculated "O" mixed liquor COD was more accurate than the measured. In several systems studied, the mass assimilation and/or metabolism rate was so high that the delay of several minutes
for mixing and sample filtering was sufficient to cause considerable error in the "O" mixed liquor sample COD. It was therefore decided to use the calculated "O" mixed liquor COD in all $T_{bOD}$ calculations. The $\Delta$ COD shown on the second table of each substrate group is the difference between the calculated "O" COD and the minimum COD of the mixed liquor. The $T_{bOD}$ values shown were then calculated by equation (A).

A graph is presented of one run from each substrate (Figures number 2 through 9). These are plots of the mixed liquor COD and MLSS progression with time to provide a clearer picture of the kinetics involved. Single runs are plotted because averages of runs without similarity between the various parameters would have no meaning.

**Pure Substrates**

Tables I and II present the composition and results of the three runs made on each of the three pure substrates - glucose, glutamic acid and sodium acetate.

With the exception of glucose runs number 1 and 2, all calculated and measured "O" mixed liquor COD results were equal or within the precision of the COD determination.

The first column of Table I shows the range of culture COD obtainable with good washing technique. Run number 1 (glucose) was a homogenized culture as previously discussed. Run number 9 (sodium acetate) is a good example of poor
<table>
<thead>
<tr>
<th>Run No.</th>
<th>Substrate</th>
<th>Culture</th>
<th></th>
<th>Substrate</th>
<th></th>
<th>&quot;O&quot; Mixed Liquor</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Meas'd</td>
<td>Volume</td>
<td>Meas'd</td>
<td>Volume</td>
<td>Calc'd COD mg/l</td>
<td>Meas'd COD mg/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COD mg/l</td>
<td>ml</td>
<td>COD mg/l</td>
<td>ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Glucose</td>
<td>27</td>
<td>1425</td>
<td>1070</td>
<td>988</td>
<td>500</td>
<td>277</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>8</td>
<td>500</td>
<td>1070</td>
<td>980</td>
<td>1500</td>
<td>737</td>
</tr>
<tr>
<td>*3</td>
<td>Glucose</td>
<td>15</td>
<td>400</td>
<td>5350</td>
<td>5030</td>
<td>25</td>
<td>310</td>
</tr>
<tr>
<td>4</td>
<td>Glutamic Acid</td>
<td>17</td>
<td>800</td>
<td>500</td>
<td>522</td>
<td>800</td>
<td>270</td>
</tr>
<tr>
<td>*5</td>
<td>Glutamic Acid</td>
<td>16</td>
<td>775</td>
<td>500</td>
<td>537</td>
<td>800</td>
<td>281</td>
</tr>
<tr>
<td>6</td>
<td>Glutamic Acid</td>
<td>12</td>
<td>500</td>
<td>250</td>
<td>265</td>
<td>250</td>
<td>96</td>
</tr>
<tr>
<td>7</td>
<td>Na Acetate</td>
<td>10</td>
<td>975</td>
<td>250</td>
<td>235</td>
<td>960</td>
<td>122</td>
</tr>
<tr>
<td>*8</td>
<td>Na Acetate</td>
<td>16</td>
<td>275</td>
<td>250</td>
<td>235</td>
<td>275</td>
<td>126</td>
</tr>
<tr>
<td>9</td>
<td>Na Acetate</td>
<td>36</td>
<td>475</td>
<td>5000</td>
<td>4620</td>
<td>25</td>
<td>265</td>
</tr>
<tr>
<td>Run No.</td>
<td>Substrate</td>
<td>Loading T&lt;sub&gt;B&lt;/sub&gt;OD/MLSS gm/gm</td>
<td>Time to Minimum Hours</td>
<td>Max. Rate &lt;span class=&quot;subscript&quot;&gt;dc&lt;/span&gt; (mg/l) hr&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>Δ COD mg/l</td>
<td>T&lt;sub&gt;B&lt;/sub&gt;OD mg/l</td>
<td>% of Theor. T&lt;sub&gt;B&lt;/sub&gt;OD</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
<td>-------------------------------</td>
<td>----------------------</td>
<td>-------------------------------------------------</td>
<td>-----------</td>
<td>-------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Glucose</td>
<td>0.27</td>
<td>1 1/2</td>
<td>0.20</td>
<td>250</td>
<td>960</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>0.71</td>
<td>13</td>
<td>0.049</td>
<td>720</td>
<td>960</td>
<td>90</td>
</tr>
<tr>
<td>3*</td>
<td>Glucose</td>
<td>0.42</td>
<td>4</td>
<td>0.11</td>
<td>285</td>
<td>4850</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>Glutamic Acid</td>
<td>0.30</td>
<td>3</td>
<td>0.10</td>
<td>253</td>
<td>506</td>
<td>101</td>
</tr>
<tr>
<td>5*</td>
<td>Glutamic Acid</td>
<td>0.43</td>
<td>4</td>
<td>0.094</td>
<td>262</td>
<td>516</td>
<td>103</td>
</tr>
<tr>
<td>6</td>
<td>Glutamic Acid</td>
<td>0.09</td>
<td>1 1/4</td>
<td>0.073</td>
<td>81</td>
<td>240</td>
<td>96</td>
</tr>
<tr>
<td>7</td>
<td>Na Acetate</td>
<td>1.0</td>
<td>32</td>
<td>0.026</td>
<td>105</td>
<td>210</td>
<td>84</td>
</tr>
<tr>
<td>8*</td>
<td>Na Acetate</td>
<td>0.28</td>
<td>8</td>
<td>0.037</td>
<td>108</td>
<td>216</td>
<td>86</td>
</tr>
<tr>
<td>9</td>
<td>Na Acetate</td>
<td>0.42</td>
<td>8</td>
<td>0.051</td>
<td>229</td>
<td>4580</td>
<td>92</td>
</tr>
</tbody>
</table>
washing. The minimum COD of this run was 23 mg/l. However, the culture COD of 36 mg/l was used as the minimum for calculation of the $T_{b,OD}$.

The third and fourth columns of Table I show the recovery efficiency of the COD test on these substrates. The theoretical $T_{b,OD}$ is calculated on the assumption that the nitrogen content of a substrate goes to $N^3$. This same assumption is made with regard to the COD determination. However, the consistently higher substrate COD (than theoretical) of glutamic acid indicates the possibility that the amino group is oxidized to free nitrogen and water.

A direct relation between "Loading" and "Time to minimum COD" was observed as shown on Table II. This relation would probably have been even more direct if just the active fraction of MLSS could had been considered.

Comparison of the unit assimilation rate ($\frac{dc}{Mdt}$) shown on Table II offered no continuity. This was to be expected since total MLSS were used in the computation rather than only the active fraction of solids and no control had been exercised on the cultures prior to use in the experiments.

$T_{b,OD}$ of the substrates as percent of theoretical $T_{b,OD}$ varied from 84 to 103%. However, when the measured $T_{b,OD}$ was shown as percent of substrate COD, the variation was only 89 to 99%. This illustrates the dependence of the $T_{b,OD}$ test on the recovery efficiency of the COD test in measuring soluble organic materials.
Figures 2, 3, and 4 are graphs of the $T_{bOD}$ progression for glucose (run #3), glutamic acid (run #5), and sodium acetate (run #8) respectively. The most striking element of these graphs is the linearity of the $T_{bOD}$ progression. This is in direct contrast to the progression proposed in Figure 1 based on the "first order" assumption.

Another unexpected deviation from the proposed curve of Figure 1 is the very sharp break in the slope at the minimum COD point. This portion of the curve is dotted on Figure 2 as no points were taken in this area. However, other glucose runs indicate this break should be as sharp as that shown for glutamic acid, Figure 3.

Growth of the culture is represented by the MLSS plot against time at the top of each graph. Contrary to $T_{bOD}$ progression, the culture growth followed the expected pattern. Growth normally peaked at the minimum COD point although, in some few cases, maximum MLSS was reached prior to this point.

Pure Substrate Mixtures with EDTA

These experiments were initiated in order to study the results of a pure substrate in combination with an organic substance not biologically degradable. Four runs were made, two each with EDTA in combination with glucose and with glutamic acid. Data and results are shown on Table III and IV. Figures 5 and 6 are COD progression curves and MLSS data for runs 10 and 12 respectively.
\[ T_{bOD} = \Delta \text{COD} \times \text{DILUTION FACTOR} \]
\[ = 285 \times \frac{425}{25} \]
\[ = 4850 \text{ mg/l} \]

RECOVERY = 91\% OF THEORETICAL

FIG. 2 - GLUCOSE T_{bOD} PROGRESSION
(Run no. 3)
\[ T_{bOD} = \Delta \text{COD} \times \text{DILUTION FACTOR} \]
\[ = 262 \times \frac{1575}{800} \]
\[ = 516 \, \text{mg/l} \]

RECOVERY = 103\% OF THEORETICAL

FIG. 3 - GLUTAMIC ACID \( T_{bOD} \) PROGRESSION (Run no. 5)
\[ T_{b\text{OD}} = \Delta \text{COD} \times \text{DILUTION FACTOR} \]
\[ = 108 \times \frac{550}{275} \]
\[ = 216 \text{ mg/l} \]

RECOVERY = 86% OF THEORETICAL

FIG. 4 - SODIUM ACETATE T_{b\text{OD}} PROGRESSION (Run no. 8)


TABLE III - PURE SUBSTRATE-EDTA MIXTURE SYSTEMS

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Substrate</th>
<th>Culture</th>
<th>Meas'd COD mg/l</th>
<th>Volume ml</th>
<th>Substrate</th>
<th>Theor.** TₜOD-mg/l</th>
<th>Meas'd COD-mg/l</th>
<th>Volume ml</th>
<th>&quot;O&quot; Mixed Liquor</th>
<th>Calc'd COD-mg/l</th>
<th>Meas'd COD-mg/l</th>
<th>MLSS mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>*10</td>
<td>Gluc.-EDTA</td>
<td></td>
<td>14</td>
<td>975</td>
<td></td>
<td>268</td>
<td>508</td>
<td>750</td>
<td>229</td>
<td>250</td>
<td>380</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Gluc.-EDTA</td>
<td></td>
<td>14</td>
<td>475</td>
<td></td>
<td>1070</td>
<td>2070</td>
<td>200</td>
<td>624</td>
<td>612</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>*12</td>
<td>Glut. Acid-EDTA</td>
<td></td>
<td>17</td>
<td>500</td>
<td></td>
<td>100</td>
<td>225</td>
<td>856</td>
<td>148</td>
<td>163</td>
<td>860</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Glut. Acid-EDTA</td>
<td></td>
<td>17</td>
<td>375</td>
<td></td>
<td>200</td>
<td>428</td>
<td>375</td>
<td>222</td>
<td>242</td>
<td>930</td>
<td></td>
</tr>
</tbody>
</table>

** Theoretical TₜOD includes only the Glucose or Glutamic Acid fraction of the mixture.
TABLE IV - PURE SUBSTRATE-EDTA MIXTURE TbOD DATA

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Substrate</th>
<th>Loading TbOD/MLSS gm/gm</th>
<th>Time to Max. Rate -dc MdE hr-1</th>
<th>( \Delta ) COD mg/l **</th>
<th>TbOD **</th>
<th>% of Theor. TbOD **</th>
<th>TbOD with Meas'd &quot;O&quot; M.L. COD mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>*10</td>
<td>Glucose-EDTA</td>
<td>0.25</td>
<td>2</td>
<td>0.11</td>
<td>75</td>
<td>172</td>
<td>64</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>0.37</td>
<td>3 1/2</td>
<td>0.10</td>
<td>235</td>
<td>792</td>
<td>74</td>
</tr>
<tr>
<td>*12</td>
<td>Glut. Acid-EDTA</td>
<td>0.07</td>
<td>1</td>
<td>0.077</td>
<td>50</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>0.11</td>
<td>2</td>
<td>0.082</td>
<td>81</td>
<td>162</td>
<td>81</td>
</tr>
</tbody>
</table>

** Columns Based on Calculated "O" Time Mixed Liquor COD.
**M.L.Soluble COD-mg/l**

**MLSS-mg/l**

\[ \text{TbOD} = \Delta \text{COD} \times \text{DILUTION FACTOR} \]

\[ = 75 \times \frac{1725}{750} \]

\[ = 172 \text{ mg/l} \]

RECOVERY = 64% OF THEORETICAL

**FIG. 5 - GLUCOSE-EDTA MIXTURE**

**TbOD PROGRESSION (Run no. 10)**
M.L.Soluble COD-mg/l

MLSS-mg/l

\[ T_b \text{OD} = \Delta \text{COD} \times \text{DILUTION FACTOR} \]

\[ '' = 50 \times \frac{1356}{856} \]

\[ '' = 79 \text{ mg/l} \]

RECOVERY = 79% OF THEORETICAL

FIG. 6 - GLUTAMIC-ACID-EDTA MIXTURE

\[ T_b \text{OD} \text{ PROGRESSION (Run no.12)} \]
These experiments were unique in that the calculated "O" mixed liquor COD was considerably less than the measured. Further, the recovery of both glucose and glutamic acid was considerably lower than that for the pure substrates alone. To be consistent, however, the calculated value for initial mixed liquor COD was used to determine the substrate T₅OD. Use of measured values, in the case of both glutamic acid runs, give 100% recoveries of the substrate.

Following completion of these four experiments, two more runs were made using EDTA only as a substrate with each of the mass cultures involved. The "O" mixed liquor COD was 166 mg/l for the glucose culture-EDTA system and 173 mg/l for the glutamic acid culture-EDTA system. Initial MLSS were 400 and 640 mg/l respectively. After 48 hours aeration the glucose-EDTA mixed liquor COD had increased from 166 to 173 mg/l and the glutamic acid-EDTA system COD had decreased from 173 to 169 mg/l. These are all within the precision of the COD test and were considered as representing no change. The test certainly indicated that essentially all COD removal in the previous experiments was from assimilation of the primary substrate (either glucose or glutamic acid) and the EDTA was not degraded.

These latter experiments with EDTA were continued for a prolonged period with surprising results. The glutamic acid culture was apparently able to assimilate the EDTA at a very...
low rate. After 27 days the mixed liquor COD of this system was reduced from 173 mg/1 to 108 mg/1. The glucose adapted system on the other hand, was unable to metabolize the EDTA and this system COD increased from 166 mg/1 to 188 mg/1 in 27 days. The increase was probably due to the products of lysed organisms being released to the substrate. In both systems the MLSS concentration decreased approximately 130 mg/1.

It was thought that the increase of measured over calculated initial mixed liquor COD in the pure substrate-EDTA systems may have been caused by a slight toxicity. EDTA will form a complex with calcium and magnesium ions and it is conceivable that most of these ions in the system could have been tied up by the EDTA. If this were true, the activity of some of the bacterial enzyme systems would certainly be inhibited. This effect was apparently more pronounced on the glucose acclimated culture.

It is thought that EDTA was not a good selection for the purpose intended. In spite of the drawbacks noted above, the same linear COD reduction was noted in these four experiments as in those on pure substrates only. The same abrupt change in slope was also noted at the minimum COD point.

Industrial Wastes

Data for three industrial wastes investigated are shown on
Table V and VI. Since the theoretical $T_{bOD}$ and COD of these wastes were not known, results are compared to the arithmetic averages of these parameters, $T_{bOD}$ and COD, as measured.

The formaldehyde-methanol waste data for run 15 are plotted on Figure 7. This waste differed from the other substrates studied in that it appeared to have a fraction that was readily available for metabolism and another fraction that was not so easily metabolized. The maximum unit rate shown for the system in Figure 7 was 0.09 gm $T_{bOD}$/gm MLSS/hr. After the sharp break in the slope of the $T_{bOD}$ progression, the unit rate was only 0.001 gm $T_{bOD}$/gm MLSS/hr. This represents almost a hundred fold decrease in assimilation rate. This was thought to be a sequential substrate removal phenomena wherein metabolism of one portion of the substrate was greatly reduced by some metabolic block. Further investigation of this phenomena was beyond the scope of this study.

Figures 8 and 9 are plots of COD progression and MLSS data for the phthalic anhydride waste (run #18) and ethylene glycol waste (run #21) respectively. They both exhibit the characteristic linear COD progression, initially, and flat plateau after the minimum COD point. However, they do not show any sharp break in slope, at the minimum COD, exhibited by the pure substrates. Rather, they have a smoother transition from the linear maximum slope to the flat slope of the minimum COD level.

Precision of the $T_{bOD}$ test on such a variable array of
<table>
<thead>
<tr>
<th>Run No.</th>
<th>Waste</th>
<th>Culture Meas'd COD mg/l</th>
<th>Culture Volume ml</th>
<th>Substrate T_bCOD mg/l</th>
<th>Substrate Meas'd COD-mg/l</th>
<th>Substrate Volume ml/ml T.W.</th>
<th>&quot;O&quot; Mixed Liquor Calc'd COD-mg/l</th>
<th>&quot;O&quot; Mixed Liquor Meas'd COD-mg/l</th>
<th>&quot;O&quot; Mixed Liquor MLSS mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Formaldehyde-Methanol</td>
<td>11</td>
<td>900</td>
<td>10,200</td>
<td>18,000</td>
<td>45/900</td>
<td>454</td>
<td>454</td>
<td>340</td>
</tr>
<tr>
<td>*15</td>
<td>&quot;&quot;</td>
<td>11</td>
<td>525</td>
<td>10,200</td>
<td>17,600</td>
<td>23/460</td>
<td>418</td>
<td>403</td>
<td>2050</td>
</tr>
<tr>
<td>16</td>
<td>&quot;&quot;</td>
<td>14</td>
<td>450</td>
<td>10,200</td>
<td>18,500</td>
<td>18/450</td>
<td>378</td>
<td>371</td>
<td>1330</td>
</tr>
<tr>
<td>17</td>
<td>Phthalic-Anhydride</td>
<td>14</td>
<td>800</td>
<td>6,320</td>
<td>6,970</td>
<td>50 -</td>
<td>423</td>
<td>424</td>
<td>790</td>
</tr>
<tr>
<td>*18</td>
<td>&quot;&quot;</td>
<td>16</td>
<td>400</td>
<td>6,320</td>
<td>6,670</td>
<td>20/200</td>
<td>233</td>
<td>232</td>
<td>890</td>
</tr>
<tr>
<td>19</td>
<td>&quot;&quot;</td>
<td>18</td>
<td>400</td>
<td>6,320</td>
<td>6,740</td>
<td>20/200</td>
<td>237</td>
<td>218</td>
<td>2170</td>
</tr>
<tr>
<td>20</td>
<td>&quot;&quot;</td>
<td>4</td>
<td>400</td>
<td>6,320</td>
<td>6,800</td>
<td>40/200</td>
<td>456</td>
<td>443</td>
<td>1760</td>
</tr>
<tr>
<td>*21</td>
<td>Ethylene-Glycol</td>
<td>10</td>
<td>400</td>
<td>1,950</td>
<td>2,450</td>
<td>40/200</td>
<td>170</td>
<td>176</td>
<td>1190</td>
</tr>
<tr>
<td>22</td>
<td>&quot;&quot;</td>
<td>8</td>
<td>400</td>
<td>1,950</td>
<td>2,420</td>
<td>20/100</td>
<td>103</td>
<td>98</td>
<td>3060</td>
</tr>
<tr>
<td>23</td>
<td>&quot;&quot;</td>
<td>7</td>
<td>200</td>
<td>1,950</td>
<td>2,460</td>
<td>25 -</td>
<td>261</td>
<td>254</td>
<td>5780</td>
</tr>
</tbody>
</table>

** Volume of raw waste/total volume of diluted waste added to culture.
<table>
<thead>
<tr>
<th>Run No.</th>
<th>Waste</th>
<th>Loading T&lt;sub&gt;b&lt;/sub&gt;OD/MLSS gm/gm</th>
<th>Time to Minimum Hours</th>
<th>Max. Rate (-dc/Δt) hr&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Δ COD mg/l</th>
<th>T&lt;sub&gt;b&lt;/sub&gt;OD mg/l</th>
<th>% of Plateau BOD</th>
<th>Plateau BOD mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Formaldehyde-Methanol</td>
<td>0.79</td>
<td>8</td>
<td>0.10</td>
<td>267</td>
<td>10,700</td>
<td>105</td>
<td>5,190</td>
</tr>
<tr>
<td>*15</td>
<td></td>
<td>0.10</td>
<td>1</td>
<td>0.094</td>
<td>213</td>
<td>9,110</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>0.16</td>
<td>4 1/2</td>
<td>0.095</td>
<td>214</td>
<td>10,700</td>
<td>105</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>Phthalic-Anhydride</td>
<td>0.49</td>
<td>10</td>
<td>0.056</td>
<td>384</td>
<td>6,530</td>
<td>103</td>
<td>2,820</td>
</tr>
<tr>
<td>*18</td>
<td></td>
<td>0.23</td>
<td>4</td>
<td>0.092</td>
<td>208</td>
<td>6,240</td>
<td>99</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>0.093</td>
<td>1</td>
<td>0.079</td>
<td>207</td>
<td>6,210</td>
<td>98</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>0.24</td>
<td>2</td>
<td>0.20</td>
<td>424</td>
<td>6,300</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>*21</td>
<td>Ethylene-Glycol</td>
<td>0.099</td>
<td>4</td>
<td>0.034</td>
<td>118</td>
<td>1,770</td>
<td>91</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>0.026</td>
<td>1 1/2</td>
<td>0.019</td>
<td>81</td>
<td>2,010</td>
<td>103</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>0.040</td>
<td>3 1/4</td>
<td>0.032</td>
<td>230</td>
<td>2,070</td>
<td>106</td>
<td>-</td>
</tr>
</tbody>
</table>
\[ T_{b\text{OD}} = \Delta \text{COD} \times \text{DILUTION FACTOR} \]
\[ \therefore = 213 \times \frac{985}{23} \]
\[ \therefore = 9110 \text{ mg/l} \]

RECOVERY = 90% OF \( T_{b\text{OD}} \)

FIG. 7 – FORMALDEHYDE-METHANOL WASTE \( T_{b\text{OD}} \) PROGRESSION
(Run no. 15)
$T_{bOD} = \Delta COD \times \text{DILUTION FACTOR}$

" $= 208 \times 600/20$

" $= 6240 \text{ mg/l}$

RECOVERY = 99% OF $\overline{T_{bOD}}$

FIG. 8 PHTHALIC ANHYDRIDE WASTE $T_{bOD}$ PROGRESSION (Run no. 18)
$T_{b\text{OD}} = \Delta \text{COD} \times \text{DILUTION FACTOR}$

\[ = 118 \times \frac{600}{40} \]

\[ = 1770 \text{ mg/l} \]

RECOVERY = 91% OF $T_{b\text{OD}}$

FIG. 9—ETHYLENE GLYCOL WASTE $T_{b\text{OD}}$ PROGRESSION (Run no. 21)
wastes as these is shown on Table VI, column 6. The standard deviation was 6.2% and the 95% confidence limit was $100^\pm 3.8\%$ for the ten industrial waste experiments. However, it must be emphasized that while this represents good reproducibility, accuracy of the test is wholly dependent on the accuracy of the COD determination on a particular waste or substrate.

The short term plateau BOD is shown for the formaldehyde-methanol waste and the phthalic anhydride waste on Table VI. These values represent the minimum oxygen requirement for assimilation of these wastes with maximum net cell synthesis. The $T_b$OD represents, on the other hand, the maximum oxygen demand of the waste.

All three systems exhibited a tendency toward increasing pH as the waste was metabolized. These pH changes were small, normally fluctuating between 7.6 and 8.6.
VI. DISCUSSION OF RESULTS

The prime characteristic of these experiments which shows the T_bOD test to be of use is the linearity of COD regression. Since the remaining concentration of biologically oxidizable material does not affect its rate of absorption or assimilation by the aerated mass culture (except at very low concentrations), the time required for completion of the test can be kept at a minimum. Test runs number 1, 6, 10, 12, 13, 15, 19, 20, and 22 show that it is entirely feasible to remove all biologically oxidizable material from the mass culture substrate in approximately an hour by the proper selection of loading. There were in fact, indications in run number 19 that the removal of 207 mg/l COD was achieved in considerably less than an hour. Practical limitations of the character of the culture, such as filterability, washing and settling characteristics, etc. will govern this time, to some extent. Dilution and toxicity of the waste will also affect the time element.

Certainly the linear assimilation noted in these experiments fills another niche in the evidence against the "first order" theory of Streeter and Phelps (2). However, it must be stressed that disappearance of substrate was measured here and not oxygen uptake. These are two entirely different parameters and not necessarily coincident.

It must also be noted that the pure substrates studied gave fully linear COD progressions, while the industrial wastes did not. The industrial waste substrates exhibited linear assimilation for the major fraction followed by a
"first order" type progression for the last small fraction of substrate removed. This was probably due to the heterogeneous mixtures of the wastes and the presence of materials having varying biological assimilation rates.

Figure 10 illustrates the relation of the unit assimilation rate $\frac{-dc}{Mdt}$ with the TBOD concentration on glutamic acid run number 5 (Figure 3). For TBOD concentrations over 6 mg/l, the rate was practically constant between 0.09 and 1.0 gm TBOD/gm MLSS/hour. Since the assimilation rate is zero when the TBOD concentration is zero, the curve must go through the origin. Therefore, at something less than 6 mg/l TBOD concentration, there must be a sharp break in the curve. This portion of the curve is shown in dashed line. Consideration of only the active fraction of the mass culture would raise the curve and steepen its slope. However, the flatness of the curve would still indicate that something other than food was limiting in the system. Further, the increase in cell weight noted during substrate disappearance does not necessarily imply an increase in cell numbers or even an increase in the net metabolizing capacity of the culture. If it were assumed that the added cell weight did not represent an increase in metabolic capacity, the curve in Figure 10 would be parallel to the abscissa at TBOD concentrations over 6 mg/l.

Although the assimilation rate is unique for each system,
FIG. 10 - RATE-CONCENTRATION CURVE
(Plotted from Glutamic Acid
$T_b$ OD Progression—Fig. 3)
it appears that the active fraction of a culture could be determined by comparing its assimilation rate to that of a young culture which has been controlled to the extent that a very high proportion of active organisms are present. This can be illustrated on Table II, glucose runs 1 and 2. The unit assimilation rate on run 1 was four times greater than on run 2. This would indicate that the culture used on run 1 consisted of four times greater active fraction than that used in run 2. Development of this concept would be useful in the operation and solids control of a waste treatment plant.

Mixed liquor solids increase for the glucose systems roughly checked with that predicted from the short term BOD plateau theory and Methods I and II of the T_bOD test. This points to the possibility of determining the oxygen uptake rate of a system by tracing the mixed liquor (unfiltered) COD regression with time. The change in unfiltered mixed liquor COD, over a time interval, is then a measure of the net disappearance of oxidizable organics from the system, whether from the substrate or cell material. This change in COD should then be equivalent to the oxygen uptake of the system for that time interval.

The total time required for completion of the T_bOD test when properly performed need not exceed 6 to 8 hours. This time includes:
A. one hour for culture pretreatment, filter weighing and assembly, and sampling of culture and substrate,

B. two hours for mixed liquor aeration, which allows sufficient time for a second sample after the minimum COD point is reached to confirm that no further COD reduction was made,

C. two hours for reflux of COD flasks,

D. one hour for cooling, dilution and COD titration.
Several areas of future work have been opened with this study. Determination of active fraction of the culture should be fruitful for research and practical applications. The relation of oxygen uptake to substrate disappearance and MLSS data would give a better understanding of the biological processes involved. The possibility of air stripping of volatile fractions must be delineated.

Work with low initial MLSS and high substrate concentration would more clearly define unit assimilation rates. In this way, the initial inactive fraction of the culture would be masked by the high relative synthesis of the system.

Additional work to make the Chemical Oxygen Demand test more definitive, including the extent of nitrogen oxidation, is also desirable.
VIII. CONCLUSIONS

1. The Mass Culture Aeration T\textsubscript{bOD} test (Method III) is a very useful analytical procedure. It provides a rapid (6 to 8 hours) determination of the total biological oxygen demand of a system.

2. The test is presently limited by the efficiency of the COD determination. Current work on the COD determination indicates the potassium dichromate-silver sulphate method with HgSO\textsubscript{4} modification (1, 13, 14) is best suited for this test.

3. Proper washing of the culture and use of the calculated "O" mixed liquor COD is necessary for precise and accurate results.

4. Assimilation of substrate does not follow "first order" kinetics in an aerated mass culture system, except possibly at very low substrate concentrations.

5. The T\textsubscript{bOD} test contains an inherent safety factor for the oxygen requirements of a waste.

6. Further study is needed to realize all of the potential of this test for defining process kinetics and understanding mass culture mechanics.
BIBLIOGRAPHY

1. Standard Methods for the Examination of Water and Waste-
   Water. 11th Edition, American Public Health Association,

2. Phelps, E. B., Stream Sanitation, Wiley and Sons, New
   York, (1944).

3. Busch, A. W., "B. O. D. Progression in Soluble Sub¬
   strates, I." Sewage and Industrial Wastes 30, 11, 1336
   (November 1958).

4. Myrick, N. and Busch, A. W., "B. O. D. Progression in
   Soluble Substrates, II, The Selective Stimulation of
   Respiration in Mixed Cultures of Bacteria and Protozoa."  
   Proceedings of the 14th Annual Industrial Waste Confer¬
   ence, Purdue University, (May 1959).

5. Busch, A. W. and Myrick, N., "B. O. D. Progression in
   Soluble Substrates, III, Short Term B. O. D. and Bio¬
   oxidation Solids Production." Proceedings of the 15th
   Annual Industrial Waste Conference, Purdue University,
   (May, 1960).

   " Proceedings of 12th Oklahoma Industrial Wastes Confer¬

7. Grady, L. Jr., Busch, A. W. "B. O. D. Progression in
   Soluble Substrates, VI, Cell Recovery Techniques in
   the T bOD Test." Proceedings of the 18th Annual Indus¬
   trial Waste Conference, Purdue University (April, May,
   1963).

   Procedure for Determination of the Biological Treat¬
   ability of Industrial Wastes." Journal Water Pollution
   Control Federation 32, 8, 841 (August 1960).

   tial Substrate Removal in Heterogeneous Populations.
   " Proceedings of the 35th Annual Meeting of the Water
   Pollution Control Federation, Toronto, Canada, (October,
   1962).

10. Moore, W. A., Kroner, R. C., Ruchhoft, C. C., "Dichromate
    " Analytical Chemistry 21, 8, 953 (August, 1949).


