A STUDY OF THE RELATIONSHIP BETWEEN BIOCHEMICAL OXYGEN DEMAND AND TOTAL ORGANIC CARBON LEVELS IN RAW AND TREATED SEWAGE

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ABSTRACT

A strong linear correlation was found to exist between the 5 day biochemical oxygen demand and the total organic carbon content of a number of standard carbon solutions, raw sewages and treated effluents. Correlation coefficients ranging from 0.705 to 0.996 were determined. In addition, a number of sources of error in the standard BOD procedure were delineated. It was concluded that the measure of organic carbon by the TOC procedure was a more realistic approach to the measurement of the pollutional potential of a waste than the standard BOD procedure.

ACKNOWLEDGEMENTS

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TO HEATHER

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INTRODUCTION

INTRODUCTION

The concept of using a biologically mediated oxygen consuming reaction as a quantitative measure of waste water pollutional potential was established more than 80 years ago. Through the years, the analytical procedures employed have been extensively studied and modified. The analytical technique has been refined to the point that today a standardized procedure for the biochemical oxygen demand (BOD) test is employed in most parts of the world.

Unfortunately, many workers in the pollution control field have come to misunderstand the original intent of the biochemical oxygen demand procedure and have come to use the assay as a measure of organic material or the organic carbon content of a particular waste. Gaudy (1) has observed that "as a measure of organic carbon, it is at best a very indirect method".

The classical design formula, operational guidelines and treatment plant performance criteria are all based on the standard biochemical oxygen demand test. The test has been found to be very slow, requiring a 5 day incubation period, and the data produced is of questionable value for operational and monitoring purposes. The purpose of this study was to determine if the use of an alternate parameter to the biochemical oxygen demand could

serve as the basis upon which the design, operation and monitoring of a biological waste treatment facility could be based. The particular parameter that was evaluated was the total organic carbon (TOC) analysis. The approach taken was to evaluate the total organic carbon analysis in comparison with the standard biochemical oxygen demand procedure and as well, attempt to define some of the variables affecting each of the analyses. It was felt that if the strong relationship between BOD and TOC would be shown that the profession would be more amenable to changeover to this new parameter.

HISTORICAL

HISTORICAL

In 1868 Edward Franklin (2) first noted that oxygen depletion in a stored sample containing organic material was a time dependent reaction. He believed the depletion to be a purely chemical phenomenon. Dupre (3), in 1884, suggested that the "microphytes" in the water were consuming the oxygen for use in their respiratory and metabolic activities, and thus laid the groundwork for the present day biochemical oxygen demand test.

Phelps (4) in 1909 presented his classical empirical monomolecular rate law for biological deoxygenation. The equation expressing the biochemical oxygen demand process was shown as:-

bacteria + substrate + growth factors + O_2 →
increased bacteria + energy + CO_2 + H_2O

This equation implies that the reaction is stoichiometric with the microrganisms themselves acting as a single chemical component in the overall reaction. Through the years, many mathematical models for BOD exertion and associated interpretations of BOD data have based their premise upon this incorrect assumption.

In 1925, Streeter and Phelps (5) first concluded that the rate of the biological reduction of organic matter was proportional to the concentration of the remaining unoxidized organic material. The law was expressed mathematically as:-

$$-\frac{dL}{dt} = K_1 L$$

in integrated form:-

$$L_t = L_o e^{-K_1 t} \qquad \text{or} \qquad Lt = Lo\ 10^{-k_1 t}$$

The symbol Lo represents the total oxidizability (BOD) of the organic matter initially present in the sample. This term has become known as the ultimate BOD. L_t is the amount of oxidizability remaining to be expressed at the corresponding time "t". "K_1" (natural logarithims) or "k_1" (common logarithims) is the proportionality or velocity constant which is sometimes referred to as the specific rate constant.

This work along with the classical work of Theriault (6) has become the basis for the dissolved oxygen "sag equation" still widely used in stream pollution work today. About the same time Reed and Theriault (7) published their classic treatise on the statistical treatment of velocity data. This work is still considered as the most comprehensive and accurate approach to the estimation of the velocity constants of the monomolecular model for BOD.

It is interesting to note that 15 years later, Phelps the originator of the monomolecular rate law theory, conceded that there is no real justification for viewing the rate of decrease of BOD as monomolecular but stated that for practical purposes it could be justified (8). During this period, little BOD research was undertaken mainly due to intensive work on the new activated sludge process. However, two important works were presented in the biological literature which were overlooked by Phelps and which may have resolved his uncertainity with the exponential function formulation. Barker and Clifton (9) and Logan (10) demonstrated inexorably that bacterial decomposition of organic substrates is a two stage reaction. These stages have described by Busch (11) as firstly, one in which oxygen uptake occurs during the conversion of the available substrate into cellular material and storage products and secondly, the utilization of oxygen in excess of the endogenous rate for bacterial predator activity.

Obviously, the work showing a two stage or "plateau" region for the BOD is not in accord with the first order reaction rate theory which was in general acceptance up to this date. The "plateau" theory stimulated extensive research into BOD kinetics which is continuing today. Many workers (12, 13, 14, 15, 16, 17, 18) began to demonstrate that

the monomolecular equation was at variance with actual occurences in the BOD test. Since the original publication of the "plateau" theory, many theories on the kinetics of BOD exertion have been proposed. Second order reaction kinetics have been shown by some (19, 20, 21, 22) to apply while Wilderer and Hartmann (23) have suggested that the process could best be described by using the equation of a third order reaction. Thus it is seen that serious concerns with the standard BOD procedure and its use were becoming apparent.

The BOD test had largely been standardized prior to the work of Barker and Clifton (9) and Logan (10). The standard dilution technique as outlined in the 13th edition of Standard Methods (24) has changed little since it was first introduced into the 8th edition (25) in 1936. The standardization of the analytical procedures were largely due to the work carried out in England by the British Royal Commission on sewage disposal and in the United States by Theriault and Hommon (26). The five day incubation time and the twenty degree incubation temperature were introduced at this time, and as time went on details as to the composition of the dilution water, effects of dilution and design of air tight seals were delineated. Theriault (27) has previously published an indepth review of the development of the standard dilution BOD procedure as adopted.

Since its inception, the five day incubation period has been cited by many as a major drawback of the analysis especially if the parameter is to be used for process control or monitoring. It is felt that by the time the information is received, it is too late to be of any major benefit in a particular situation. Caldwell and Langelier (28) experimented with a three day test and felt that it was unsuitable as did Ingols (29) in his attempts to shorten the incubation period. Meanwhile, Gotaas (30) reported in his research with regards to the two stage theory and felt that shorter incubation times would produce more meaningful data. Norgaard (31), Busch and Sawyer (32), Zehnpfenning and Nichols (33), and Garrett and Sawyer (34) produced data which showed that shorter incubation times were realistic especially in view of the two stage theory of oxidation.

The data of Tidwell and Sorrels (35) led them to the conclusion that a two day BOD was more reliable than the five day procedure. More recently, Hiser and Busch (36), Busch et al (37) and Muller and Schroeder (38, 39) have proposed new shorter approaches to the measure of the biochemical oxygen demand of a waste water.

Another serious source of error in the biochemical oxygen demand procedure was found to be the occurence of nitrification.

This is a two step reaction in which ammonia nitrogen is oxidized to nitrite by Nitrosomonas spp. and others. The nitrite produced can be further oxidized to nitrate by organisms such as Nitrobacter spp. These nitrifiers use carbon dioxide or bicarbonate as their sole carbon source and the attendant reactions consume considerable oxygen. The reactions basically proceed as follows:-

$$2NH_3 + 3O_2 \xrightarrow{\text{Nitrosomonas spp.}} 2NO_2^- + 2H^+ + 2H_2O$$

$$2NO_2^- + O_2 \xrightarrow{\text{Nitrobacter spp.}} 2NO_3^-$$

If these reactions were to go to completion, 3.43 grams of molecular oxygen would be required per gram of ammonia nitrogen oxidized to nitrite and 1.4 grams of oxygen would be required per gram of nitrite nitrogen oxidized to nitrate. Since some of the initial ammonia nitrogen ends up as cellular protoplasm, a correction factor must be applied to account for this in terms of oxygen consumed. Montgomery et al (40) has predicted that the nitrogenous oxygen demand (NOD) can quite accurately be estimated as:-

$$NOD = 3.22\ (NH_3\text{-}N \rightarrow NO_2^-\text{-}N) + 1.11\ (NO_2^-\text{-}N \rightarrow NO_3^-\text{-}N)$$

This would indicate that a waste containing 20 mg/l of ammonia nitrogen has a nitrogenous oxygen demand of about 87 mg/l.

In some wastes, nitrification is not a major factor in the five day procedure since the initial population of the nitrifying organisms is low and takes six to seven days before appreciable numbers develop to make the nitrogenous oxygen demand an important factor (41). However, in long term biochemical oxygen demand testing and in cases where high initial populations of nitrifiers are present, such as in biological treatment plant effluents, the nitrogenous oxygen demand becomes a major factor affecting the magnitude of the biochemical oxygen demand.

Many methods for the control of nitrification have been presented in literature. In 1946 Sawyer and Brodney (42) recommended pasteurization as a control technique. Acidification (43), chlorination-dechlorination (42), methylene blue addition (44), thiourea and allythiourea (40, 45), ammonium chloride addition (46) and 2-chloro-6 (trichloromethyl) pyridine (TCMP) addition (41, 47, 48, 49) have all been suggested at one time or another as a means of overcoming nitrification in the biochemical oxygen demand test. The work of J. C. Young (41, 47) would seem to indicate that the TCMP addition procedure is the superior technique to employ. Campbell et al (50, 51) have elucidated the mechanism of action and sites of inhibition of TCMP for

the chemoautotrophs Nitrosomonas spp. and Nitrobacter spp.

It is interesting to note that regardless of the fact the effects of nitrification in the biochemical oxygen demand have been known for over 60 years (52) that the 13th edition of Standard Methods makes no reference to it nor does it refer to any technique for its supression.

It was becoming more and more evident that workers in the pollution control field were finding faults with the biochemical oxygen demand test not only because of the test procedures but also because of misinterpretation and misunderstanding of the data that were produced. All too often, the biochemical oxygen data were being interpreted simply as a quantitative assessment of the amount of organic material in a sample. The dissatisfaction with the biochemical oxygen demand test had become so intense that in 1971 the Biochemical Oxygen Demand Task Group of Committee D19 on Water took action to discontinue without replacement the ASTM test for biochemical oxygen demand of industrial water and wastewater (D2329-68) (53). The action was drastic considering that the biochemical oxygen demand procedure has an important place in many state laws regarding discharges, however, the decision was based on years of practical concern about the nature of the biochemical oxygen demand procedure and the impracticality of preparing the method to reasonably

meet the objectives asked of it.

Coincident with this pressure for amendments in the biochemical oxygen demand procedure was the emergence of new instrumental techniques for the direct measure of organic carbon compounds.

Mohlman and Edwards (54) first recognized the extreme importance of this parameter over 40 years ago when they stated the following, "in view of the fundamental importance of carbon compounds in all problems of sewage treatment and disposal, it is unfortunate that we have not had a satisfactory method for routine determination of carbon in sewage and industrial wastes . . . It is therefore our belief that if a satisfactory method for carbon (determination) could be perfected, the results would be correlated more closely with the actual oxygen requirements of the sewage than any correlation possible between nitrogen and oxygen requirements."

Many methods were developed through the years employing biological (BOD) and chemical (COD) methods of indirectly measuring the carbon content of a sample through oxygen consumption. We have shown, at least for the biochemical oxygen demand, some of the pitfalls of this very indirect technique. Up until 1963, the Van Slyke-Folche wet carbon

combustion technique (55) and the Mohlman and Edwards test (54) were the chief methods used to measure carbon content. The tests were however, found to involve long and tedious methods. These methods basically involved the oxidation of all organic carbon to carbon dioxide and water and titrimetically measuring the carbon dioxide generated which had been trapped in a standard caustic solution. Gas chromotography had also been employed, however, these procedures require elaborate sample clean up and were all too often overly specific to a particular carbon compound to be of any value on a multi-component system such as a wastewater. A rapid instrumental technique for the determination of carbon in dilute aqueous solutions was recently introduced by Van Hall et al (56, 57). The technique employed involves the rapid combustion of a sample in a stream of oxygen in a heated tube containing a catalyst. The CO_2 produced is then measured by a nondispersive infrared analyser sensitive specifically to carbon dioxide. The early procedures were capable only of measuring total carbon with the inorganic carbon fraction being previously removed by acidification to pH2 and scrubbing with nitrogen gas for three to five minutes (58).

However, in 1970 a method was developed for removing inorganic carbon using an instrumental thermal technique

which made the analytical technique even simpler and less time consuming. Since that time, modifications and refinements to the instrument have provided the means for a reliable and reproducable method for analysing organic carbon in aqueous solutions. A few investigators have attempted to show correlation between BOD and COD and TOC values, however, for the most part, these reports were either carried out using the old technique of scrubbing to remove carbon dioxide (59, 60) or were carried out by the companies producing the instruments (61, 62). The most noteworthy scientific study to date was that of Emery et al (63) which showed that good correlation between BOD and TOC could be obtained. This work however, was carried out on impounded river water and not sewage. In his summation, Emery calls for further investigation into the relationships between BOD and TOC such that reliable prediction factors can be prepared.

MATERIALS AND METHODS

MATERIALS AND METHODS

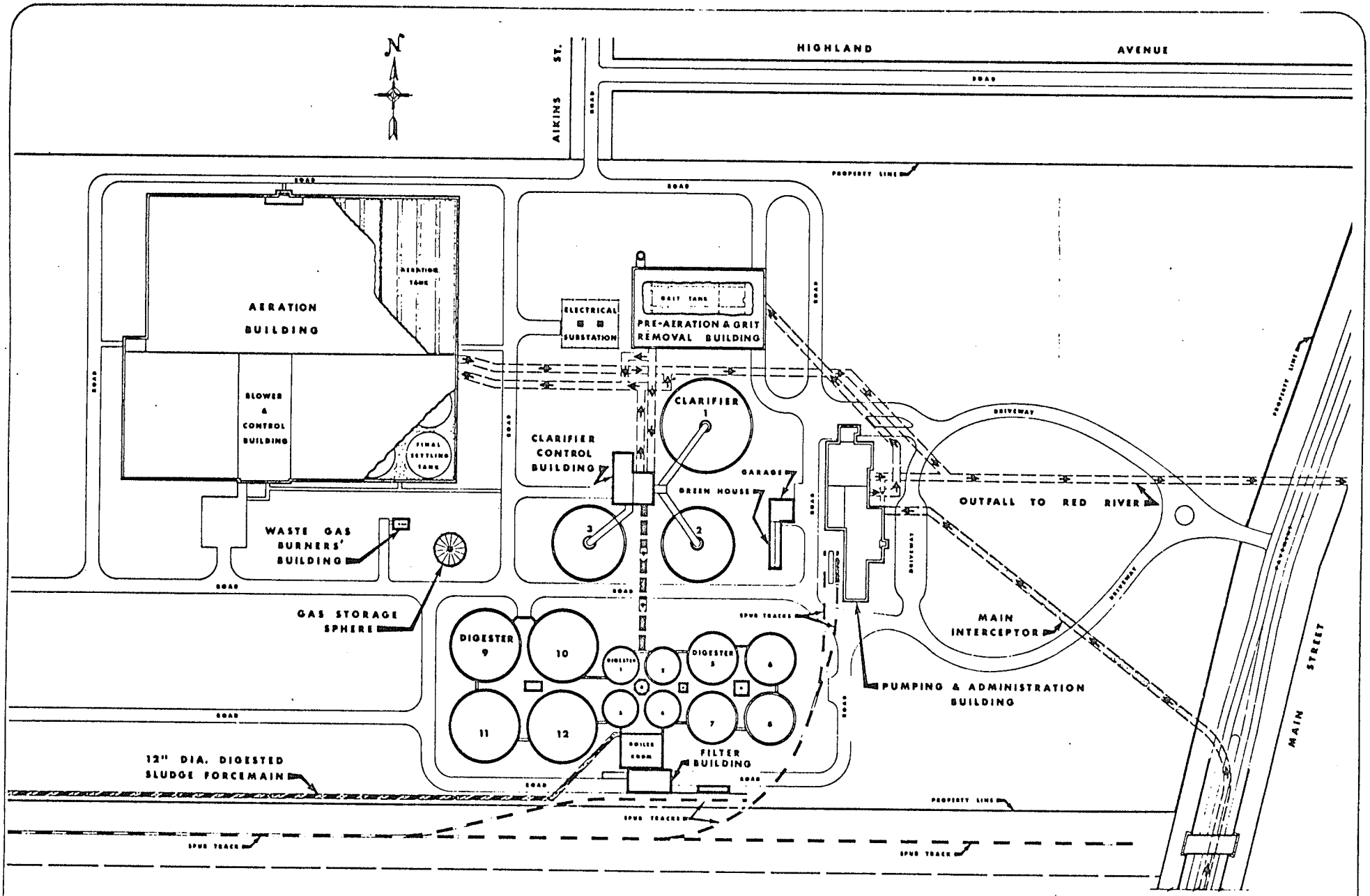
GENERAL EXPERIMENTAL OUTLINE

The relationship between the total organic carbon level and the BOD of various standard carbon solutions and raw and treated wastewater was examined. Standard solutions of organic carbon and various wastewaters were subjected to both TOC and BOD analysis. The results of the analysis were correlated using statistical methods. As well, various tests were conducted in an effort to determine the magnitude of various reaction coefficients and also the effects of such things as seeding, incubation time and nitrification in the BOD test. Such factors as reproducibility and blending effects as well as precision and accuracy were examined in the TOC.

PLANT LAYOUT, SAMPLING TECHNIQUES

The majority of the samples tested in this program were collected at various points throughout the North End Water Pollution Control Centre in the City of Winnipeg. Figure 1 shows a layout of the plant. The waste being treated at this plant is a combination of domestic industrial waste with the proportions of each, in terms of strength, being in the order of 60% domestic to 40% industrial. The industrial wastes are chiefly food processing wastes of packing

FIGURE 1. Layout of North End Sewage Treatment Plant.



NORTH END SEWAGE TREATMENT PLANT

houses, breweries, dairies and vegetable processors. Wastes are also received from the petro-chemical, metal plating, paint and paper processing industries.

Unless otherwise indicated, all samples obtained in this study were collected by means of Markland Duckbill Automatic Samplers[1] which were to set to collect samples every 15 minutes to produce a 24 hour composite. As well as the samples collected at the North End Plant, samples were also collected from the Charleswood Sewage Lagoons and from the South End Water Pollution Control Centre. The wastes received at these sewage works is strictly domestic wastewater. These samples were also 24 hour composites collected every 15 minutes.

TOC ANALYSER

The instrument used in this work was a model 915 Beckman Carbonaceous Analyser[2]. The system essentially involves the volatilization and oxidization of the sample in the presence of a heated catalyst in a oxygen atmosphere. The product of the combustion of the carbonaceous compounds is carbon dioxide which is swept from the system by the oxygen

1. Markland Specialty Engineering Ltd., Etobicoke, Ontario.
2. Beckman Instruments Inc., Fullerton, California

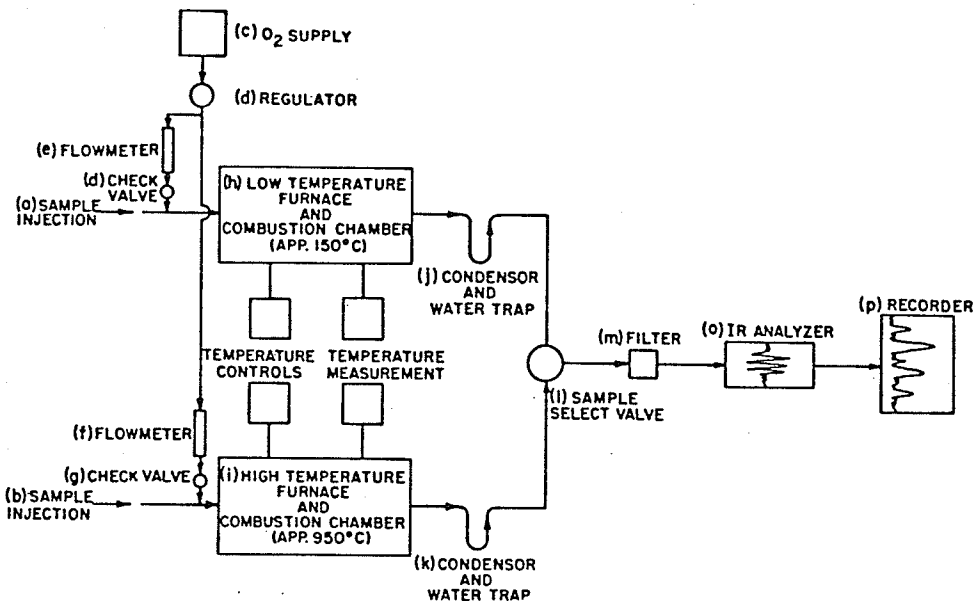
carrier gas and detected by a nondispersive infrared analyser sensitive to carbon dioxide. A signal from the infrared analyser is relayed to a recorder. By the use of a prepared standard curve a quantitative measure can be obtained of total organic carbon by using the difference between total carbon and inorganic carbon as will be shown.

Figure 2 shows the operational details of the total organic carbon analyser. Two furnaces are being used in the system. The low temperature furnace is maintained at a temperature of 150°C. The low temperature combustion tube is packed with quartz chips wetted with 85% phosphoric acid. For this testing a sample volume of 20 microliters was used. The acid packing causes the release of carbon dioxide from any inorganic carbonates contained in the sample and since the temperature is only 150°C no combustion of organic materials takes place. The resulting carbon dioxide and water vapour are swept from the tube by the pure oxygen carrier gas (50 to 150 ml/minute), through a condenser to remove the water vapour and into the nondispersive infrared analyser where the CO_2 is measured.

A second 20 microliter sample is then taken and injected into the high temperature combustion tube. This tube is made

FIGURE 2. Schematic diagram of total organic carbon analyser.

TOC ANALYZER



Flow diagram of total organic carbon analyzer.

of a special alloy and is maintained at the temperature of 950°C. The tube is packed with asbestos fibre impregnated with cobaltous oxide. Immediate combustion of all carbonaceous material takes place and the resulting carbon dioxide and water vapour are swept out of the tube in a similar fashion as that indicated above. Once a total carbon and an inorganic carbon reading have been obtained the difference is taken and reported as total organic carbon or TOC.

EFFECT OF BLENDING

Since microliter volumes were being used, the maximum particle size that could be tolerated was 170 microns, this being the inside diameter of the syringe tip. It was necessary therefore to ensure that a representative measurement of the particulate matter contained in the sample was being obtained. Emery et al (63) used both sonic and standard blending techniques in an effort to reduce particle size and found both to be acceptable.

Tests were carried out on samples of secondary effluent and raw sewage to determine if blending could be used to produce a homogenous solution. The test involved blending samples in a Sorval Omni Mixer[1] for periods of one to five

1. Ivan Sorvall Inc., Newtown, Conneticut

minutes. Following the blending procedure triplicate TOC analyses were carried out on each sample. The syringe used in this and in all following experiments was a Hamilton #705[1] fitted with a metal Leur tip.

PRECISION TESTING

In an effort to ensure that reasonable precision was being obtained, a series of replicate tests was conducted on standard carbon solutions, raw sewage and secondary effluent samples. Precision tests on the samples were carried out by making 25 replicate injections and statistically evaluating the results. The raw sewage and final effluent samples were blended for 3 minutes prior to injection.

Also a series of 120 injections of standard solutions of potassium hydrogen phthalate, sodium carbonate and sodium bicarbonate were made. Statistical analyses of the results were carried out.

ACCURACY TESTING

Sewage samples were tested using the standard addition technique to ensure that reasonable accuracy was being achieved. The samples analysed were raw sewage and secondary effluent. Potassium hydrogen phthalate at a concentration of 30 mg/l was used as the internal standard.

1. The Hamilton Co. Inc., Whittier, California.

The standard addition technique was used throughout the course of the study to ensure acceptable accuracy was being maintained. Tests were also carried out to determine the limit of sensitivity of the instrument. This was accomplished by increasing the gain of the instrument to the point at which the noise to signal ratio becomes the maximum tolerable for measurements of reasonable precision.

BOD TESTING

The BOD test method utilized throughout this work is outlined in the 13th edition of Standard Methods (24). The associated D.O. determinations were carried out using a Weston and Stack model 350 digital D.O. meter[1].

It was felt that if a meaningful analysis of the BOD/TOC relationship was to be made that some necessary background data of BOD kinetics would be of great value. To this end, experiments were conducted to elucidate the magnitude of the velocity constants and the effects of seed changes and nitrification.

VELOCITY CONSTANTS

Tests were carried out to determine velocity constants for the BOD reaction on samples of filtered and unfiltered raw sewage and secondary effluent. The testing involved

1. Weston and Stack Inc., Malvern Pa.

setting up BOD's on each of the samples and incubating them for periods of 2, 5, 8 and 20 days. The data from the BOD determinations were then used to calculate the velocity constants from the first order equation using the method outlined by Sheehy (64). The information obtained would allow for the prediction of theoretical ultimate oxygen demands and could be used as a cross check with the actual determined (20 day) values.

SEED TESTS

The purpose of seeding is to introduce into the sample a biological population capable of oxidizing the organic matter in the wastewater. Where such microorganisms are already present as in domestic sewage, seeding is unnecessary and should not be employed (24). However, an attempt was made to determine if changes in the seed type would result in variation of the BOD results.

A series of experiments was conducted in which various types of seed were employed. The seeds evaluated for raw sewage were the indigenous raw sewage organisms and mixed liquor organisms from the secondary treatment process. The seed suspension was prepared as outlined in Standard Methods. The BOD's were seeded at a rate of 3 ml of seed suspension per litre of sewage. BOD tests were carried out on raw sewage and

filtered raw sewage. Incubation times of 2, 5, 8 and 20 days were employed, and following incubation samples were withdrawn and spread plated out on trypicase soy agar. The plates were incubated for 24 hours at 20°C, following which colony counts and morphology were obtained.

On the final effluent, the seeds used were the indigenous final effluent organisms and raw sewage organisms. Testing was carried out on filtered and unfiltered effluent samples. The test procedure was the same as that employed with the raw sewage.

NITRIFICATION

Since the nitrogenous oxygen demand can under certain circumstances make a major contribution to the BOD, an attempt was made to determine the magnitude of the problem under the conditions used in these experiments. Filtered and unfiltered samples of raw sewage and final effluent were subjected to 2, 5, 8 and 20 day BOD analysis. Initially, and at the end of each incubation period samples were withdrawn from the BOD bottles and examined for ammonia, total kjeldahl nitrogen (TKN), nitrite and nitrate. A parallel series of experiments was carried out in which the samples were spiked with 10 mg/l of TCMP to inhibit nitrification (41). The ammonia levels were measured by

means of an ammonia specific ion electrode connected to an Orion model 701 Digital Milivolt Meter[1]. The total kjeldahl nitrogen, nitrite and nitrate analyses were carried out using a Technicon Auto Analyser[2]. A detailed description on the methods employed for the nitrogen analyses is included in the appendix of this study.

BOD, TOC CORRELATIONS

Correlation of BOD and TOC was carried out on the data obtained from a number of experiments carried out on standard carbon solutions and wastewaters. Correlations were attempted on glucose-glutamic acid solutions, raw sewage, primary effluent, final effluent, and secondary effluent. The BOD analysis used for this test was the standard 5 day procedure as outlined in Standard Methods. The TOC determinations were the average of 3 - 20 microliter injections of samples blended 2 minutes.

Linear regression analysis was carried out using a programmable desktop computer. The regression curves shown are the lines of best fit as computed from the data. All

1. Orion Research Inc., Cambridge, Mass.

2. Technicon Inst. Corp., Tarrytown, N.Y.

standard deviation values shown are at the 95% confidence level.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

EFFECT OF BLENDING

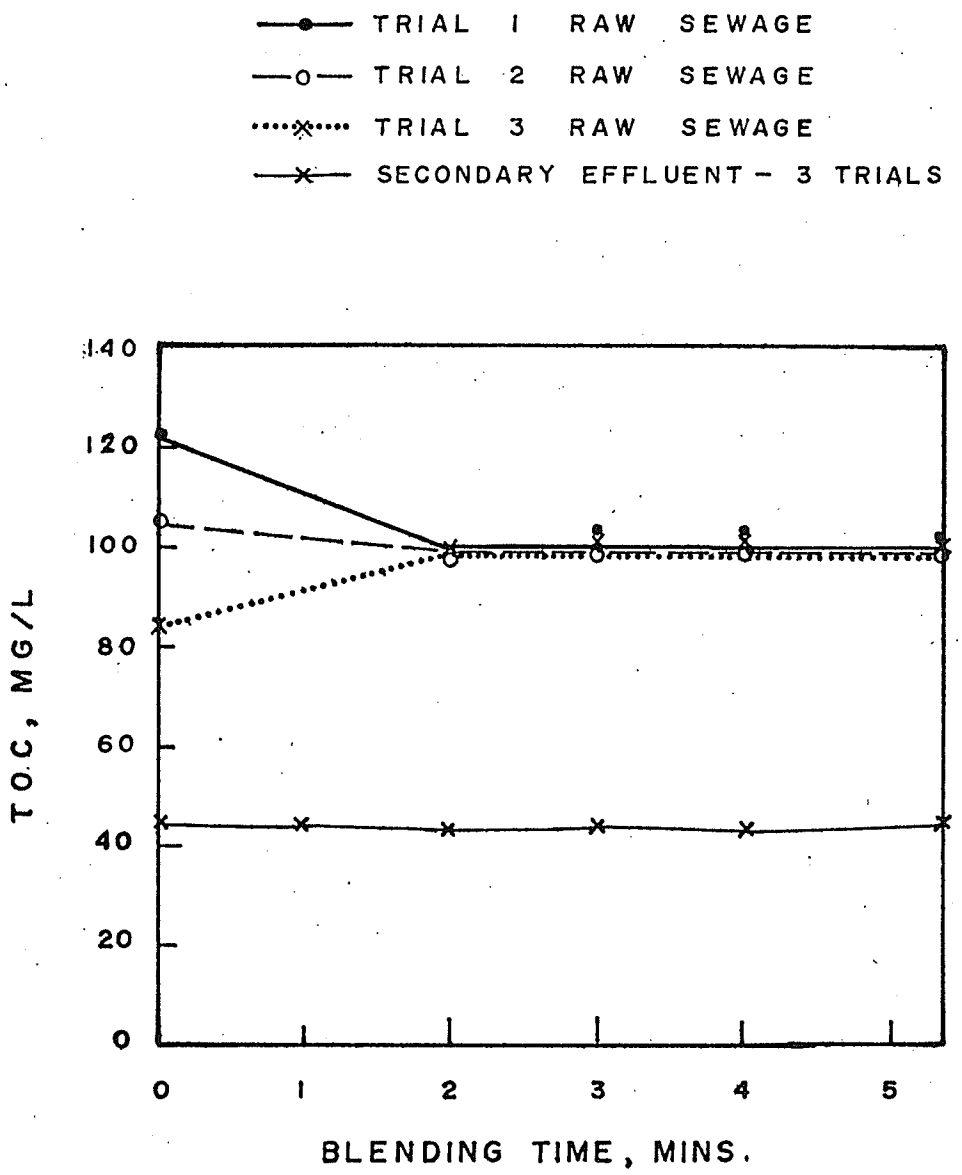
It was found that blending of the samples is necessary, especially samples such as raw sewage which lack homogeneity because of their suspended solids content. The blending was found to be not as critical for secondary effluent samples where suspended solids levels were found to be much lower.

A blending time of 2 minutes was found to be sufficiently long to produce a homogenous solution. Figure 3 shows the effect of blending on raw sewage and secondary effluent total organic carbon levels.

A blending time of 2 minutes was employed in all of the testing carried out in this program. It would seem advisable not to extend the blending time over the 5 minute mark for fear that any volatile components in a wastewater could be stripped off by the agitation. Because of the wide range of volatile compounds that could possibly exist in sewage, no attempt was made to determine if loss of volatiles was occurring in these experiments.

One series of tests was carried out using a potassium hydrogen phthalate standard carbon solution and it was found that no change in carbon concentration occurred after

FIGURE 3. The effect of blending on TOC values for raw sewage and secondary effluent.


TRIAL 1 RAW SEWAGE
TRIAL 2 RAW SEWAGE
TRIAL 3 RAW SEWAGE
SECONDARY EFFLUENT – 3 TRIALS
140
120
100
80
60
40
20
0
T.O.C., MG/L
0
1
2
3
4
5
BLENDING TIME, MINS.

5 minutes of blending.

PRECISION TESTING

The results of 25 replicate injections of standard glucose solution showed that 96% of the values were within one standard deviation of the mean at the 95% confidence level and that all values were within 2 standard deviations of the mean.

Similar testing on the raw sewage and final effluent showed that 80% of the values were within 1 standard deviation of the mean at the 95% confidence level and that all values were within 2 standard deviations.

Linear regression analyses of peak height on organic carbon concentration for 120 injections of potassium hydrogen phthalate, carbonate, bicarbonate solutions showed a correlation coefficient of 0.998 with a standard deviation of less than 1.0.

From these values it can be seen that the technique has a very high degree of precision. In an effort to ensure that the precision was maintained throughout the course of this experiment, all injections were made in triplicate.

ACCURACY TESTING

Tests carried out using the standard addition technique showed very consistent results. In all samples analysed whether they were standard solutions, domestic sewage, or treated effluent, the results obtained on the unspiked and spiked samples minus the added concentration were within 1 standard deviation at the 95% confidence level.

The good agreement obtained between the spiked and non-spiked samples would indicate that the oxidation of the carbon was both rapid and efficient.

The lower limit of analysis and sensitivity of the instrument was found to be 1 mg/l of carbon with a precision ±0.2 mg/l.

SEED TESTING

The results of the seed testing showed that the source of the seed organisms had little effect on the BOD values. It was found that the mixed liquor organisms produced equivalent results with the raw sewage indigenous organisms and that seeding with raw sewage organisms showed no advantage over the indigenous secondary effluent population. The BOD values in all cases were comparable at 2, 5, 8 and 20 days. Figure 4 shows typical results obtained.

No differences in the filtered BOD's were found that could be attributed to the seed type. Figure 5 shows a typical

FIGURE 4. The effect of various seed types on BOD values on raw sewage and secondary effluent.

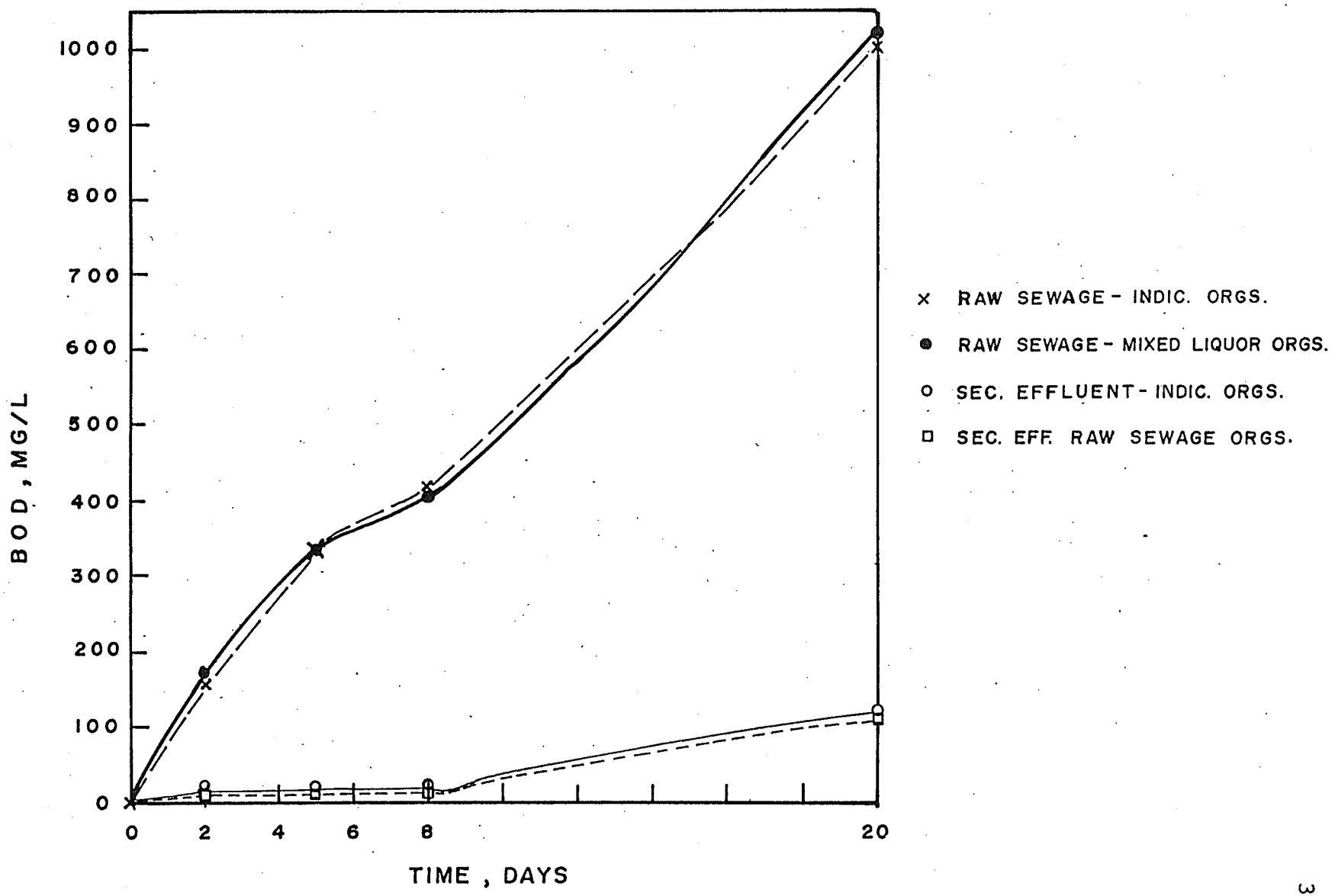
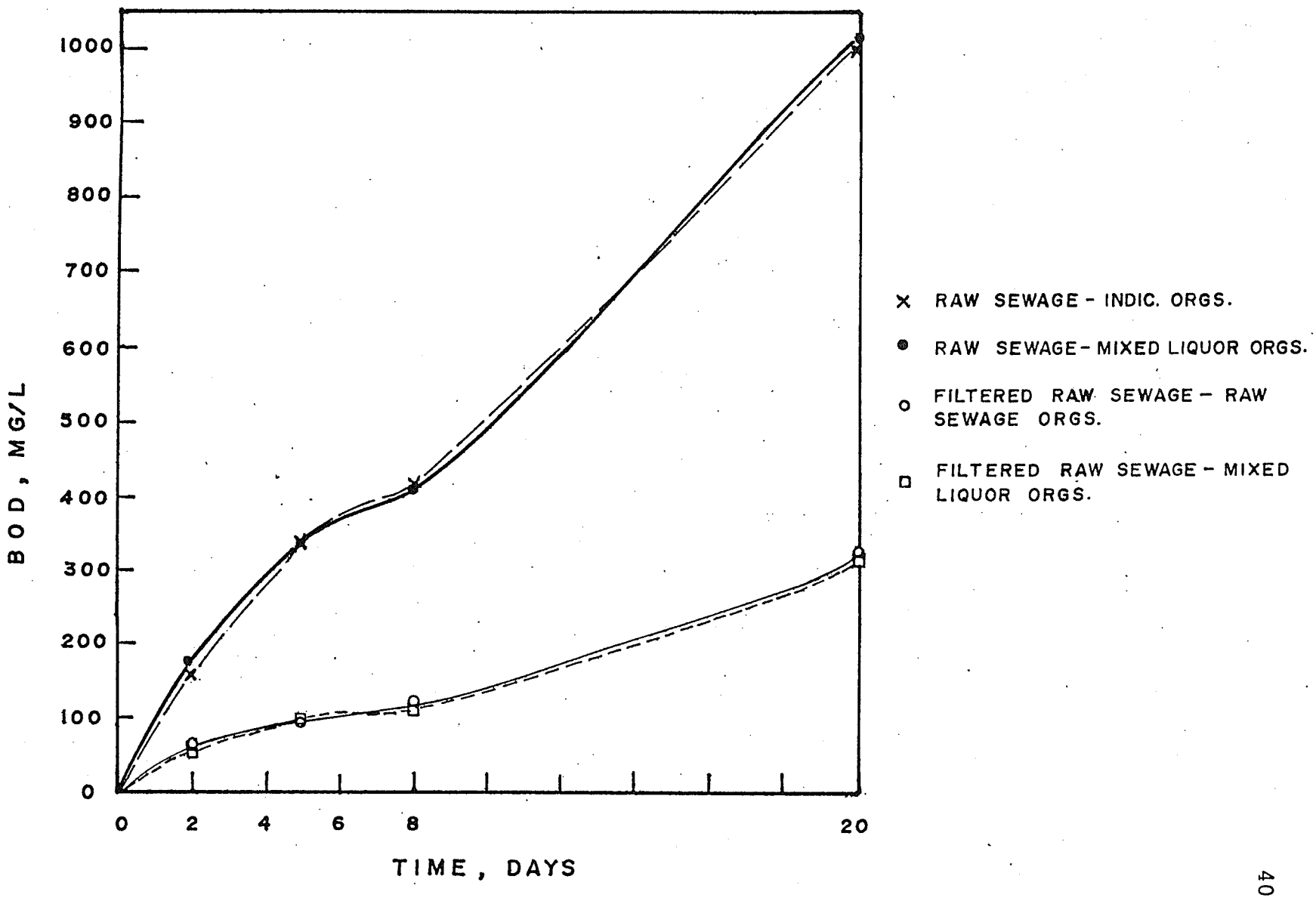

1000
900
800
700
600
500
400
300
200
100
0
BOD, MG/L
0
2
4
6
8
20
TIME, DAYS
× RAW SEWAGE - INDIC. ORGS.
● RAW SEWAGE - MIXED LIQUOR ORGS.
○ SEC. EFFLUENT - INDIC. ORGS.
□ SEC. EFF. RAW SEWAGE ORGS.

FIGURE 5. Effect of various seed types on BOD values on filtered and unfiltered raw sewage.


1000
900
800
700
600
500
400
300
200
100
0
BOD, MG/L
0
2
4
6
8
20
TIME, DAYS
RAW SEWAGE - INDIC. ORGS.
RAW SEWAGE - MIXED LIQUOR ORGS.
FILTERED RAW SEWAGE - RAW SEWAGE ORGS.
FILTERED RAW SEWAGE - MIXED LIQUOR ORGS.

plot of filtered BOD's with both seed types. It was initially felt that perhaps the final effluent may contain high numbers of nitrifying organisms due to nitrification ongoing in the activated sludge process. This consequently would produce higher 5 day BOD values since the nitrification could begin immediately. This however, was not found to be the case since those samples seeded with raw sewage organisms produced parallel results to those samples containing the indigenous secondary effluent organisms. The nitrogen testing and the inhibition studies carried out indicated that both seeds contained a significant population of nitrifying organisms since the process commenced almost immediately. The results of these tests will be discussed in more detail in a later section.

It was found that the numbers of organisms showed a slight increase during the first two days of incubation. The numbers then began to decline at between 2 and 5 days and showed a steady decline up to 20 days. This is in accord with the "plateau" theory which states that predator activity begins after approximately 2 days incubation. This predator activity results in reduced numbers of bacteria (11). Further, it was found

that the species diversity in the bottles began to change. Initially, 5 major morphological types of colonies were found in the plated out seeds. The number of morphological types of colonies declined with time until at 20 days. Only one type comprised about 99% of the growth (Fig. 6).

VELOCITY CONSTANTS

It was found that the velocity constants showed considerable variation from one day to the next. The range of values encountered was from 0.08 to 0.15 for the raw sewage and the secondary effluent. Steele (65) has stated that the value is rarely uniform and can vary from less than 0.05 to more than 0.20.

The variation in k rates found leaves considerable room for speculation as to why such differences in the reaction rate occurs. Two of the most important factors are of course the nature of the organic material present and the ability of the seed organisms present to degrade the material. A near doubling of the k rate for example from 0.08 to 0.15 will yield ultimate carbonaceous demand (UOD) levels of 332 and 243 respectively, for a waste assumed to have a BOD_5 of 200 mg/l (Figure 7). Conversely, if one assumes a UOD of 300 mg/l, 5 day BOD values of 180 mg/l

FIGURE 6. Change in bacterial numbers and colony types with time.

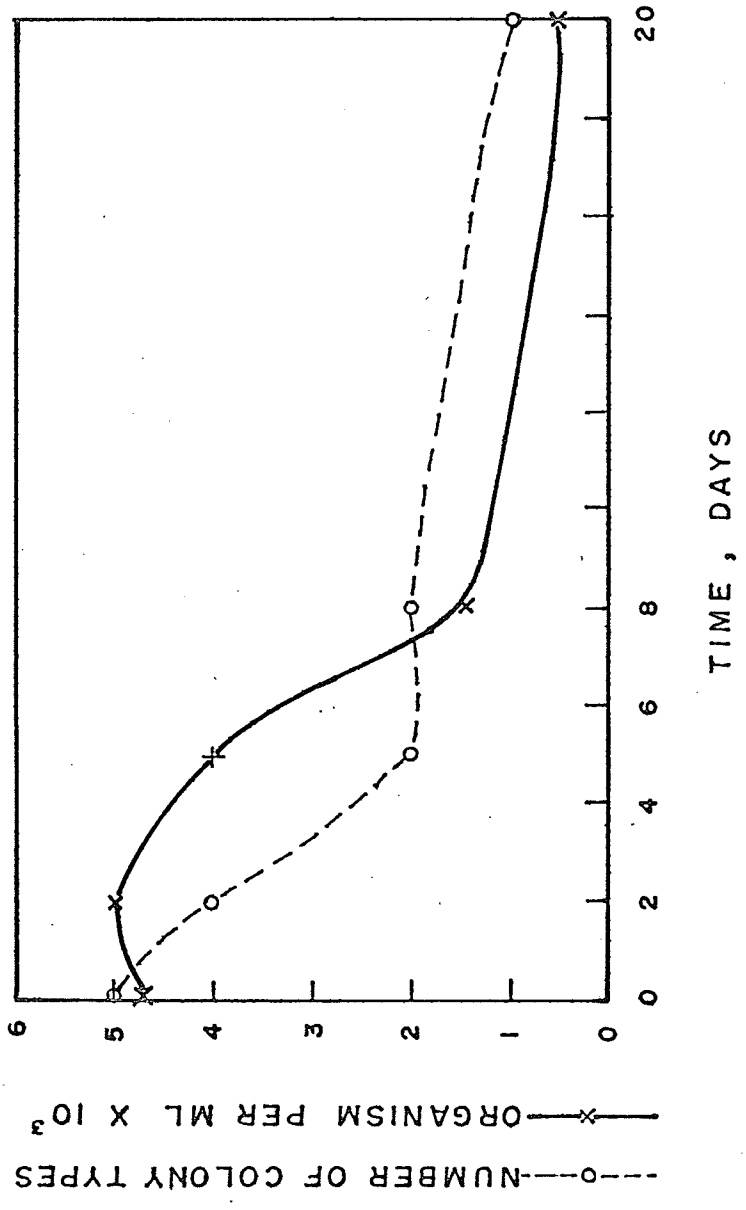
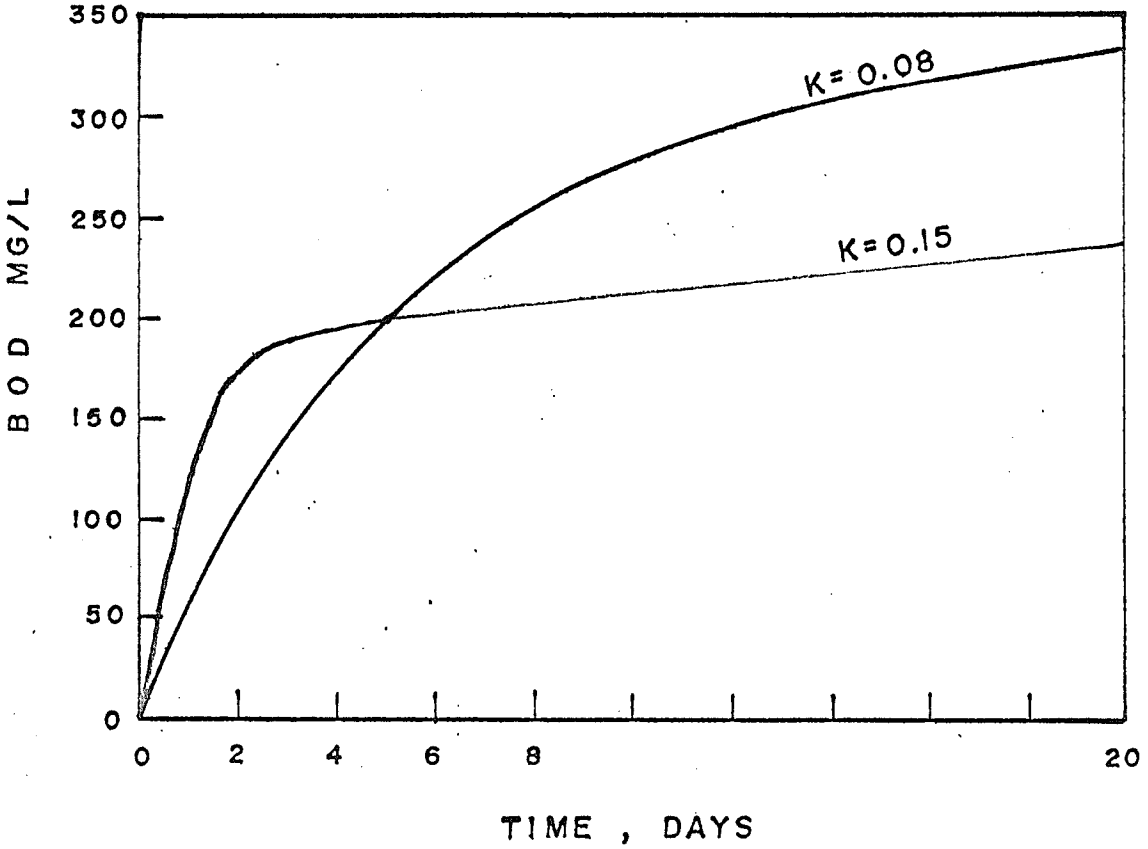

6
5
4
3
2
1
0
0
2
4
6
8
20
TIME, DAYS
—×— ORGANISM PER ML X 10^3
---o--- NUMBER OF COLONY TYPES

FIGURE 7. Effect of changing velocity constant on ultimate oxygen demand assuming a 5 day BOD of 200 mg/l.


350
300
250
200
150
100
50
0
BOD MG/L
K= 0.08
K= 0.15
0
2
4
6
8
20
TIME , DAYS

with a k of 0.08 and 240 mg/l with a k of 0.15 are obtained (Figure 8). Thus it can be seen that the variation in the velocity constants found in this study show that considerable discretion must be used in the interpretation of the BOD results. It will be shown later that large discrepancies in the actual versus theoretical ultimate oxygen demands can be found and do not seem to be related to seed effects or nitrification.

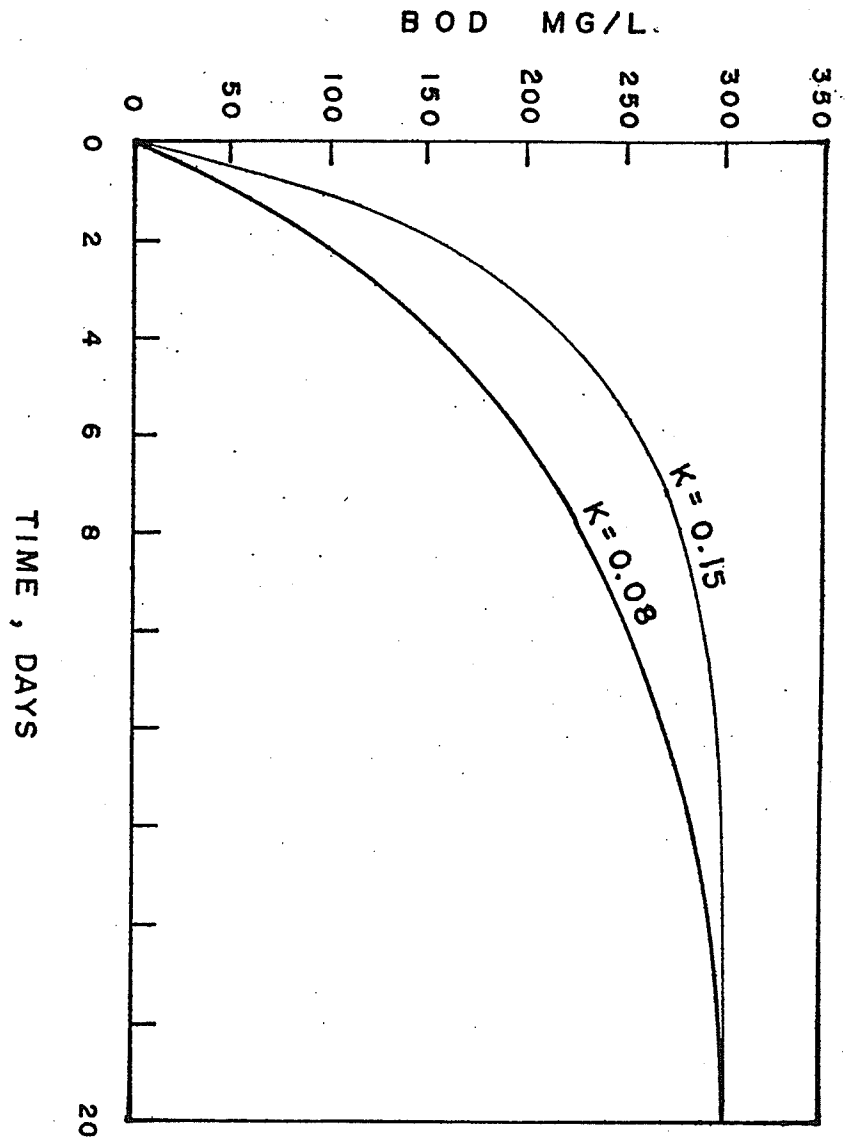
NITRIFICATION

Tests were conducted to evaluate the degree of nitrification that occurred during incubation in inhibited and uninhibited, filtered and nonfiltered samples of raw sewage and secondary effluent.

It was found that considerable nitrification took place in both filtered and unfiltered raw sewage and secondary effluent samples that had not been previously spiked with TCMP. The addition of TCMP at a concentration of 10 mg/l seemed to effectively control the nitrification process.

As previously stated, it was hypothesized that the samples seeded with secondary effluent organisms or with mixed liquor organisms would show nitrification at an early stage due to the presence of adequate numbers of nitrifying organisms.

FIGURE 8. Effect of changing velocity constant on 5 day BOD level assuming an ultimate oxygen demand of 300 mg/l.


BOD MG/L.
0
50
100
150
200
250
300
350
TIME, DAYS
0
2
4
6
8
20
K=0.15
K=0.08

However, it was found that this did not occur and in fact what occurred was that both the samples seeded with secondary effluent organisms or mixed liquor organisms and those seeded with raw sewage organisms began to show signs of nitrification any time after 2 days. The testing showed that parallel results were obtained with both seed types up to 20 days (Figure 9).

It was found that with the stronger wastes that the nitrification could be detected as occurring after 2 days (Figure 10) while the lower strength wastes, such as that obtained on Sundays, and the secondary effluents did not show obvious signs of nitrification until after 5 days (Figure 11). It is highly probable that this is due to a dilution phenomenon since the higher strength wastes obviously must have higher dilutions made to them. The nitrification may become more apparent under these conditions, that is the magnitude of the change between the 2 day value and the 5 day value is much larger with the high strength wastes, therefore, making any differences apparent. This phenomenon has been reported elsewhere (63, 66) and is called "sliding" BOD. It is felt that the nitrification process in all samples begins after 2 days incubation but does not constitute a major interference in low strength wastes and effluents until sometime after 8 days.

FIGURE 9. Effect of seed types on inhibited and uninhibited BOD levels. Nitrification inhibited with 10 mg/l of TCMP.

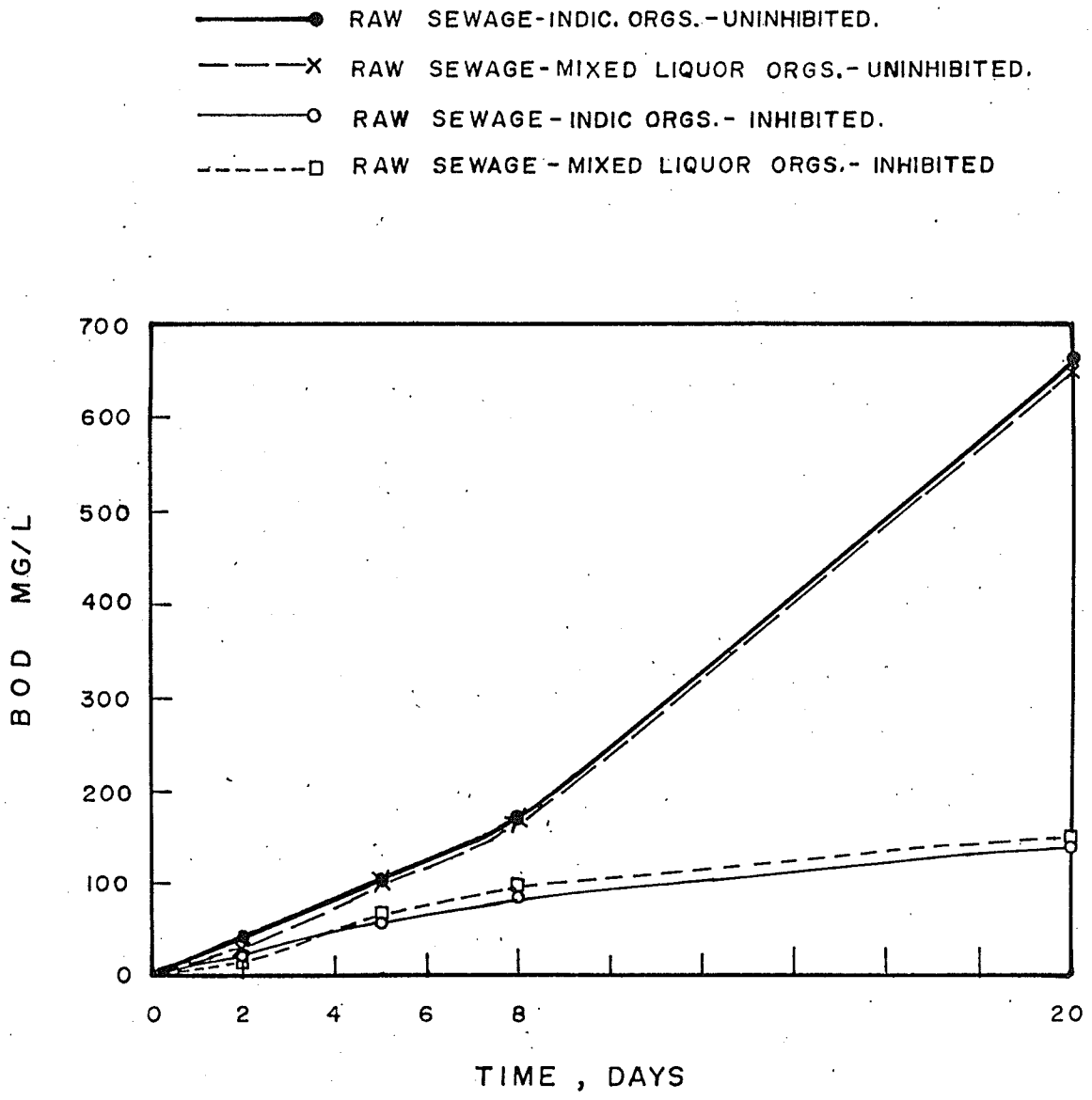

RAW SEWAGE-INDIC. ORGS.-UNINHIBITED.
RAW SEWAGE-MIXED LIQUOR ORGS.-UNINHIBITED.
RAW SEWAGE-INDIC ORGS.- INHIBITED.
RAW SEWAGE-MIXED LIQUOR ORGS.- INHIBITED
BOD MG/L
700
600
500
400
300
200
100
0
0
2
4
6
8
20
TIME , DAYS

FIGURE 10. Extent of nitrification on inhibited and uninhibited BOD tests on high and low strength raw sewage. Nitrification inhibited by 10 mg/l of TCMP.

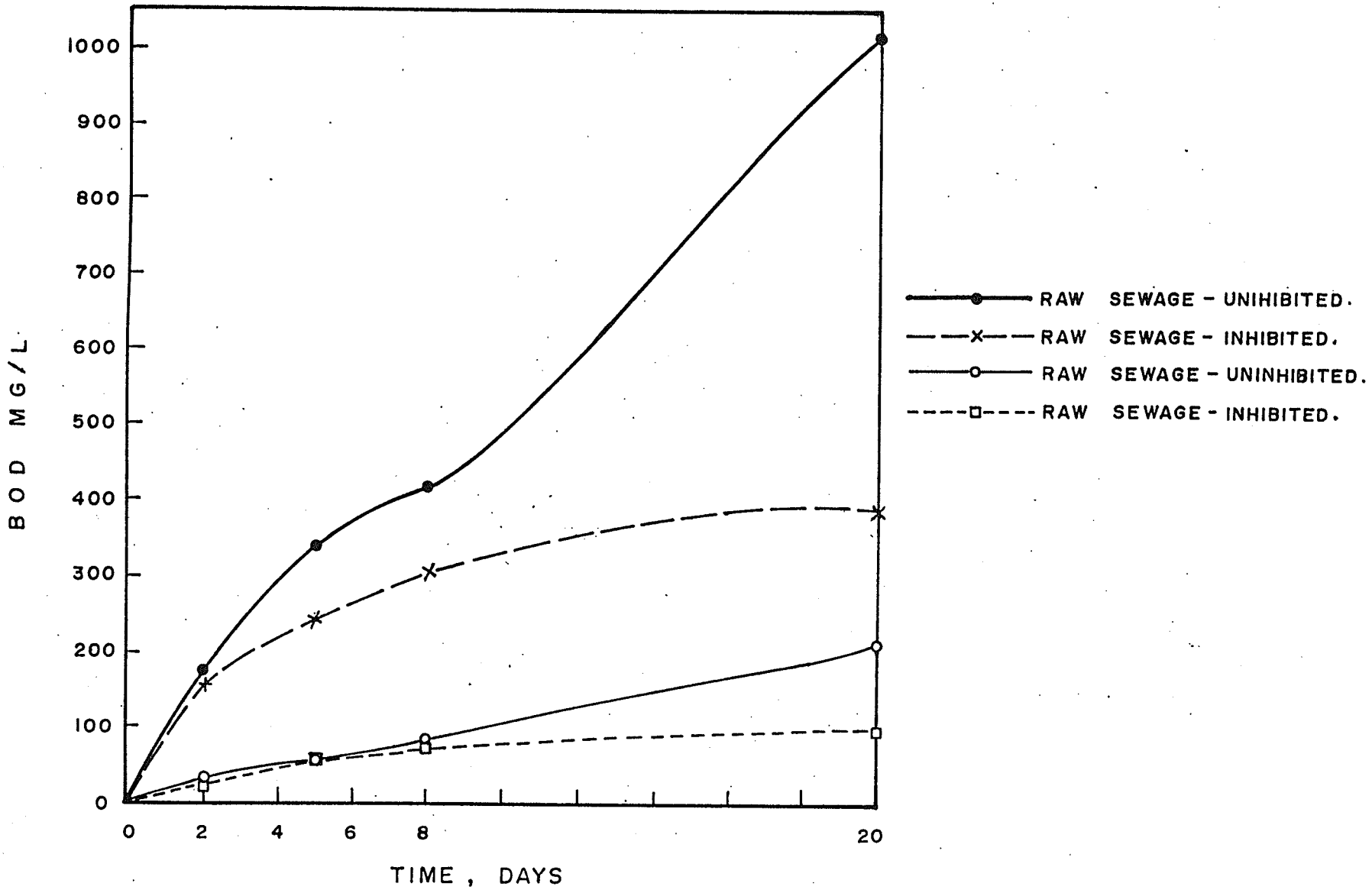
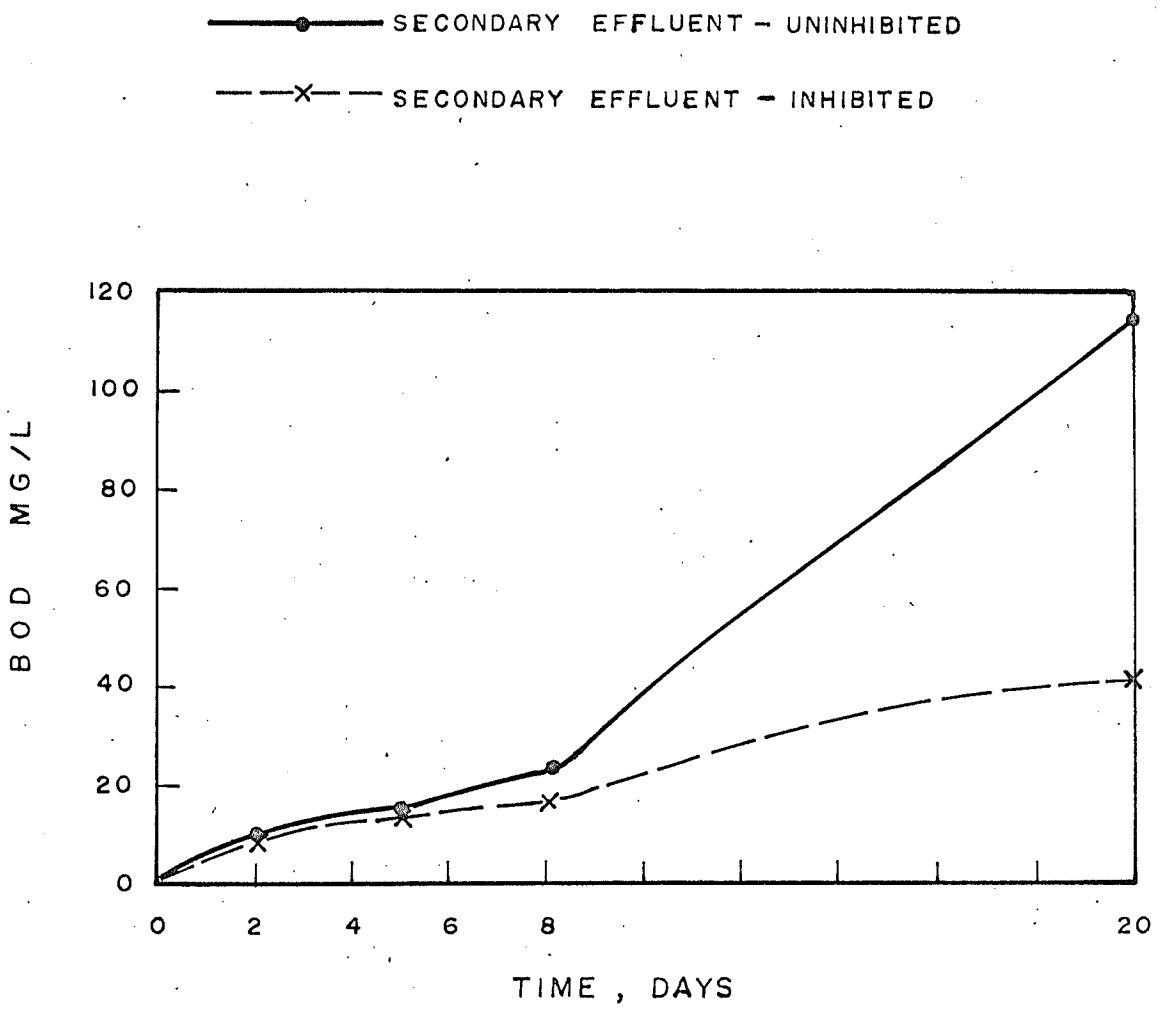
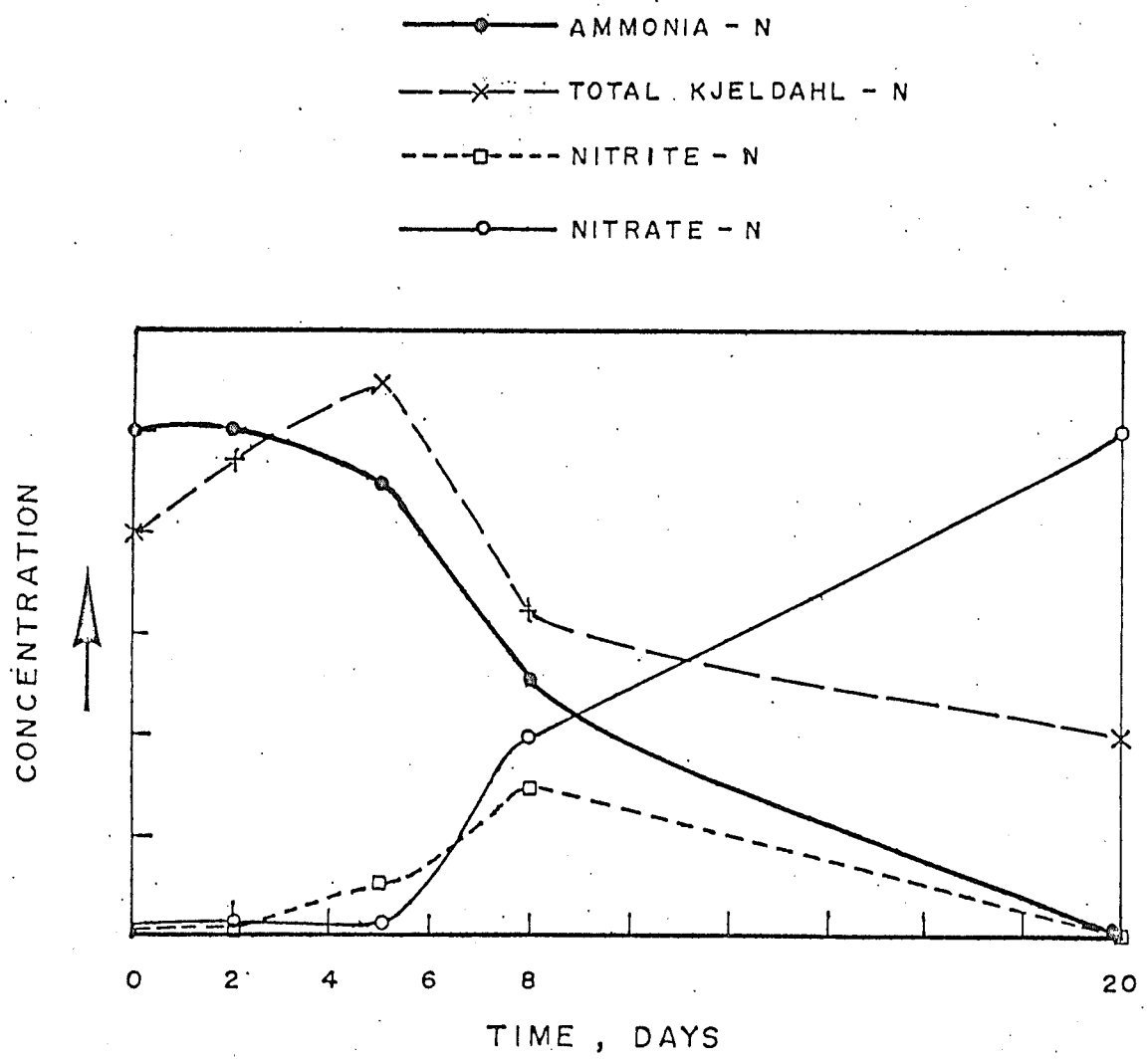

1000
900
800
700
600
500
400
300
200
100
0
BOD MG/L
0
2
4
6
8
20
TIME , DAYS
RAW SEWAGE - UNIHIBITED.
RAW SEWAGE - INHIBITED.
RAW SEWAGE - UNINHIBITED.
RAW SEWAGE - INHIBITED.

FIGURE 11. Extent of nitrification in inhibited and uninhibited BOD tests on secondary effluent. Nitrification inhibited by 10 mg/l of TCMP.


SECONDARY EFFLUENT - UNINHIBITED
SECONDARY EFFLUENT - INHIBITED
BOD MG/L
120
100
80
60
40
20
0
0
2
4
6
8
20
TIME , DAYS

Analyses of the nitrogen compounds in the BOD bottles also showed that nitrification began to occur any time after 2 days in the unspiked samples. Further the nitrogen testing showed that the TCMP acted as an effective control technique for the suppression of nitrification. An attempt was made to produce a nitrogen balance for the reactions taking place in the BOD bottles but met with little success. Several dilutions of the wastes had to be made for BOD purposes and this resulted in solutions containing very low concentrations of nitrogen. For this reason, it was felt that the values could not be used to produce a meaningful nitrogen balance, however, the values did reveal the overall trend. In the uninhibited samples it was found that the ammonia levels began to fall after 2 days and at 20 days the concentration of ammonia was undetectable. Further the nitrate levels increased dramatically throughout the 20 day incubation period. The nitrite levels rose to a peak at 8 days and then dropped to undetectable levels at 20 days. The total kjeldahl nitrogen levels rose to a peak at 5 days and declined thereafter. Figure 12 shows the trends that were revealed by the nitrogen analyses. The inhibited samples showed that all nitrogen compounds remained stable, relative to the uninhibited levels, through the 20 day incubation period.

FIGURE 12. The trend of nitrogen changes in uninhibited sewage 20 day BOD tests.


AMMONIA - N
TOTAL KJELDAHL - N
NITRITE - N
NITRATE - N
CONCENTRATION
0
2
4
6
8
20
TIME , DAYS

ULTIMATE OXYGEN DEMAND PREDICTIONS

An attempt was made to predict ultimate oxygen demand levels both carbonaceous and carbonaceous plus nitrogenous. This was done by using long term inhibited and uninhibited BOD studies, the Streeter-Phelps equation (5), and the equations developed by Montgomery (40).

It was found that with raw sewage in all cases the ultimate total oxygen demand as determined by the total uninhibited 20 day BOD values did not equal the inhibited 20 day BOD value plus the nitrogenous oxygen demand as calculated by the Montgomery equation. Typical values are shown in Table 1. It is noted that the discrepancies between the actual determined UOD and the calculated UOD vary from 29 to 72% for the raw. The secondary effluent values showed much better comparison and in general the lower the strength of the wastes the better the comparison obtained.

The UOD (carbonaceous) values calculated using the 5 day BOD and the Streeter-Phelps equation show reasonably good comparison. The calculated UOD value and the theoretical UOD value from the 5 day value plus the Montgomery NOD also show relatively good agreement.

The extremely high UOD values found on the high strength raw sewage could not be readily explained. It was again

TABLE 1

Typical values showing the relationship between actual determined total ultimate oxygen demands, calculated total ultimate oxygen demands, and theoretical ultimate oxygen demands.

SEWAGE TYPE	UOD_T*	UOD_t x	+	NOD	=	CALC. UOD_T	UOD_t°	+	NOD	=	THEOR. UOD_T
Raw Sewage	1010	390	+	87		477	425	+	87		512
Raw Sewage	660	140	+	85		225	166	+	85		251
Raw Sewage	216	100	+	55		155	95	+	55		150
Secondary Eff.	180	51	+	45		96	42	+	45		87
Secondary Eff.	115	42	+	41		83	52	+	41		93
Secondary Eff.	84	48	+	34		82	71	+	34		105

* ACTUAL DETERMINED TOTAL UOD

x ACTUAL DETERMINED CARBONACEOUS

° THEORETICAL CARBONACEOUS UOD FROM VELOCITY CONSTANTS

felt that the very high dilution used could account for a large part of the error since small changes in the final dissolved oxygen levels leads to large errors in the BOD. This sliding BOD theory was reinforced by the fact that the lower strength wastes repeatedly showed better correlation with the theoretical values and calculated values than did the high strength wastes.

BOD/TOC CORRELATIONS

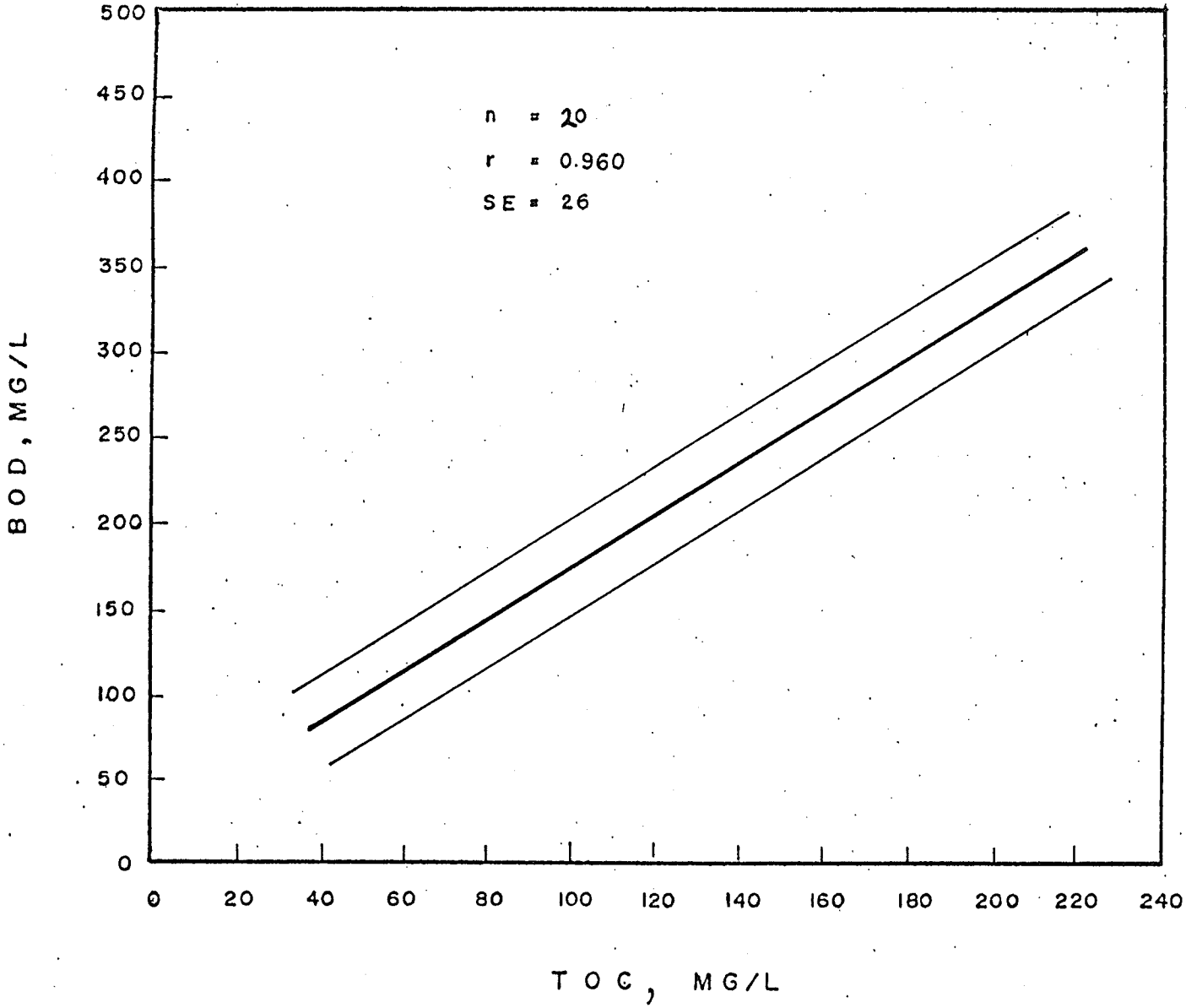
It was previously shown that a great deal of dissatisfaction is being directed towards the BOD test. For process control and for effluent monitoring the time involved between sample collection and final result makes the BOD test of questionable value. A minimum of 6 days (5 days incubation, 1 day set up and final analysis) takes place between collection and result. It has also been shown above that the BOD values are subject to many interferences that can severely alter their magnitude. It is felt that the TOC analyses would provide quicker, less expensive, more meaningful information on which to base operational changes or from which to assess the pollutional strength of a waste. It was felt that if the TOC could be shown to have a strong correlation to the BOD test that members of the profession would be more likely to begin using this new parameter. Also a strong positive correlation would allow workers in the field to begin to better relate to the TOC

analysis in terms of the pollutional strength of a waste. It must be stressed however, that a lack of correlation does not negate the importance of the TOC as will be shown later. The TOC was correlated to 5 day BOD values. Although it has been shown above that the BOD_5 can have considerable error associated with it, it was felt that with any particular waste that such factors as seed effects and nitrification would cause a "consistent" error and thus not greatly affect the correlation. Robbins et al (69) on his work with swine waste has shown that good BOD - TOC correlations exist and that where correlations were poor the source of errors was felt to be anamolies in the BOD analysis.

STANDARD SOLUTION CORRELATIONS

It was found that very strong correlation between BOD and TOC existed on standard solutions of glucose-glutamic acid. The correlation coefficient was found to be 0.995 with a standard error of 15. A correlation coefficient of 0.960 and a standard error of 26 was obtained when BOD/TOC correlations were carried out on standard glucose solutions (Figure 13). The values show that excellent correlation exists between TOC and BOD when carried out on readily biodegradable soluble organic compounds.

FIGURE 13. Regression analysis of 5 day BOD on TOC on a standard carbon solution. Outside lines are 95% confidence bands.


n = 20
r = 0.960
SE = 26
BOD, MG/L
TOC, MG/L
500
450
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
180
200
220
240

SEWAGE AND EFFLUENT CORRELATIONS

A statistical analysis of the relationship between sewage BOD and TOC was carried out. The results showed that a sound correlation exists between raw sewage BOD_5 and TOC. The correlation coefficient obtained on 112 sets of raw sewage data was 0.893 with a standard error of 37 (Figure 14). Regression analysis of the secondary effluent also produced a good correlation with a correlation coefficient of 0.705 and standard error of 9 on 88 pairs of data (Figure 15). Combining the raw and effluent data yielded a correlation coefficient of 0.962 and standard error of 29 (Figure 16).

It was found that when regression lines of individual groups of data such as those having TOC's of 0 - 50, 50 - 100, and 100 - 300 were plotted that the regression lines varied in slope (Figure 17). This effect it is felt again shows that the phenomenon of sliding BOD values is occurring on the higher strength (higher dilution) wastes. For practical purposes it is felt that the total BOD_5 can be best estimated from the TOC by using Figure 14 for the raw and Figure 15 for the effluent. The best estimate of raw sewage carbonaceous BOD_5 it is felt would be obtained from extrapolating Figure 15, since these values are less subject to the sliding BOD phenomenon.

FIGURE 14. Regression analysis of 5 day BOD on TOC on raw sewage. Outside lines are 95% confidence bands.

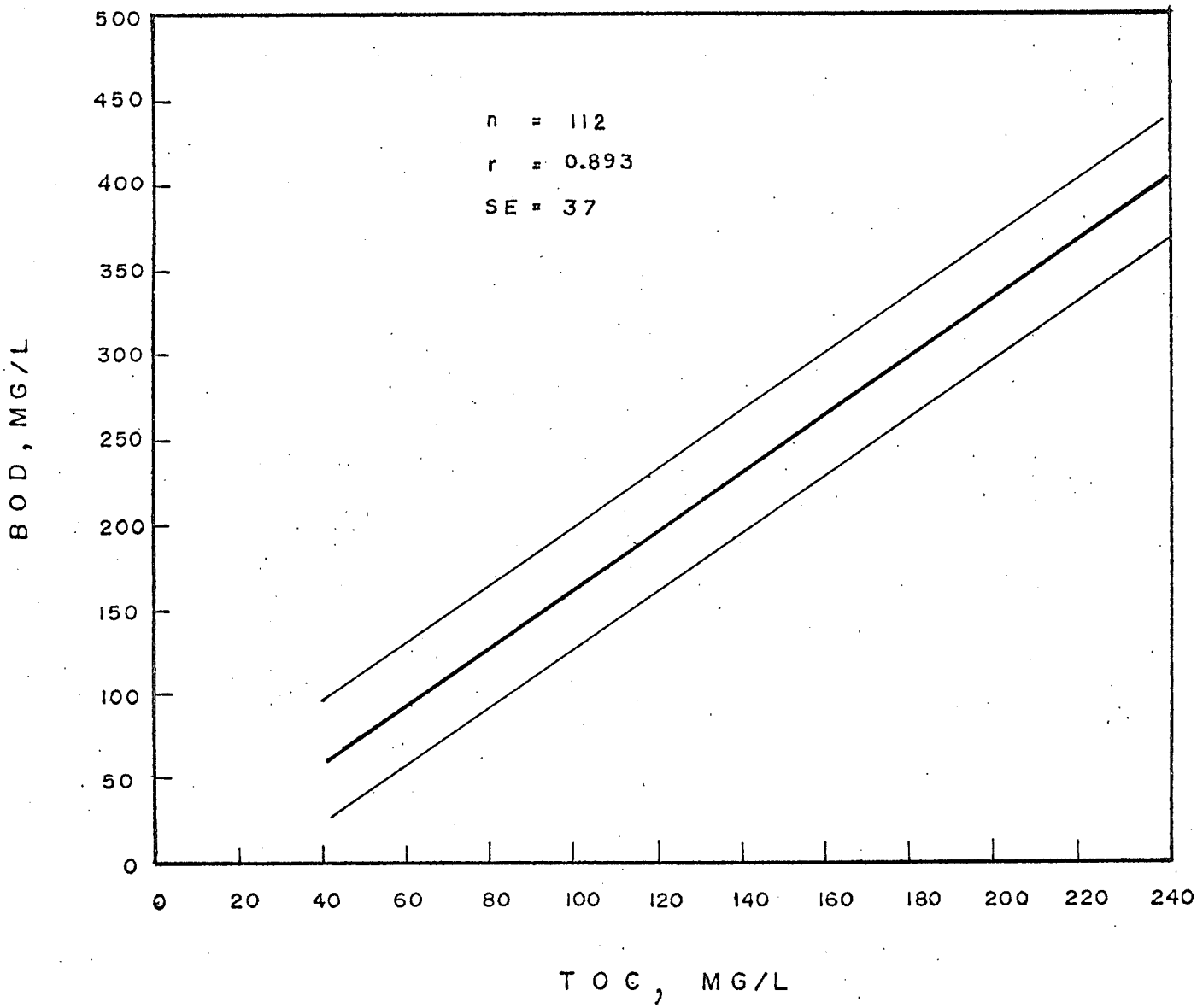

n = 112
r = 0.893
SE = 37
500
450
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
180
200
220
240
BOD, MG/L
TOC, MG/L

FIGURE 15. Regression analysis of 5 day BOD on TOC on secondary effluent. Outside lines are 95% confidence bands.

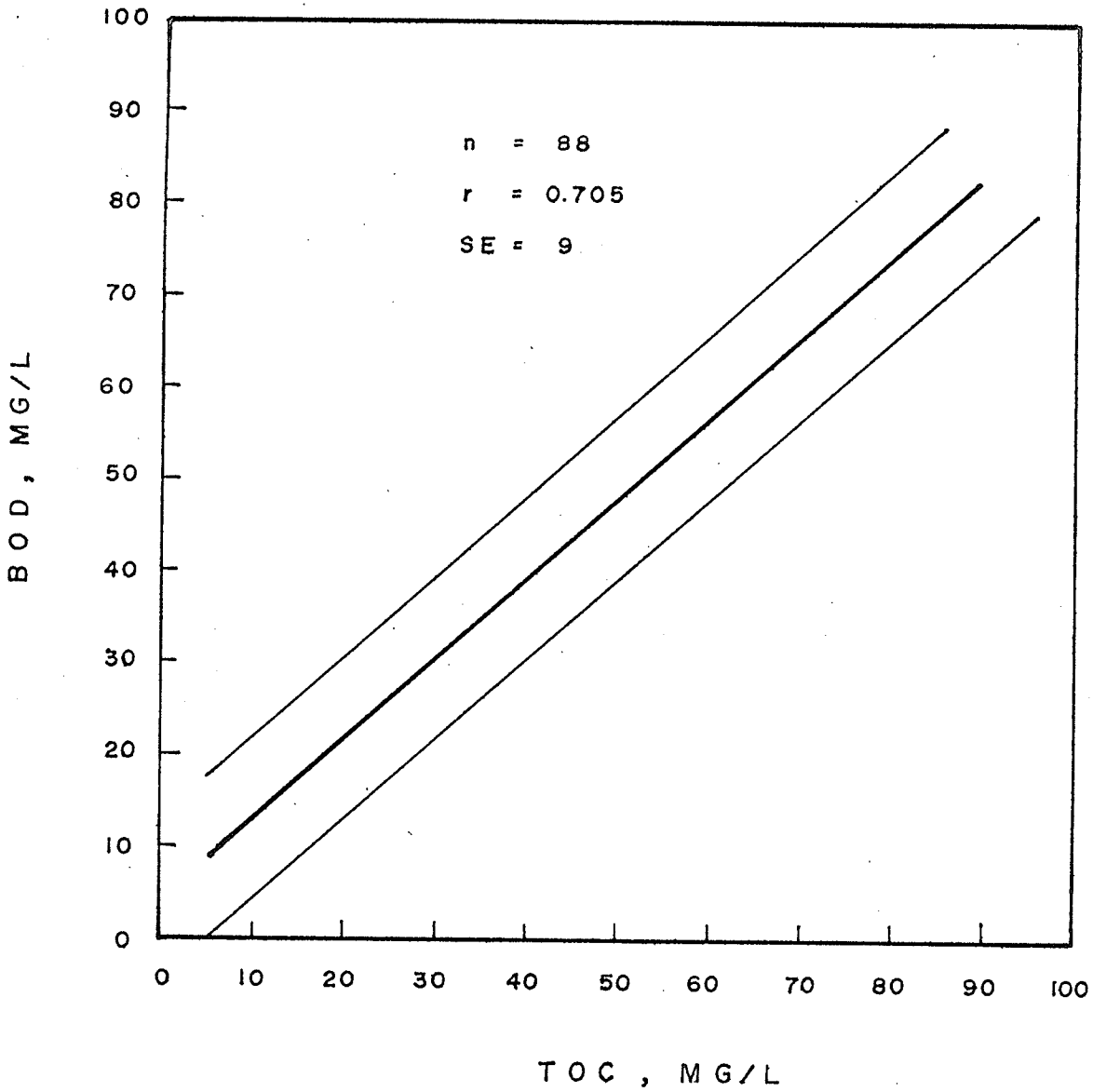

n = 88
r = 0.705
SE = 9
BOD, MG/L
TOC, MG/L
0
10
20
30
40
50
60
70
80
90
100

FIGURE 16. Regression analysis of 5 day BOD on TOC on combined data of raw sewage and secondary effluent. Outside lines are 95% confidence bands.

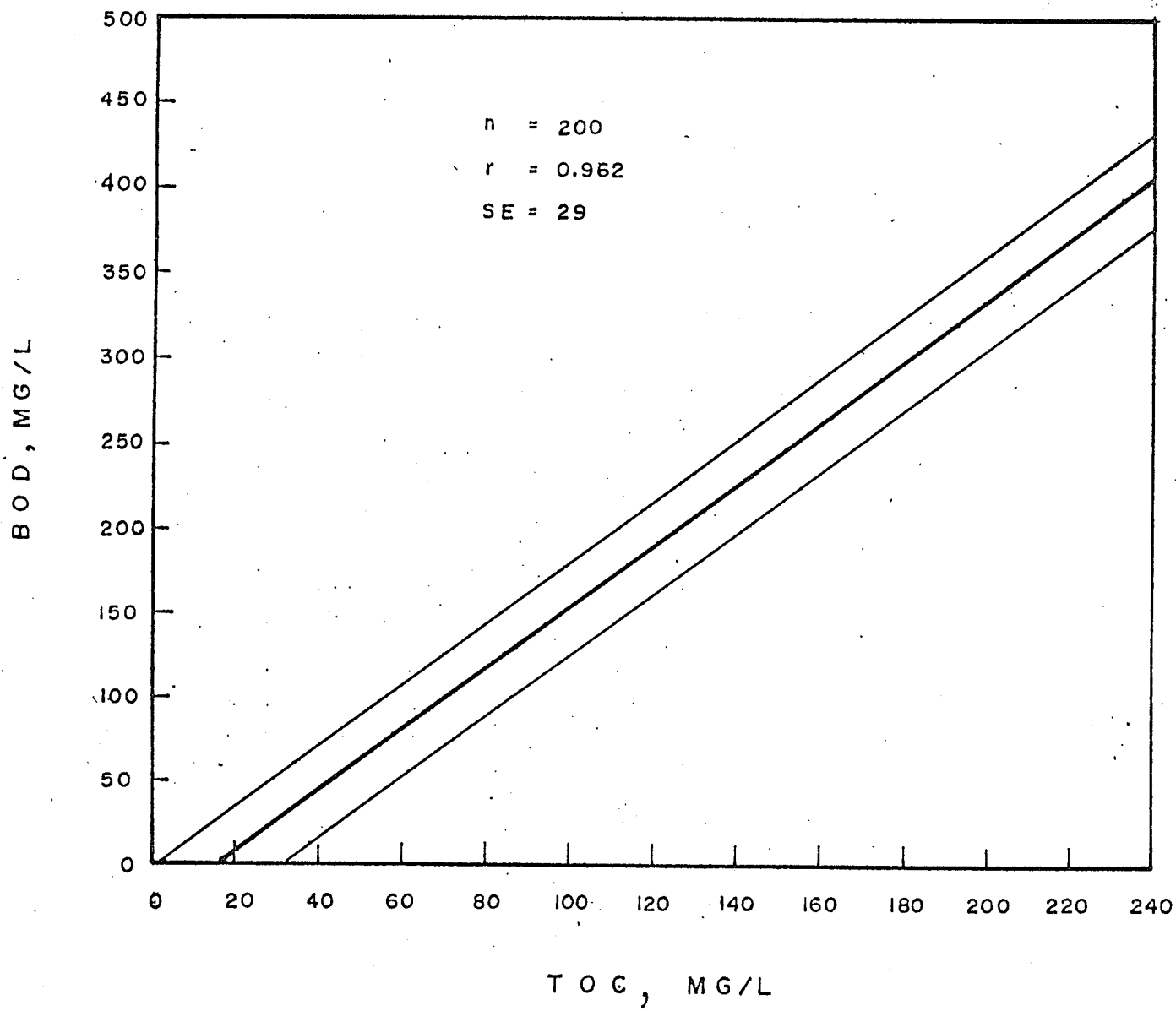
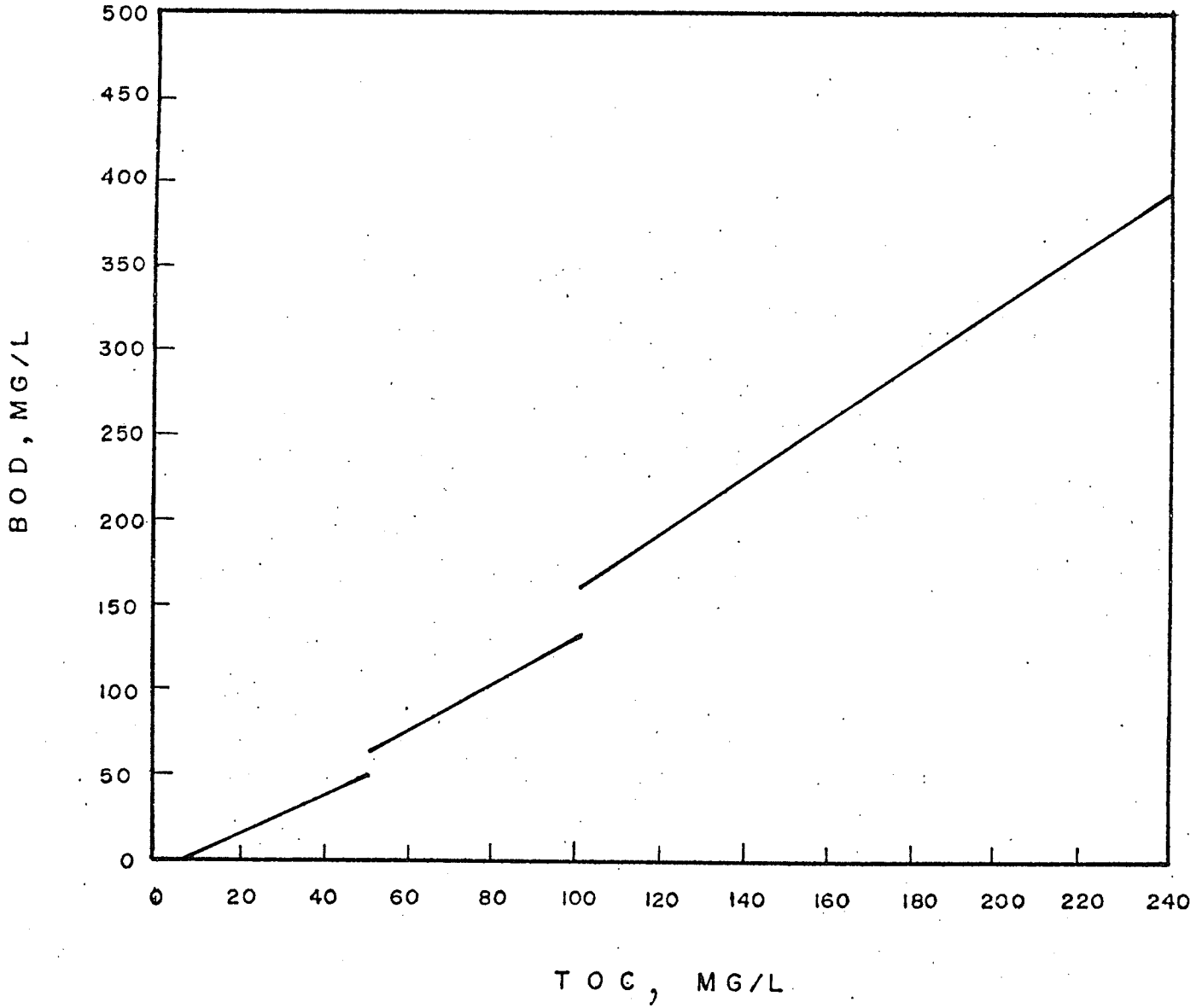

n = 200
r = 0.962
SE = 29
BOD, MG/L
TOC, MG/L
500
450
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
180
200
220
240

FIGURE 17. Regression lines produced from grouped data on various strength wastes.


500
450
400
350
300
250
200
150
100
50
0
BOD, MG/L
0
20
40
60
80
100
120
140
160
180
200
220
240
TOC, MG/L

In an effort to verify the curves an attempt was made to fit the South End Water Pollution Control Centre raw sewage and primary effluent data and Charleswood Lagoon raw sewage and effluent data to the curves. It was found that both the effluent data and the South End raw sewage data fit, within 1 standard deviation, the curves produced from the North End Plant data. The Charleswood Lagoon raw data however, did not seem to fit. The regression line produced by the data was quite flat and nowhere near the other regression lines found. Samples of Charleswood Raw sewage were consequently subjected to a complete analysis to determine if any inhibitory elements were present. It was found that the samples contained in excess of 1 mg/l of copper. Subsequently it was discovered that a length of copper tubing had inadvertently been used in the sampling system and was contaminating the samples. Copper has long been known as a major interfering element in the BOD analysis (66).

It was felt that this problem served to demonstrate that even a lack of correlation did not invalidate the TOC values but made them of vital importance. The experience did tend to point out one of the major weak points in a biological assay system and that is the problem of inhibition of biological activity. Had the contamination problem at Charleswood not been discovered , years may have been spent collecting and

analysing samples for BOD. The invalid BOD results obtained may well have served as the basis upon which a major design decision would have been based.

CONCLUSION

CONCLUSION

It has been successfully shown that TOC analysis can be a useful wastewater analysis parameter. This does not mean that the BOD concept does not have a place in the pollution control field but shows that an alternate parameter yielding more meaningful information is now available. Intermittent determination of the biodegradability of a waste using a modified 2 day BOD or Warburg analysis should yield sufficient data on which to assess the biodegradability or the treatability of a waste. The TOC parameter can be used on a more frequent basis to determine organic carbon concentrations such that influent waste strength, effluent strength, treatment efficiencies and design parameters can be obtained.

Thus while the concept of BOD remains an important factor in the pollution control field, changing technology has made the BOD procedure less important. Gaudy (1), an acknowledged expert in his field, has summarized the change of importance of the BOD procedure saying there is no real need to employ the 5 day BOD test as a functional loading parameter in the design of a biological treatment plant and there is no technologically justifiable need to relate the performance of a plant to the removal of 5 day BOD. What we are more interested in is the dynamics of the organic carbon compounds within a system.

APPENDIX

using the electrode

pH adjustment of solutions

Before measurement, all samples and standards must be made basic. Add 1 ml of 10 M NaOH per 100 ml of (neutral) solution. To make 10 M NaOH add water to 400 g of NaOH to make 1 liter, or dilute commercially available 50% NaOH with an equal volume of water.

checking electrode operation

Fill a 1-liter beaker with distilled water. Add approximately 10 ml of 10 M NaOH and mix, using a magnetic stirrer. Place the ammonia electrode in the solution. Add 1 ml of a 0.1 M ammonium chloride standard solution to obtain an ammonium level of 10^{-4} M. Using the mv scale on an expanded scale pH/mv meter, record the electrode potential. Provide a good mixing rate; wait at least 30 seconds before reading.

Now add 9 ml of the standard solution and record the potential. It should read approximately 59 mv more negative than the previous reading. (See Figure 4).

Note that it is not necessary to have exactly 1 liter of water, the only requirement is that the *change* in concentration be ten-fold between additions. By adding standards of other concentrations, the response of the electrode can be evaluated over a greater range.

standardizing solutions

Ammonia standards are not stable due to loss of ammonia gas to the air. Primary standards of ammonium chloride are recommended (Orion Cat. No. 95-10-06, 0.1 M ammonium chloride or Cat. No. 95-10-07 ammonium chloride, 1000 ppm as N). Immediately before use, add base to the standard to convert ammonium ion to ammonia, 1 ml of NaOH per 100 ml of standard.

To prepare a stock 100 ppm (as NH_3) standard, place 58.8 ml of the 0.1 M ammonium chloride standard in a 1-liter volumetric flask (use a 50 ml pipet and a 10 ml pipet graduated in tenths to deliver 8.8 ml), add water to volume. More dilute standards are prepared by serial dilution. Immediately before use add 1 ml of 10 M NaOH per 100 ml of standard.

storage

Between measurements the electrode should be kept in alkaline standardizing solution. When not in use, the electrode should be placed in a 0.1 M ammonium chloride solution (without sodium hydroxide). *Do not store in air.*

If the electrode is accidently left in air, rather than in a solution, that portion of the internal filling solution between the inside of the membrane and the sensing

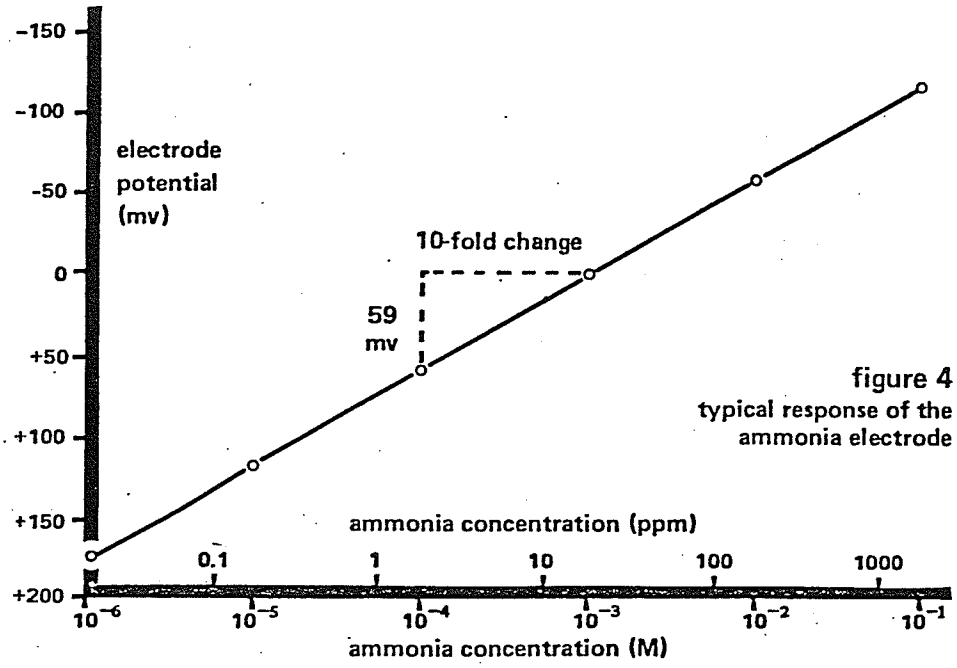


figure 4
typical response of the ammonia electrode

element will dry out. To restore electrode operation hold the electrode by the outer body and, grasping the electrode cable directly above the cap, pull up on the cable so as to lift the sensing element off the membrane. Fresh internal filling solution will now flow under the membrane. The electrode will now be immediately ready for use.

If the electrode is to be returned to the storage box, it should be disassembled; the inner body, outer body and bottom cap rinsed with water, dried and re-assembled without filling solution or membrane.

measuring hints

Samples and standards should be at the same temperature.

Samples and standards should be stirred using a magnetic stirrer. Some magnetic stirrers generate sufficient heat to change solution temperature. This effect can be minimized by placing a piece of insulating material on the stirrer.

To prevent solution carry-over, the electrode should be rinsed with distilled water and blotted dry with a tissue between measurements.

Ammonia gas is lost to the air from the samples and standards. Between standardizations, keep beakers containing standards covered. Do not add NaOH to ammonium chloride standards and samples until they are ready to be measured. Use as large a solution volume as convenient to minimize the surface area to volume ratio. Do not stir solutions at so fast a rate as to cause a vortex to be formed. The rate of ammonia loss at room temperature from a stirred 100 ml basic solution in a 100 ml beaker is about 50% in six hours.

troubleshooting

checklist:

Were the membrane, spacer, and O-ring installed in the cap in the proper order, with the membrane right way up (page 4)?

Was the bottom cap screwed on tightly (so as to close the gap between the bottom cap and body)?

Was the internal filling solution added?

Was the top cap screwed down into place?

Is the electrode plugged into both the reference and sensing electrode input connectors?

Were samples and standards made basic?

membrane failure

Membrane failure is characterized by a shift in electrode potential, drift, and poor response. Membrane failure may be apparent on visual inspection as dark spots or discoloration of the membrane. Handling the membrane during installation may affect its hydrophobic properties, causing shortened life. Handle the membrane with the tweezers provided. A membrane will last from one week to several months depending on usage.

checking sensing element operation

The electrode sensing elements may be checked as follows:

Add 0.58 grams of NaCl to 100 ml of 4.01 and 100 ml of 7.00 pH buffer. Dissolve completely. Immerse the inner body in each of the two solutions, rinsing with distilled water between solutions. A potential difference of about 175 mv should be observed between the two solutions if the inner body sensing elements are correctly operating.

NITRATE + NITRITE IN WATER
(Range: 0-0.6ppm)

The automated procedure for the determination of nitrate plus nitrite in water utilizes the reaction whereby nitrate is reduced to nitrite by an alkaline solution of hydrazine sulfate containing a copper catalyst. The stream is then treated with sulfanilamide under acidic conditions to yield a diazo compound which couples with N-1-naphthylethylenediamine dihydrochloride to form a soluble dye which is measured colorimetrically. The final product measured represents the nitrite ion originally present plus that formed from the nitrate.[1]

Chlorine, sulfide, ferric ion, and phosphate ion interfere.

PERFORMANCE AT 20 SAMPLES PER HOUR

Using Aqueous Standards:

Sensitivity (Conc'n which gives deflection from baseline to 40%T)	0.34ppm
Coefficient of Variation (95% confidence level at 0.3ppm)	2.2%
Detection Limit	0.02ppm

REAGENTS

COLOR REAGENT: (Technicon Nos, T11-5065, T01-5017)

Sulfanilamide, $C_6H_8N_2O_2S$	20 g
Concentrated Phosphoric Acid H_3PO_4	200 ml
N-1-Naphthylethylenediamine dihydrochloride, $C_{12}H_{14}N_2 \cdot 2HCl$	1.0g
Distilled water, q.s.	2000 ml

Preparation:

To approximately 1500ml of distilled water add 200ml concentrated phosphoric acid and 20g of sulfanilamide. Dissolve completely. (Heat if necessary.) Add 1g of N-1-naphthylethylenediamine dihydrochloride, and dissolve. Dilute to two liters. Add 1.0ml Brij-35 (Technicon No. T21-0110) Store in a cold, dark place. STABILITY: one month.

STOCK COPPER SOLUTION:

Anhydrous Cupric Sulfate, $CuSO_4$	2.5 g
Distilled water, q.s.	1000 ml

Preparation:

Dissolve 2.5g cupric sulfate in distilled water and dilute to one liter

Working Copper Solution:

Dilute 6.25ml of stock copper solution to two liters with distilled water.

STOCK SODIUM HYDROXIDE, 3N

Sodium Hydroxide, NaOH	120 g
Distilled water, q.s.	1000 ml

Preparation:

Dissolve 120g of sodium hydroxide in 750ml distilled water. Allow to cool and dilute to one liter.

Working Sodium Hydroxide, 0.3N:

Dilute 100ml of 3N sodium hydroxide to one liter with distilled water.

STOCK HYDRAZINE SULFATE:

Hydrazine Sulfate, $N_2H_4 \quad H_2SO_4$	54.00 g
Distilled water, q.s.	2000 ml

Preparation:

Dissolve 54.00g of hydrazine sulfate in 1800ml distilled water. Dilute to two liters. This solution is stable for six months if stored in a tightly stoppered amber bottle.

CAUTION: Toxic if ingested.

Working Hydrazine Sulfate:

Dilute 25ml of stock hydrazine sulfate to one liter with distilled water. Store in an amber bottle. STABILITY: One month.

STANDARDS

NITRATE-NITROGEN STANDARD: 100 ppm

Potassium Nitrate, KNO_3 (Technicon No. T13-5074)	0.7218 g
Distilled water, q.s.	1000 ml

Preparation:

Dissolve 0.7218g of potassium nitrate in distilled water and dilute to one liter. Add 2ml of purified chloroform per liter as a preservative. Prepare working standards in the range of 0.02ppm to 0.6ppm in serial dilutions for calibration.

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1. Kamphake, L.J., Hannah, S.A., and Cohen, J.M., Automated Analyses for Nitrate by Hydrazine Reduction, Water Research, Vol. 1, 1967, pg. 206.

NITRITE-NITROGEN STANDARD: 100 ppm

Sodium Nitrite, $NaNO_2$ (Technicon No. T11-0169)	0.4926	g
Distilled water, q.s.	1000	ml

Preparation:

Dissolve 0.4926g of sodium nitrite in distilled water and dilute to one liter. Add 2ml of purified chloroform per liter as a preservative. Prepare working standards in the range 0.02ppm to 0.6ppm in serial dilutions for calibration.

OPERATING NOTES

1. To determine optimum temperature for the reduction process, set the temperature of the heating bath at 38°C and aspirate a .04ppm nitrite-nitrogen standard for five minutes. Follow this with a .04ppm nitrate-nitrogen standard for five minutes. The resulting two peaks must have equal optical densities. Incomplete reduction is indicated when the nitrate-nitrogen peak is lower than the nitrite-nitrogen peak. Equivalence is obtained by increasing the temperature of the heating bath. If the nitrate-nitrogen peak is higher than the nitrite-nitrogen peak, loss of nitrite is indicated and the heating bath temperature should be decreased. Repeat the aspiration of the two samples to determine whether equivalence has been achieved. Once the correct temperature has been determined for the system, there is usually no need for further adjustment.

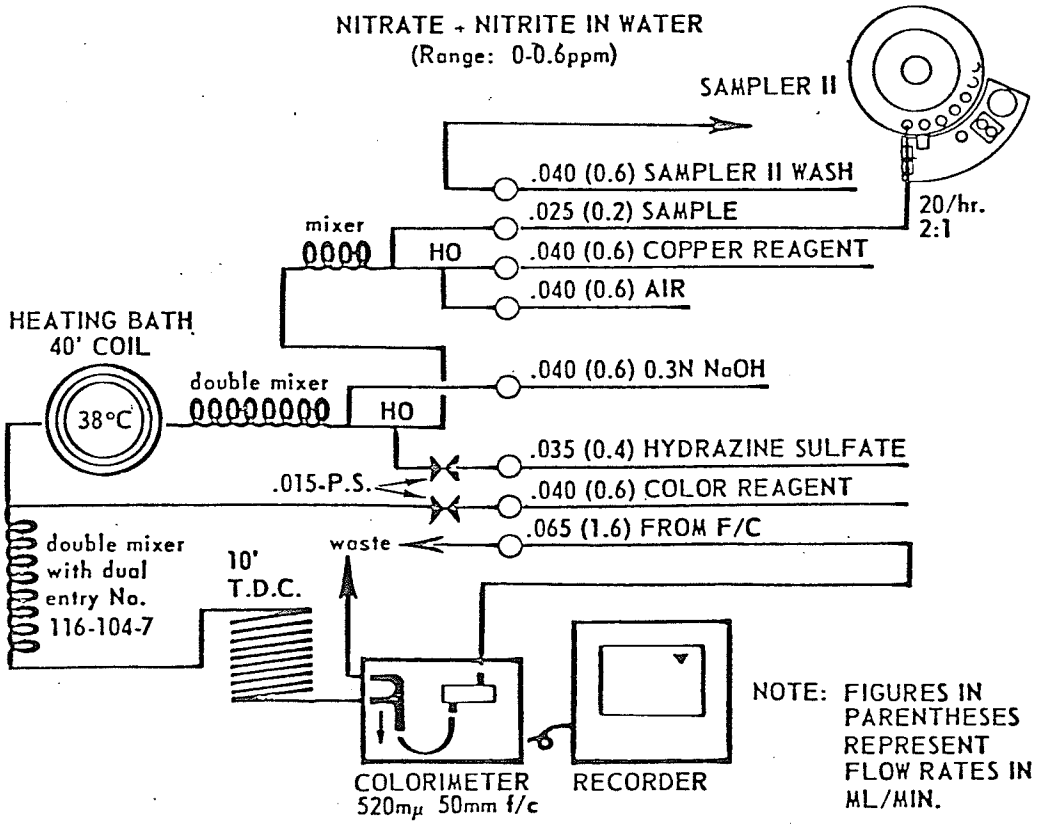
2. Samples should be processed and analyzed as soon as possible. If this cannot be done immediately, they should be refrigerated at 5-10°C or preserved with 1ml chloroform per 100ml sample.

3. In order to determine nitrate levels, the nitrite alone must be subtracted from the total (nitrate + nitrite). This can be achieved using Technicon Methodology No's. 34-69W or 35-69W or by substituting distilled water for the copper, hydrazine, and NaOH lines on the manifold. A separate calibration curve should be determined for nitrate plus nitrite and for nitrite alone.

4. Where particulate matter is present, the solution must be filtered prior to the determination. This can be accomplished by having the Technicon Continuous Filter as an integral part of the system if the sample is such that Whatman #4 or equivalent filter paper is satisfactory. (See Continuous Filter Manual, No. CFO-1).

If the samples contain high concentrations of heavy metals which are precipitated in the alkaline medium of the reduction process, 0.1N sodium hydroxide may be introduced to the sample through the mixing block on the Continuous Filter in order to form the metal hydroxide prior to sample filtration. (See Addendum to Manual No. CFO-1).

5. At the end of a day's operation, the system should be cleansed by pumping 0.1N hydrochloric acid through for five minutes followed by distilled water for ten minutes. This wash dissolves any cuprous oxide which has formed within the analytical train.



TOTAL NITROGEN (KJELDAHL)

(Range: 0-40 ppm)

The quantitative determination of total nitrogen involves digestion of organic material, using the Technicon Continuous Digestor, followed by measurement of the quantity of ammonia produced. The quantitation of ammonia is achieved utilizing the Berthelot Reaction in which the formation of a blue indophenol complex occurs when ammonia is reacted with sodium phenate followed by the addition of sodium hypochlorite.[1, 2]

PERFORMANCE AT 20 SAMPLES PER HOUR

Using Aqueous Standards:

Sensitivity (Conc'n which gives 40%T deflection)	17 ppm
Coefficient of Variation (95% confidence level at 270ppm)	0.74%
Detection Limit	0.05ppm

REAGENTS

DIGESTION MIXTURE:

Selenium Dioxide (Tech. No. T11-0117)	3.0g
Sulfuric Acid, concentrated (Sp. Gr.-1.84)	900ml
Perchloric Acid, 68-70%	20ml

Preparation:

Dissolve 3.0g selenium dioxide in approximately 50ml distilled water and add 20ml perchloric acid Add 900ml concentrated sulfuric acid and dilute to one liter. Mix and allow to cool. Adjust the volume and store in amber glass bottles.

CAUTION: This reagent is very corrosive.

SODIUM HYDROXIDE REAGENT:

Sodium Hydroxide (NaOH)	350g
Potassium Sodium Tartrate ($KNaC_4H_4O_6.24H_2O$)	50g

Preparation:

Dissolve 350g sodium hydroxide and 50g potassium sodium tartrate in about 700ml distilled water in a one liter volumetric flask. Allow to cool and dilute to volume. Store in a polyethylene bottle.

NOTE: The potassium sodium tartrate is added to prevent precipitation of heavy metal contaminants in the alkaline medium.

ALKALINE PHENOL: Technicon No. T01-0115

Sodium Hydroxide, (NaOH)	200g
Phenol, liquified, about 88%	276ml
Distilled water, q.s.	1 L

Preparation:

Dissolve 200g of sodium hydroxide in 700ml distilled water in a vessel surrounded by circulating cold water. Slowly add 276ml of liquified phenol from a separatory funnel to the sodium hydroxide solution, stirring the mixture continuously. Dilute to one liter with distilled water. Store in a polyethylene bottle.

SODIUM HYPOCHLORITE:
Technicon No. T01-0114

Preparation:

Any good commercially available household bleach having 5.0% available chlorine is suitable.

STANDARDS

In order to achieve the greatest accuracy from this system, it is essential that a carefully assayed nitrogen containing material having the same matrix as the samples is used for calibration. (e.g., It has been found that β-alanine should be used for meat samples and 2-Benzyl-2-pseudo-thiourea for milk samples.)

The following ammonium sulfate standard is suitable for checking out the system for proper functioning.

STOCK STANDARD: 10,000ppm

Ammonium Sulfate, $(NH_4)_2 SO_4$	47.168g
Sulfuric Acid, 50% v/v, q.s.	1 Liter

Preparation:

Dissolve 47.168g ammonium sulfate in 50% sulfuric acid and dilute to one liter with acid. Prepare serial dilutions in the range 0.05ppm to 40ppm.

STANDARD AND SAMPLE PREPARATION TECHNIQUE

1. Place an aliquot of the sample material in a

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1. Van Slyke, Donald D., and Hiller, Alma, J. BioChem., V102, 1933, p. 499.
2. Ferrari, A., N.Y. Acad. Sci., Vol. 87, Art. 2, 792-800, July 22, 1960

flask. This weight is chosen so the final concentration of the sample is within the analytical range of the system (i.e., 1 gr. of dry material having a theoretical nitrogen content of 0.3% in a final volume of 100ml will have a concentration of 30ppm N.)

In this weighing the sample should either be dried or the water content taken into consideration, and ground in a stainless steel Wiley mill, passing a 20-mesh screen.

2. The sample is "wetted" with distilled water (about 40% of the final volume) and allowed to stand for one or two hours.

NOTE: It is often most convenient to perform this step at the close of the working day and allow the samples to "wet" overnight.

3. Slowly add concentrated sulfuric acid to the wetted sample. Only enough H_2SO_4 is added to make a final concentration of 45-50% in the final volume.

NOTE: Be sure not to exceed 50% H_2SO_4 in the final sample concentration as this is the upper limit of acidity tygon pump tubes can tolerate).

The subsequent heat of reaction will char and solubilize the sample on gentle agitation.

4. After cooling, dilute to volume with distilled water. The cool (room temperature) sample is now ready to be placed in cups on the sampler.

OPERATING NOTES

1. It is necessary when using this sample preparation technique to use a rotary mixer and segmenting sample probe on the Sampler II. The mixer stirs the sample as it is being aspirated and before it is aspirated, thus insuring that a representative aliquot of the material in the cup is sampled.

The segmenting sample probe introduces an air bubble into the sample stream immediately upon leaving the cup. This bubble serves to keep any small particles in suspension until they reach the helix and to keep the walls of the tubing, pipettes and pump tubes from becoming contaminated with sample material.

2. The alkaline phenol reagent should be filtered through glass wool prior to use.

3. The Sampler II Wash Receptacle should contain acid of the same concentration as the prepared samples and standards.

DIGESTOR WARM-UP PROCEDURE

(1) With all reagent lines except the acidflex in water, turn on both proportioning pumps and digestor module.

(2) Check to see that the system is operating properly, e.g., water pumping from diluent pipette, proper aspiration in mixing chamber, etc. (See Continuous Digestor Manual, T-69-123).

(3) Begin pumping digestion mixture and all analytical reagents through their respective lines.

(4) After five minutes, turn on Heat switch and adjust amperage to proper settings.

NOTE: To determine proper heat settings, continuously aspirate a midrange concentration of the nitrogen containing standard through the system and vary the amperage upwards from 2 and 4 respectively. Continue to increase the amperage until maximum optical density is obtained. These will be the optimum amperage settings for any given matrix. (Settings 4 and 7 are usually adequate).

(5) Warm-up time is approximately twenty minutes (or until reagent baseline stabilizes).

DIGESTOR SHUT-DOWN

(1) Remove helix cover and turn off Heat switch.

(2) Place alkaline phenol and sodium hypochlorite lines in wash water.

(3) After temperature for all three stages reads below 150°C, place digestion mix line in wash water.

(4) When all acid has been washed clear of helix, place NaOH line in wash water.

(5) Continue to wash out system for 10-15 minutes.

(6) Shut off proportioning pumps.

(7) Break vacuum in liquid waste bottle.

(8) Shut off digestor power switch.

(9) Replace helix cover.

TOTAL NITROGEN (KJELDAHL) (Range: 0-40ppm)

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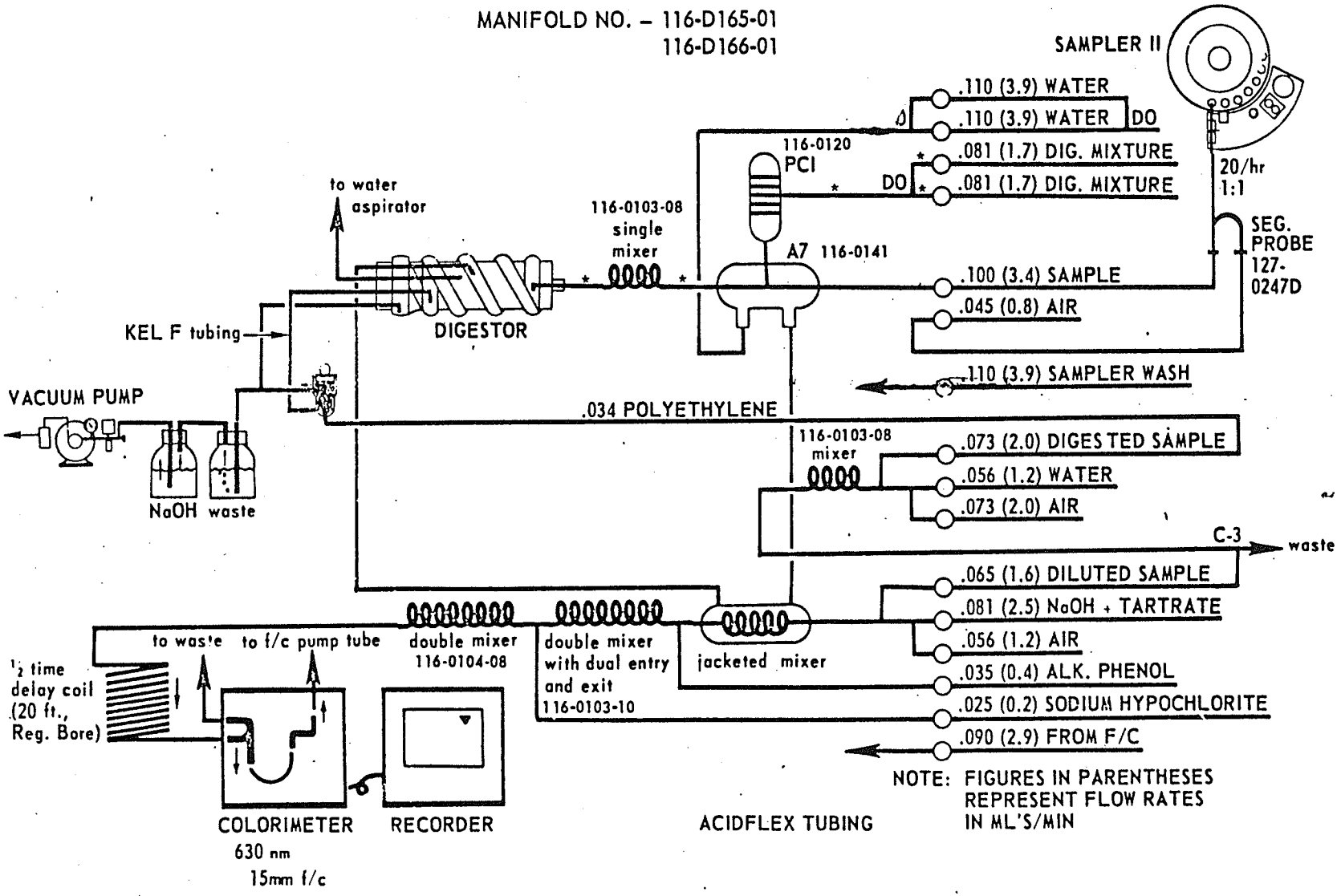
116-D166-01



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