CHANGES IN THE SPECIES AND CONCENTRATION OF CARBON, NITROGEN AND SULFUR COMPOUNDS DURING THE BOD TEST AND THEIR EFFECT ON OXYGEN UPTAKE

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Project A-073-PR
Grant Agreement No. 14-34-0001-2141

FINAL TECHNICAL REPORT
TO
U.S. DEPARTMENT OF THE INTERIOR
WASHINGTON, D.C. 20240

"The work on which this report is based was supported in part by funds provided by the United States Department of the Interior as authorized under the Water Research and Development Act of 1978 (P.L. 95-467)".

September 1984

ABSTRACT

The biochemical oxygen demand (BOD) is defined as the amount of oxygen required by bacteria in the oxidation of organic matter under aerobic conditions. Nitrification is the oxidation of nitrogen forms to nitrite and nitrate. In this process oxygen is consumed and thus interferes with the standard BOD test, when it is desired to measure carbonaceous BOD, leading to high BOD results.

The main objective of this study was to obtain a better view of the BOD test, when used as a regulatory parameter in the performance evaluation of a wastewater treatment plant. Specifically it was desired to observe the effect of nitrification and of the oxidation of other possible oxygen-consuming substances, such as sulfur compounds, and to develop a method by which the results of the standard BOD test could be corrected by stoichiometry, so that the final results of the test would only reflect the actual amount of oxygen used in the oxidation of carbonaceous organic matter, the so called carbonaceous BOD.

Several tests were run on wastewater treatment plant effluents in which the changes in oxygen concentration in the samples were measured on a day by day basis, as well as changes in sulfates, nitrites, nitrates, and alkalinity. Initially, the tests were run for ten days and tests for total Kjeldahl nitrogen and ammonia nitrogen were also performed at the beginning, at midpoint, and at the end of each run, for the purpose of computing nitrogen balances as a check for the other tests. Near the end of the study, some parallel "inhibited" BOD tests were also run.

Early in the study it was discovered that under the conditions of the standard BOD test there is no significant, if any, interfering effect of sulfur compounds in the test. The alkalinity measurements produced

constant values during all runs. Nitrification was indeed measured, although the effect was not great in the analyzed samples. Stoichiometric corrections for nitrification were applied to the BOD values obtained in the standard BOD test. This "corrected" BOD values were smaller than the standard values, as expected, and about the same in general as the results of the "inhibited" BOD test. Excellent correlation was found between the "correct" BOD values and the "inhibited" BOD values.

Based on the results of this study, it may be concluded that both the "corrected" and the "inhibited" BOD tests are better parameters than the standard test, in which to rely in the performance evaluation of wastewater treatment plants, since they give the true value of carbonaceous BOD in the treated wastewater, as a true measurement of the presence of decomposable organic matter.

Acknowledgement

This study was financed with a grant obtained from the Water Resources Division of the U. S. Geological Survey, through the Water Resources Research Institute of the University of Puerto Rico, under the direction of Dr. Rafael Muñoz Candelario, Associate Dean of Engineering for Research. Special thanks go to José R. Roubert, graduate student in the Civil Engineering Department of the University of Puerto Rico, who did most of the experimental work of this study.

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Introduction

Biochemical oxygen demand (BOD) is usually defined as the amount of oxygen required by bacteria while stabilizing decomposable organic matter under aerobic conditions (1). The BOD test is widely used to determine the pollutional strength of domestic and industrial wastewaters in terms of the oxygen that they will consume if discharged into natural water courses in which aerobic conditions exist. The test is one of the most important in stream pollution control activities. It is of prime significance in regulatory work. As an example, NPDES permits usually limit tbe BOD concentration that may be discharged into a stream.

Because of the nitrification effect some BOD tests, particularly those performed on treatment plant effluents, tend to give misleading results (4). To try to solve this problem an "inhibited" BOD test has been proposed (2) (9) and has been adopted by Standard Methods (10) to be used with certain substrates. Nevertheless, it has been reported (4) that state regulatory agencies and the Environmental Protection Agency (EPA) have refused to accept the inhibited BOD test in many recent cases. It is evident that some confusion exists with regard to this matter.

The problem could be further complicated by other oxygen-consuming organic substances, such as organic sulfur, a common component of certain proteins. Under aerobic decomposition of these substances, sulfur might be released in the form of sulfates, an oxidized specie of sulfur.

Object and Scope

The principal objectives of this project were:

1. To determine how the concentration and species of carbon, nitrogen, and sulfur compounds change (on a day by day basis) during a ten-day BOD run, for various types of substrates

2. To correlate through stoichiometry those changes with changes in dissolved oxygen concentration during the ten-day BOD run.
3. To help in better understanding the dynamics of the BOD test, used as a regulatory and monitoring tool.

Although not included in the original proposal of this study, a correlation of the results of this project with parallel "inhibited" BOD runs was attempted.

Literature Review

It has been recognized that the biological oxidation of nitrogenous materials will cause errors in the standard BOD test (2) (3). This will result in higher BOD values than the true oxygen demand excerted by organic carbonaceous materials.

Carbonaceous and nitrogenous oxygen demands must be considered separately in evaluating the performance of wastewater treatment plants. To do otherwise leads to the generation of effluent quality data that is both meaningless and misleading. (4). In fact, it is theoretically possible for plant effluents, which are in the so called incipient nitrification stage, to exhibit greater 5-day BOD values than those shown by untreated sewages. This factor has tended to place in a rather unfavorable light those operators who strive to get a reasonable degree of nitrification in their plant effluents (2).

A treatment plant that is partially nitrifying will have nitrifying bacteria associated with the volatile suspended solids in the effluent. Such a plant will show an increased effluent BOD as a result of nitrification during the standard 5-day test. Another plant, treating an identical wastewater, but not achieving partial nitrification, would display a lower

effluent 5-day BOD than the first plant. The second plant would seem to be doing a better job than the first, in terms of BOD removal, but just the opposite is true. The first plant achieving partial nitrification, is actually removing more total BOD than the other plant that is not achieving partial nitrification. (4).

Although considerable amount of work has been done on the nitrification process (2) (3) (4) (5) (6) (7) (8) (9), there has been little agreement on the proper way to handle the nitrification interference in the BOD test, although some suggestions have been made to that effect. (4). Just recently the Standard Methods (10) have adopted the "inhibited" BOD test for those cases in which it is expected to have significant numbers of nitrifying bacteria in the substrate, as it happens in sewage treatment plant effluents. Nevertheless, Dague (4) reports that he "has recently encountered several cases where the state regulatory agency and the Environmental Protection Agency (EPA) has refused to accept the inhibited 5-day BOD (carbonaceous BOD_5) test as the basis for interpreting wastewater treatment plant performance".

On the other hand, it is not well known how other oxygen-consuming substances may effect the BOD test. For instance, certain proteins contain sulfur which may be released in the form of sulfates during their biodegradation. It has been long recognized that the sulfur cycle is an important life cycle. In the decomposition of organic matter, sulfur plays an important role. The BOD test relies on the aerobic decomposition of organic matter under controlled conditions. How much sulfur is released in the form of sulfates during a BOD run and how it affects oxygen uptake is certainly an important question to be answered.

Laboratory Procedure

Unchlorinated effluents from two nearby wastewater treatment plants were used for this study. The plant of the town of Añasco is a secondary plant with trickling filters. The plant of Alturas de Mayaguez Urbanization is an extended aeration activated sludge system.

Standard BOD tests were run on multiple portions of each sample, for two different dilutions, for periods of 5 to 10 days. Each day duplicate portions of each sample were analyzed for dissolved oxygen, nitrites, nitrates, alkalinity and sulfates. For the initial ten-day runs, total Kjeldahl nitrogen and ammonia nitrogen tests were run at the beginning, at midpoint, and at the end of the ten-day period to compute nitrogen balances. It was soon discovered that near the end of the ten-day run dissolved oxygen values were very low and the system tended to denitrify, with the probable loss of gaseaous N_2. This was evidenced by the reduction of nitrate levels near the end of the run. For this reason, shorter runs were programed thereafter, with TKN and NH_3-N tests performed at the beginning and end of the runs only.

After the first few test runs, it was found that sulfate was not been released during the course of the BOD test (at least not in measurable levels). Thereafter, sulfate tests were discontinued, since its participation in the process was deemed insignificant.

Originally, it was proposed to use a newly acquired TOC analyzer to follow the transformations undergone by carbon species. Unfortunately, the instrument never performed adequately and this part of the study had to be excluded from the project.

Near the end of this study, "inhibited" BOD tests were run on parallel samples to try to correlate the results of this technic with those obtained

by the standard BOD test, both without and with a nitrification correction applied to it. Although this was not part of the original proposal, it was used as a sort of substitute for the unperformed TOC studies. As it turned out, this part of the study gave very interesting and significant results.

All tests were performed using standard technics as specified in the "Standard Methods for the Examination of Water and Wastewater", 15th edition. (10).

Computation Procedure

According to the "Standard Methods", the following formula is to be used in the computation of the biochemical oxygen demand:

$$\text{BOD in mg/l} = \frac{D_1 - D_2}{P}, \text{ when seeding}$$

is not required, as in the effluent from domestic wastewater treatment plants, or

$$\text{BOD in mg/l} = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}, \text{ when using seeded dilution}$$

water, where

D_1 = DO of diluted sample 15 minute after preparation

D_2 = DO of diluted sample after incubation

B_1 = DO of dilution of seed control before incubation

B_2 = DO of dilution of seed control after incubation

f = Ratio of seed in sample to seed in control

P = Decimal fraction of sample used

If nitrification occurs in the substrate, the value of D_2 will be low by an amount equal to the oxygen consumed in nitrification. Therefore, it is necessary to determine by estoichiometry the amount of oxygen used in nitrification. This amount is then added to the value of D_2 before the computation of the BOD value to obtain the "corrected" BOD. In fact,

if nitrification occurs in the seeded dilution water, the same adjustment must be made in the value of B_2.

The following procedure is used to determine the amount of oxygen consumed in nitrification:

1. Determine the change in the NO_2-N and NO_3-N concentration, from initial and final values during the BOD run.
2. Consider the following stoichiometric relationships in the conversion of NH_3-N to NO_2-N and to NO_3-N:

   (a) $NH_4^+ + 3/2\ O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$

   by which 18g NH_4^+ is equivalent to 14g N, which in turn is equivalent to 48g O. Therefore, 1 mg/l NH_3-N is equivalent to 3.43 mg/l O in its conversion to NO_2^-.

   (b) $NO_2^- + \frac{1}{2}\ O_2 \longrightarrow NO_3^-$

   by which 46g NO_2^- is equivalent to 14g N, which in turn is equivalent to 16g O. THerefore, 1 mg/l NO_2-N is equivalent to 1.14 mg/l O in its conversion to NO_3^-.

   (c) By simple addition, 1 mg/l NH_3-N is equivalent to 4.57 mg/l O, if it is fully oxidixed to NO_3-N.
3. Compute the dissolved oxygen used in nitrification as follows:

   NOD in mg/l = (Δ NO_2-N) x 3.43 + (Δ NO_3-N) x 4.57

   were NOD = nitrification oxygen demand

   ΔNO_2-N = change in NO_2-N concentration during a BOD run = (NO_2-N) final - (NO_2-N) initial

   ΔNO_3-N = change in NO_3-N concentration during a BOD run = (NO_3-N) final - (NO_3-N) initial.
4. Add algebraically the value of NOD to the value of D_2 before computing the "corrected" BOD.

If nitrification is observed in the dilution water, use the same procedure to adjust the value of B_2, when using seeded dilution water.

In this project, the veracity of the NO_2-N and NO_3-N values was confirmed by means of mass balances of nitrogen species which considered the change in TKN during the BOD run. The following example, taken from one of the actual tests, will serve to illustrate the procedure:

Nitrogen form	Time, days	
	0	5
TKN, mg/l	3.11	2.91
NO_2-N, mg/l	0.23	0.26
NO_3-N, mg/l	0.60	0.69
Total N	3.94	3.86

Both columns should add to the same total value. The minor discrepancy of 0.06 mg/l (with respect to the average, about 1.5%) is accepted as an analytical error. A distribution of the error is done as follows:

$$\text{Average total N} = \frac{3.94 + 3.86}{2} = 3.90$$

$$\text{Correction factor for "0" day} = \frac{3.90}{3.94} = 0.99$$

$$\text{Correction factor for "5" day} = \frac{3.90}{3.86} = 1.01$$

Multiplying the values in the table by the appropriate correction factor, we obtain:

	Time, days	
Nitrogen form	0	5
TKN, mg/l	3.08	2.94
NO_2-N, mg/l	0.23	0.26
NO_3-N, mg/l	0.59	0.70
Total N	3.90	3.90

From the above adjusted value, we compute the change in NO_2-N and NO_3-N, as follows:

$$\Delta NO_2\text{-}N = 0.26 - 0.23 = 0.03 \text{ mg/l}$$

$$\Delta NO_3\text{-}N = 0.70 - 0.59 = 0.11 \text{ mg/l}$$

from which NOD = 0.03 x 3.43 + 0.11 x 4.57 = 0.61 mg/l. As previously explained, the value of NOD = 0.61 is added to the parameter D_2 for the computation of the "corrected" BOD. If any of the above values would have come out with a negative sign, it would have been added algebraically, that is, taking into account the negative sign.

For the same test run the following results were obtained for the parameters is the BOD equation:

D_1 = 8.55 mg/l $\qquad$ B_1 = 9.4 mg/l

D_2 = 6.20 mg/l $\qquad$ B_2 = 8.9 mg/l

$P = \frac{100}{300} = 0.333$ $\qquad$ $f = \frac{200}{300} = 0.667$

Adjusted D_2 = 6.20 + 0.61 = 6.81 mg/l

$$\text{"Corrected" BOD in mg/l} = \frac{(8.55-6.81) - (9.4-8.9) \times 0.667}{0.333}$$

$$= 4.22 \text{ mg/l}$$

No nitrification was observed in the incubation of the dilution water and, therefore, a correction in the value of B_2 was unnecessary.

The standard BOD test (with no correction for nitrification) would have produced the following result:

$$\text{BOD in mg/l} = \frac{(8.55 - 6.20) - (9.4 - 8.9) \times 0.667}{0.333}$$

$$= 6.05 \text{ mg/l}$$

This represents, for this particular case, an increment of 43.4% over the corrected value.

The computation of the "inhibited" BOD follows the same procedure as the standard BOD test, except that the value of D_2 supposedly reflects the absence of nitrification due to the inhibition of the nitrifying bacteria. The D_2 value must come out higher than in the standard test. In the particular sample used as illustration in the previous examples $D_2 = 6.75$, which when introduced in the BOD equation gives:

$$\text{"Inhibited" BOD in mg/l} = \frac{(8.55 - 6.75) - (9.4 - 8.9) \times 0.667}{0.333}$$

$$= 4.40 \text{ mg/l}$$

which is lower than the standard test value, as expected. In this particular example, the "inhibited" BOD value come out slightly higher than the "corrected" value of 4.22 mg/l previously computed.

Finally, a correlation was attempted between the results of the "corrected" and "inhibited" BOD tests, by plotting each value in one test against the corresponding value in the other test and performing statistical analysis on the data.

Results

During the duration of the experimental phase of this project a total to ten ten-day BOD runs were conducted, in which dissolved oxygen, nitrites, nitrates, and alkalinity were analyzed for on a day by day basis. In some of the samples nitrification did not occur, as evidenced by constant (or nearly constant) values of nitrites and nitrates during the run. The results from these particular runs were discarded because they were not significant for the purpose of this study.

In many of the tests, the mass balances for nitrogen and consumed oxygen could not be carried to the end of the run because it was observed that denitrification was occurring in the last few days of the run. For this reason, the computations to correct BOD for nitrification were performed up to the point beyond which denitrification was becoming evident, as demonstrated by a reduction in the nitrate concentration.

The accompanying figures (1 through 5) represent in graphical form the results of the four runs considered most representative for the work performed during the course of this investigation, as well as the correlation of the values of "corrected" BOD versus "inhibited" BOD. Figures bearing the same identifying number followed by a letter (such as 1a, 1b, 1c, 1d) constitute a set related to one particular run. Letter "a" of each set is a plot of BOD versus time in days for the significant period within the ten-day run. For two of the runs (figures 1a and 2a) a standard or normal BOD curve, as measured, is presented as well as a "corrected" BOD curve in which a correction was applied to deduct the effect of nitrification. For the other two runs (figures 3a and 4a) a curve representing the "inhibited" BOD values is also included.

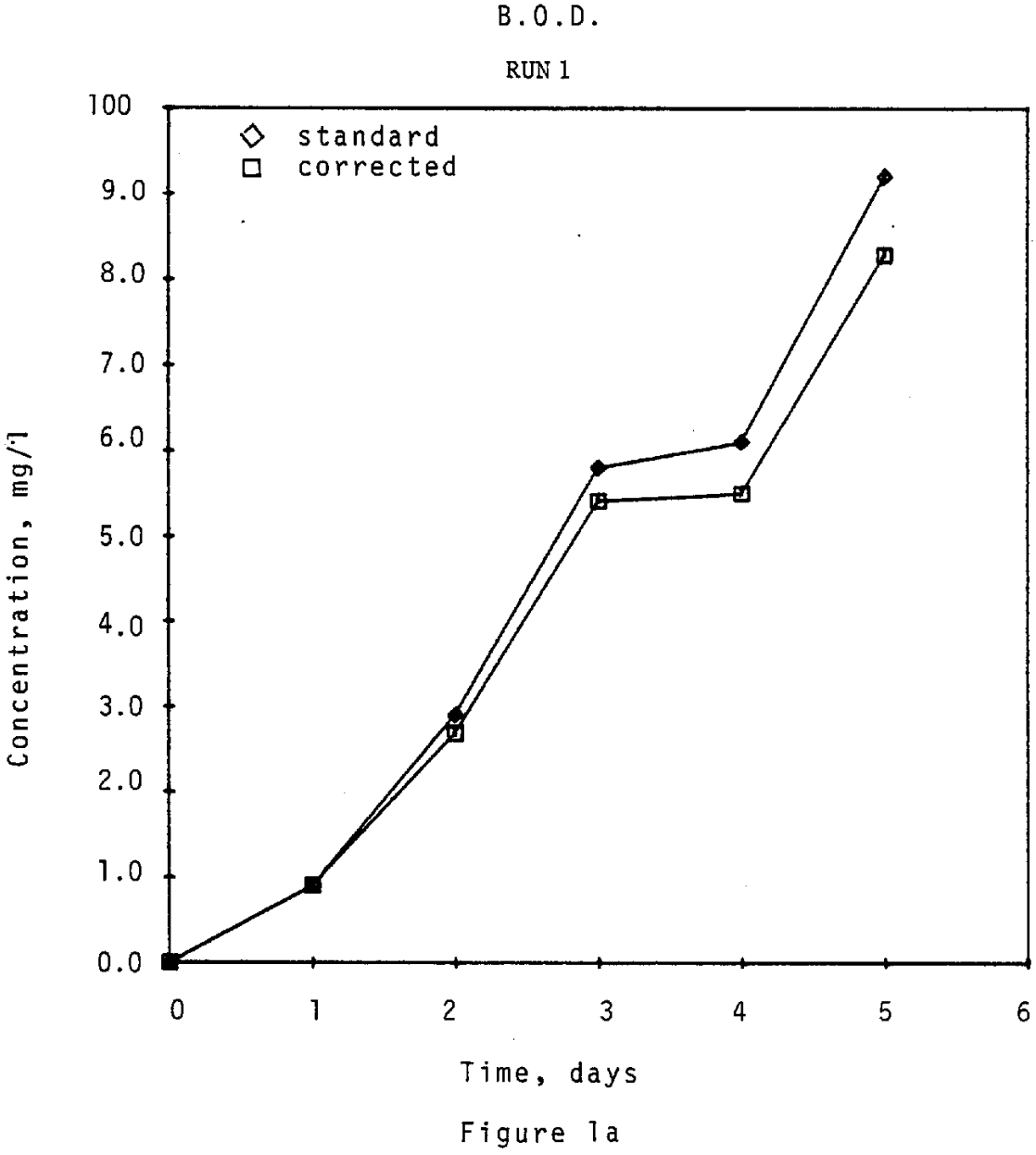
B.O.D.
RUN 1
standard
corrected
100
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Concentration, mg/l
0
1
2
3
4
5
6
Time, days

Figure 1a

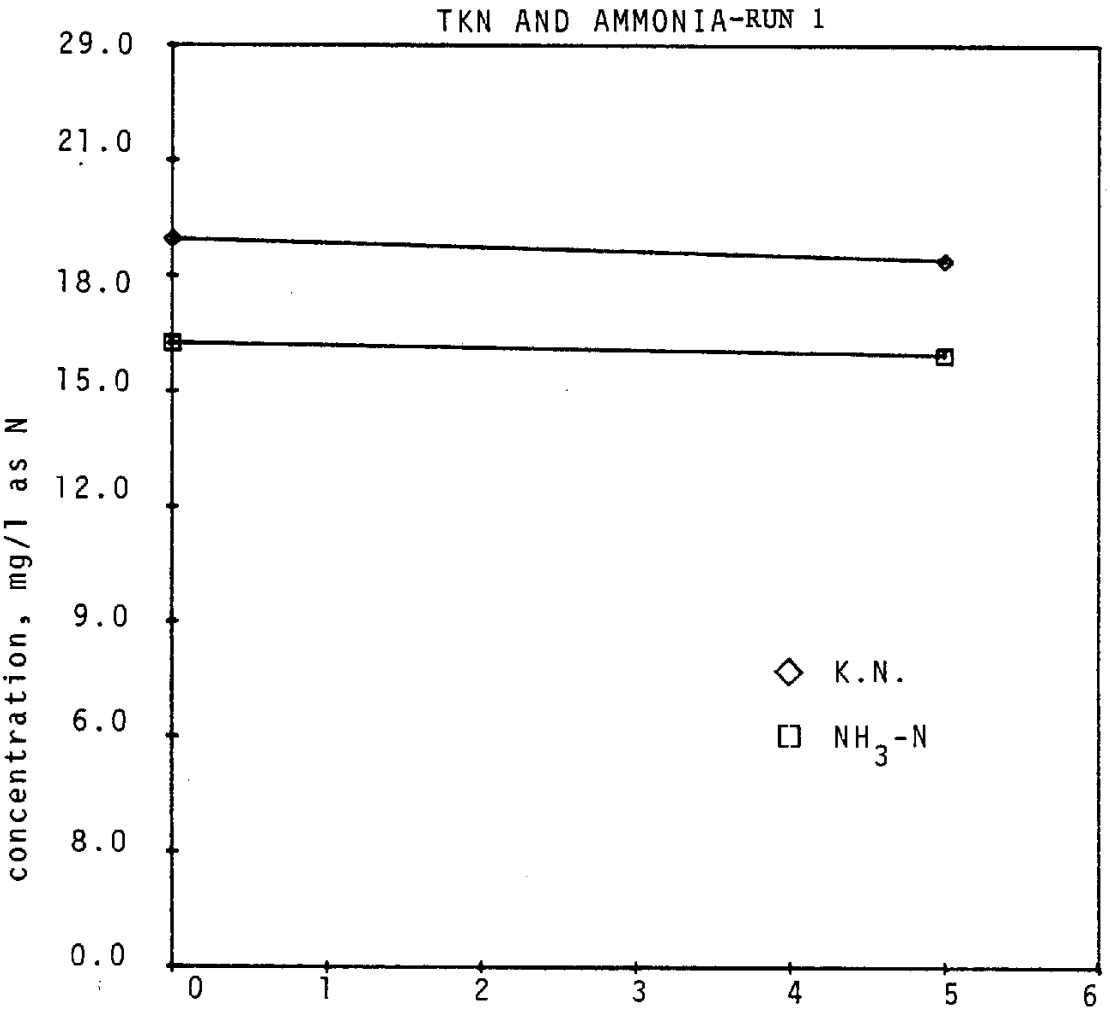
TKN AND AMMONIA-RUN 1
29.0
21.0
18.0
15.0
12.0
9.0
6.0
8.0
0.0
concentration, mg/l as N
0
1
2
3
4
5
6
K.N.
NH3-N
Time, days

Figure 1b

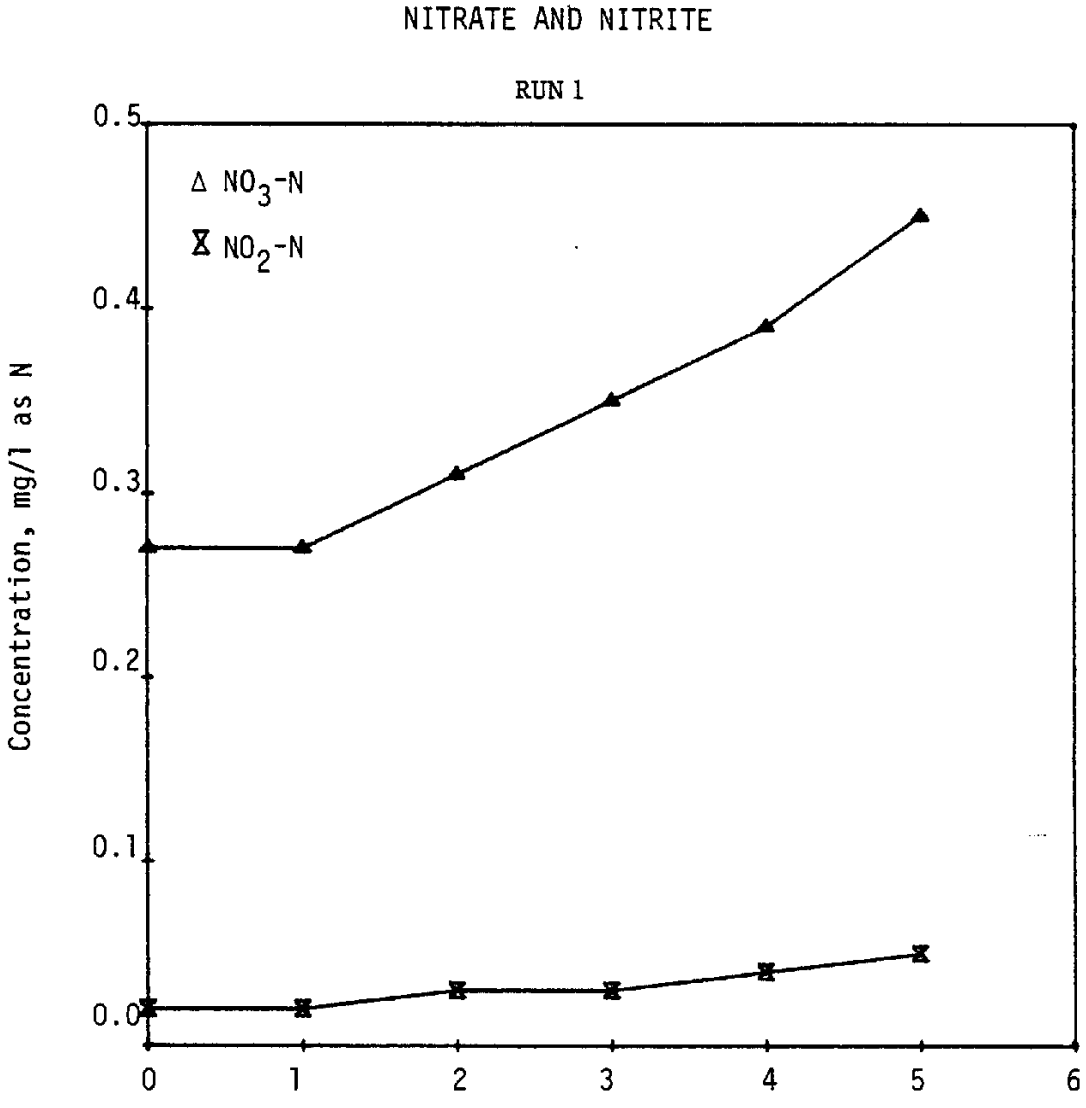
NITRATE AND NITRITE
RUN 1
Δ NO_3-N
⧖ NO_2-N
Concentration, mg/l as N
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
Time, days

Figure 1c

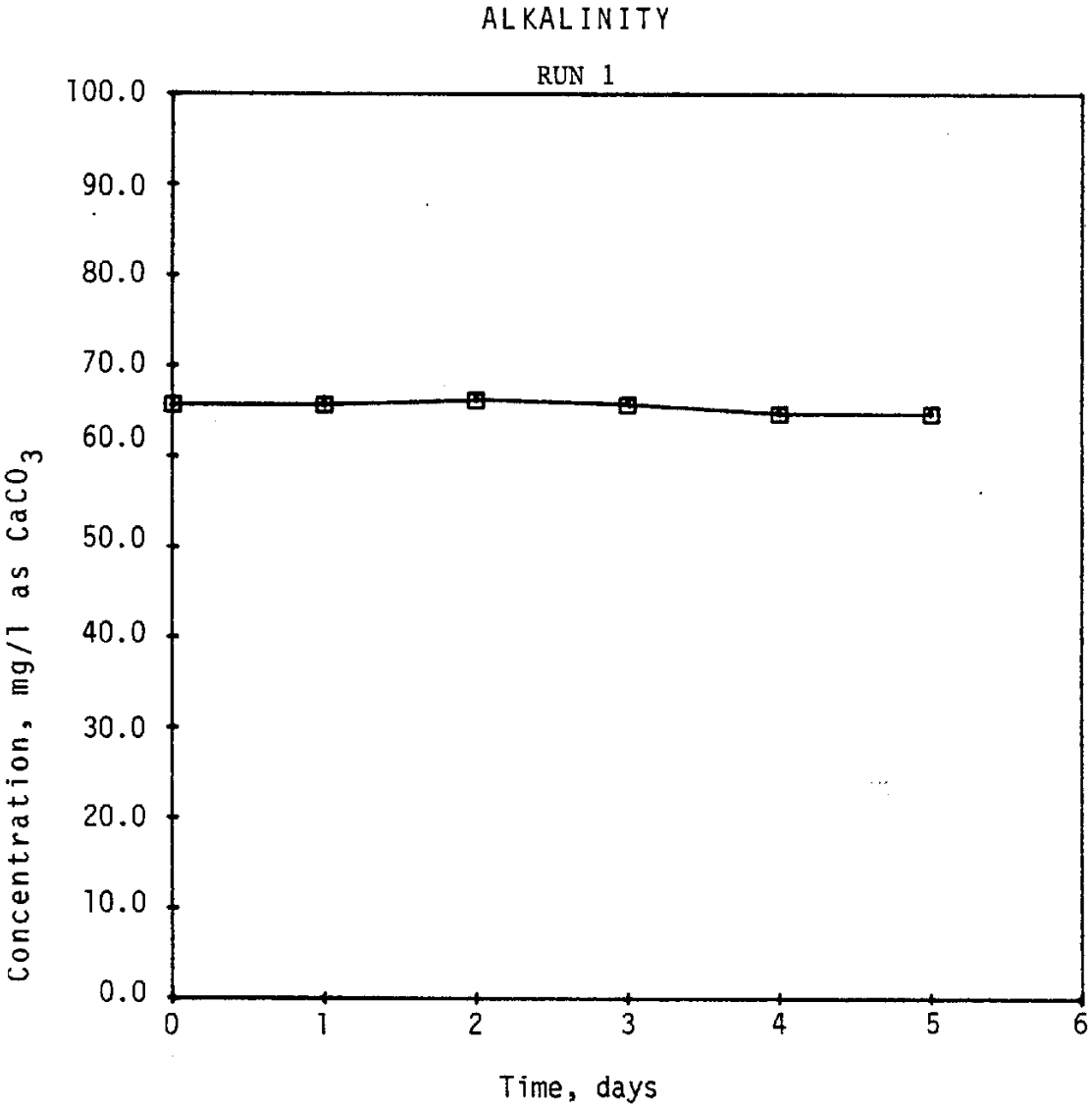
ALKALINITY
RUN 1
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Concentration, mg/l as $CaCO_3$
0
1
2
3
4
5
6
Time, days

Figure 1d

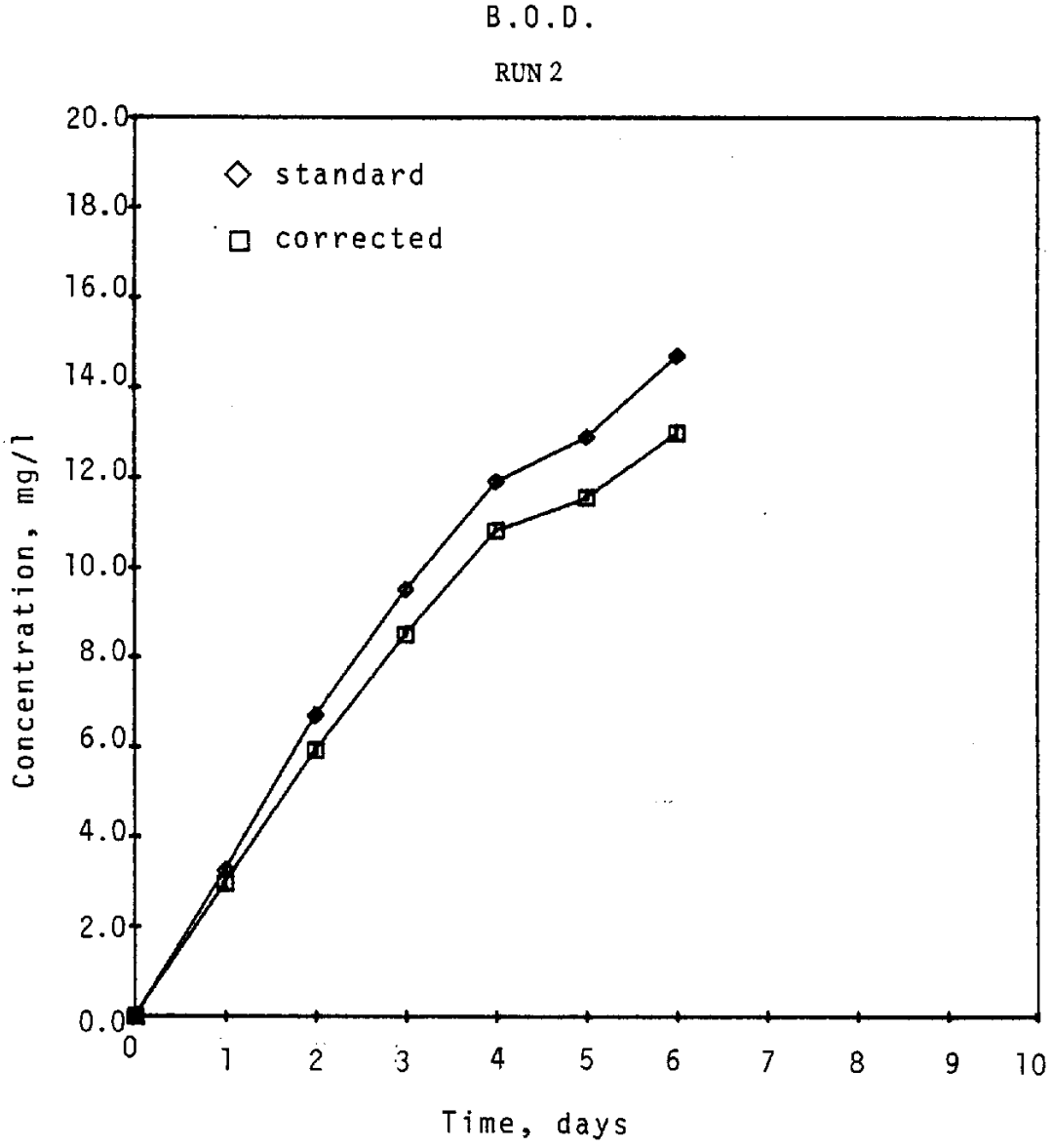
B.O.D.
RUN 2
standard
corrected
Concentration, mg/l
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 2a

T.K.N. AND AMMONIA

RUN 2

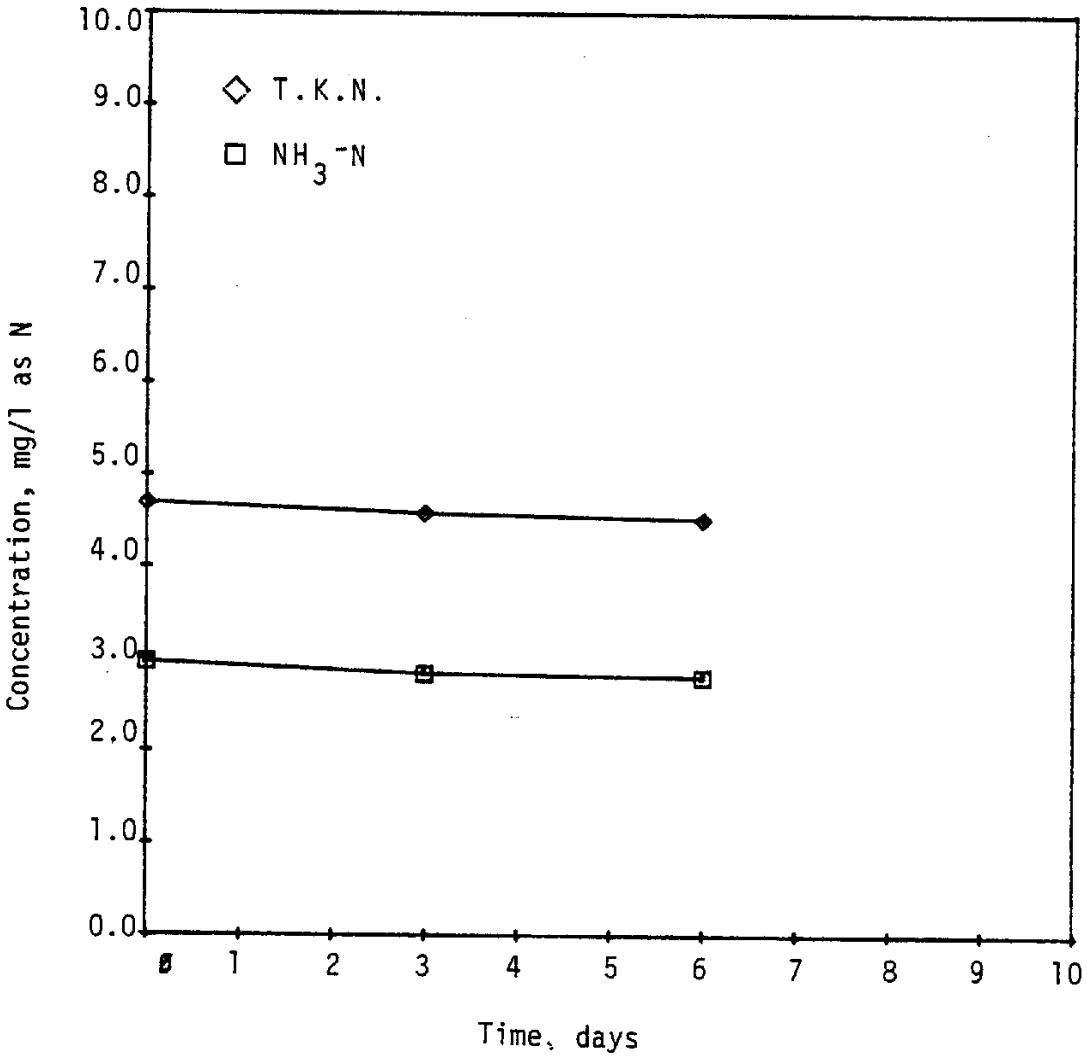

Figure 2b

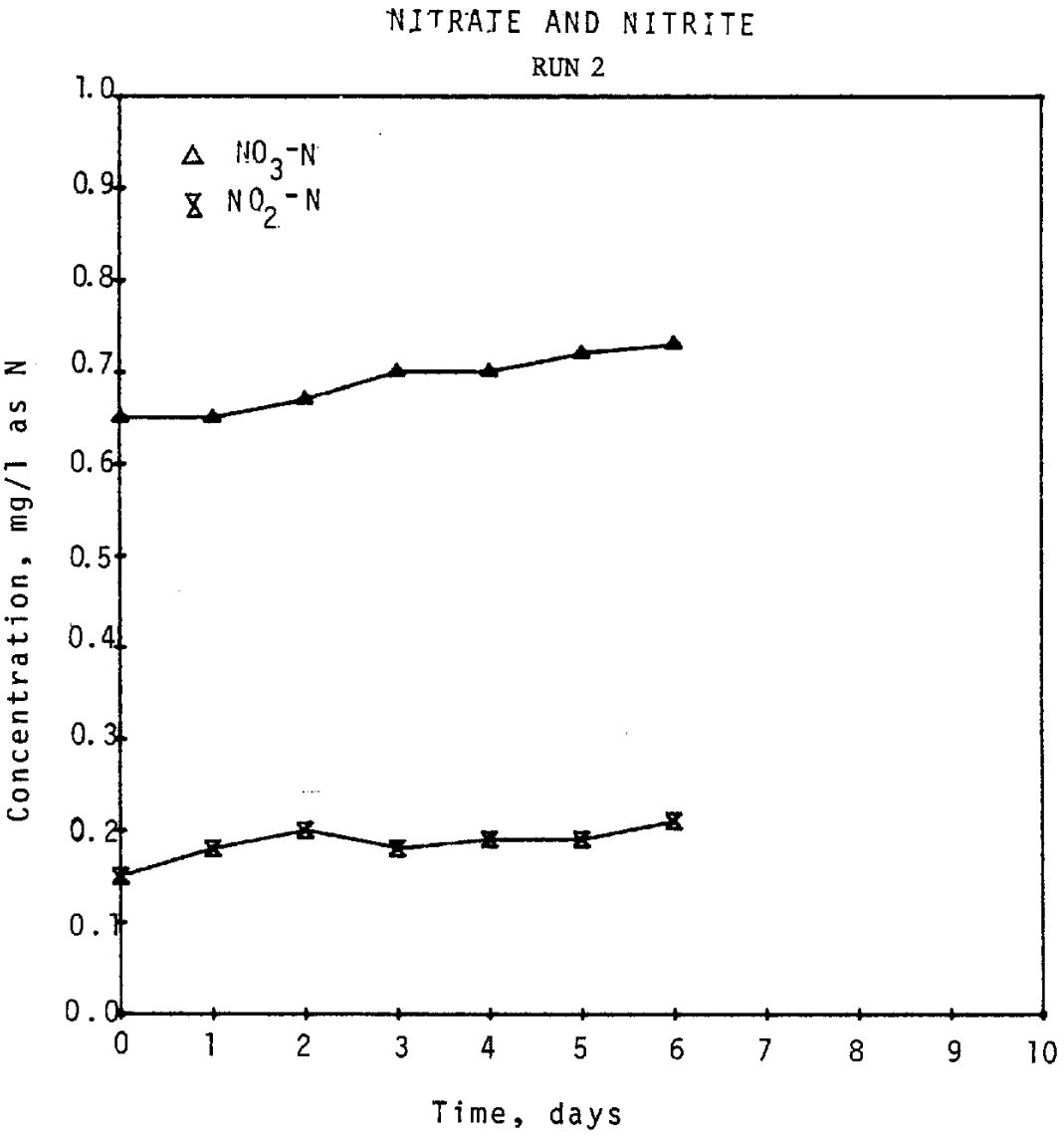
NITRATE AND NITRITE
RUN 2
△ NO_3-N
⧖ NO_2-N
Concentration, mg/l as N
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 2c

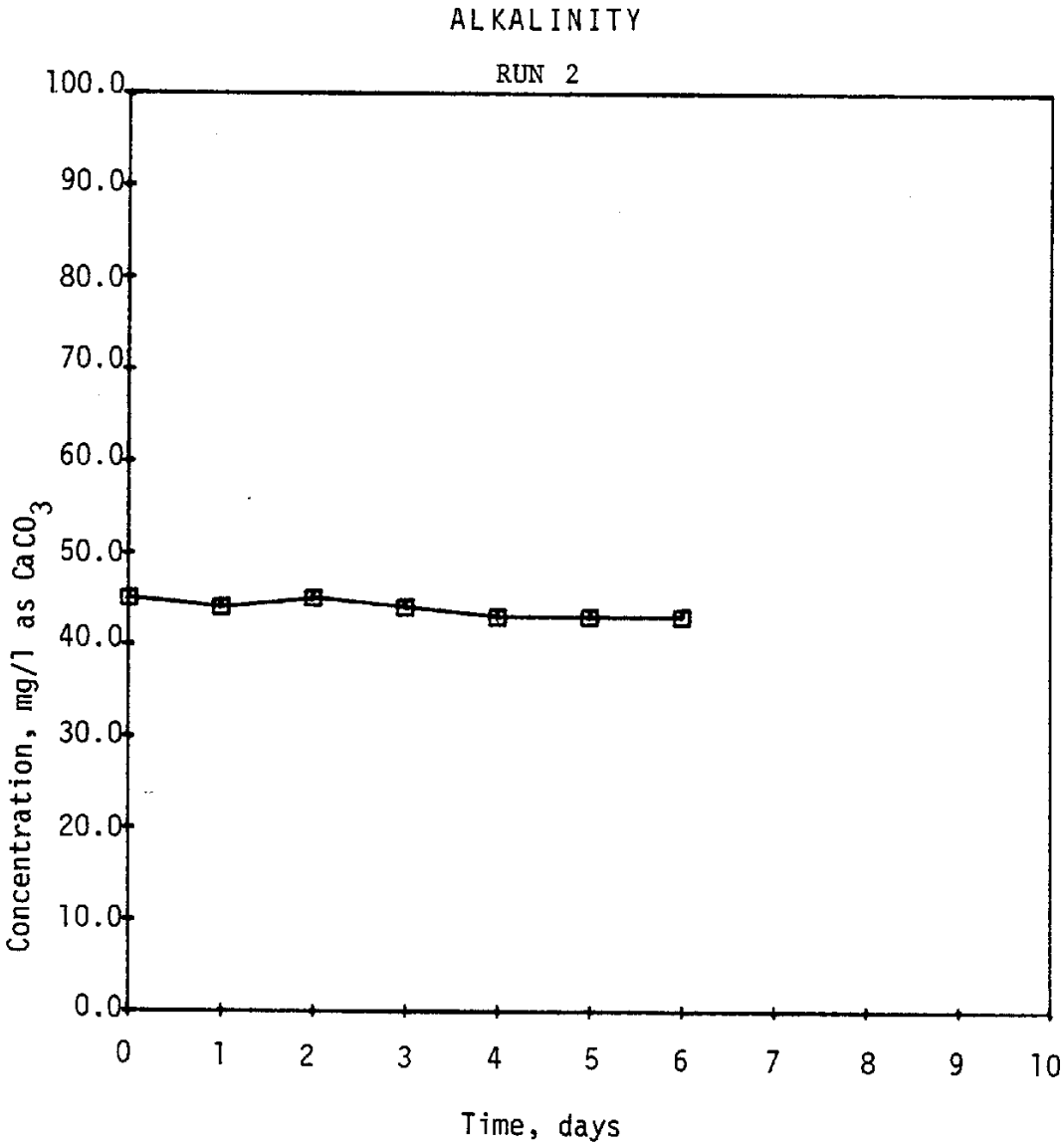
ALKALINITY
RUN 2
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Concentration, mg/l as $CaCO_3$
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 2d

B.O.D.

RUN 3

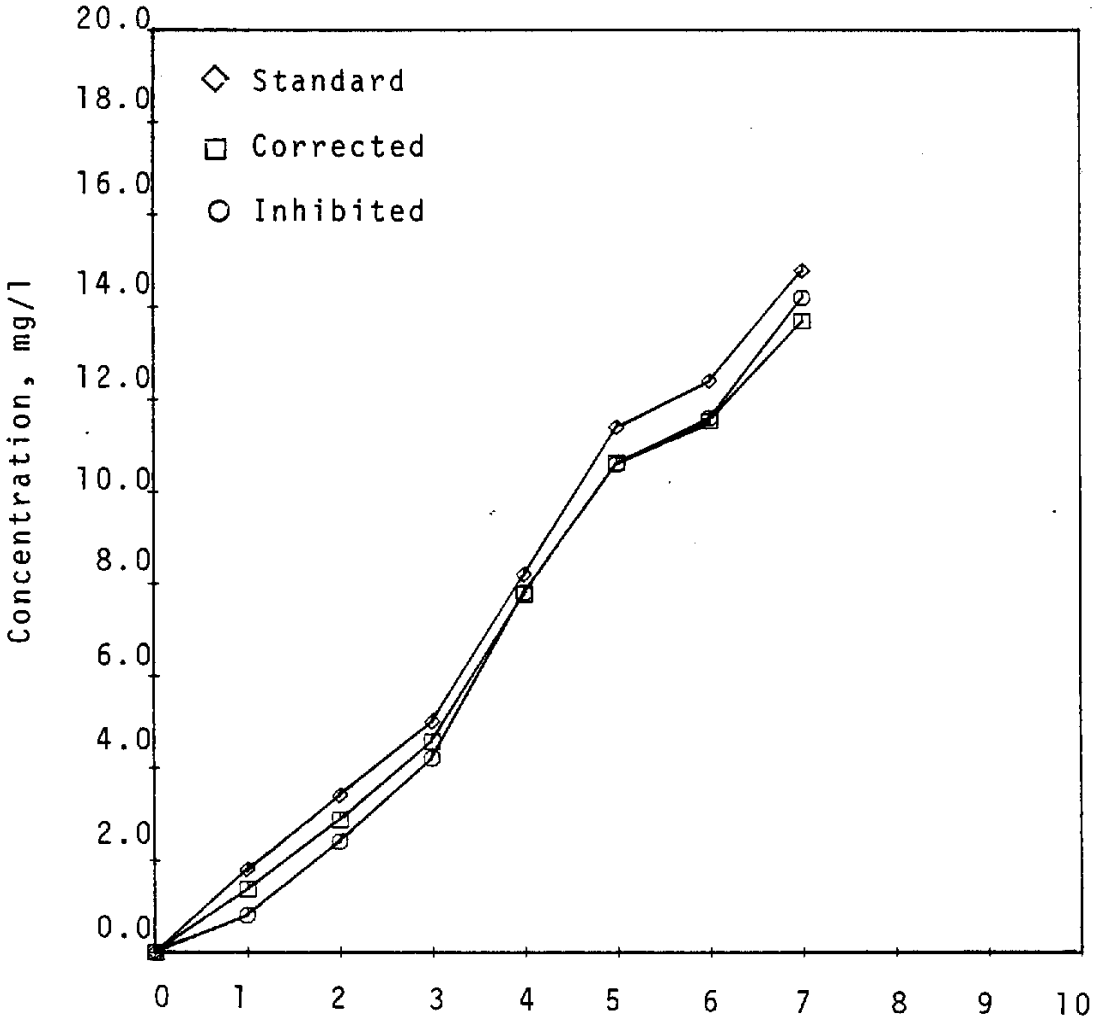

Figure 3a

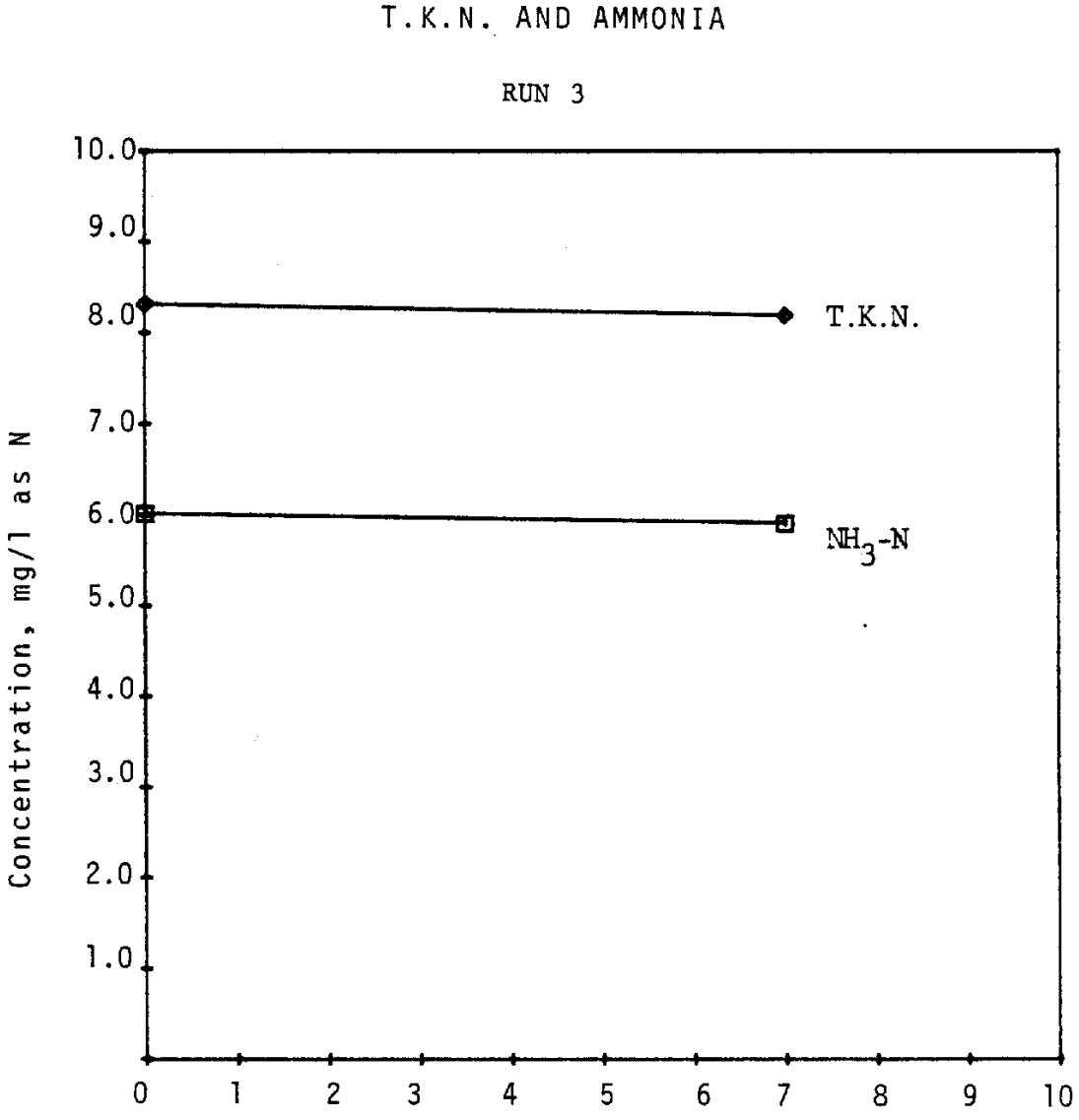
T.K.N. AND AMMONIA
RUN 3
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Concentration, mg/l as N
T.K.N.
NH3-N
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 3b

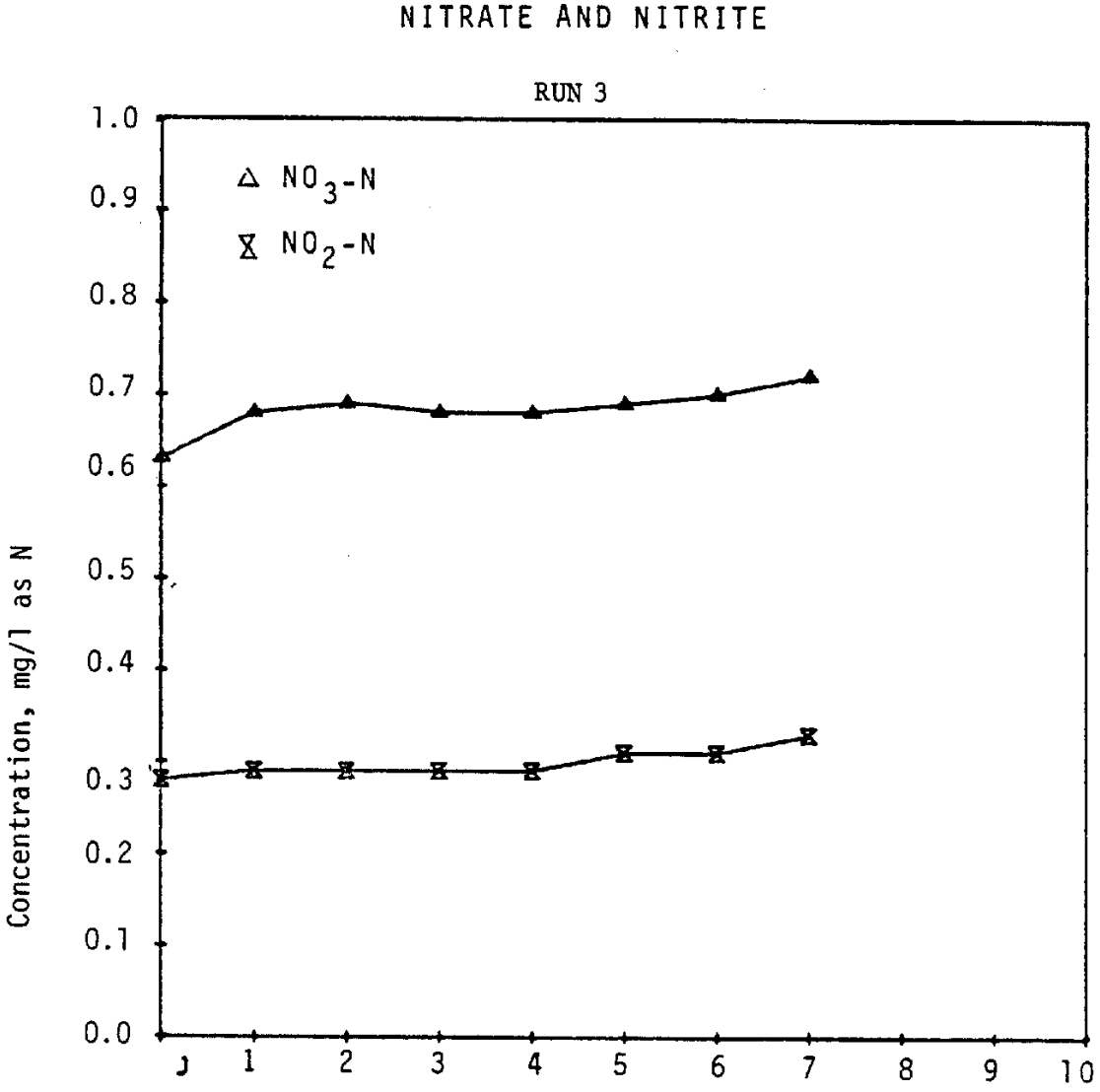
NITRATE AND NITRITE
RUN 3
△ NO_3-N
⧖ NO_2-N
Concentration, mg/l as N
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 3c

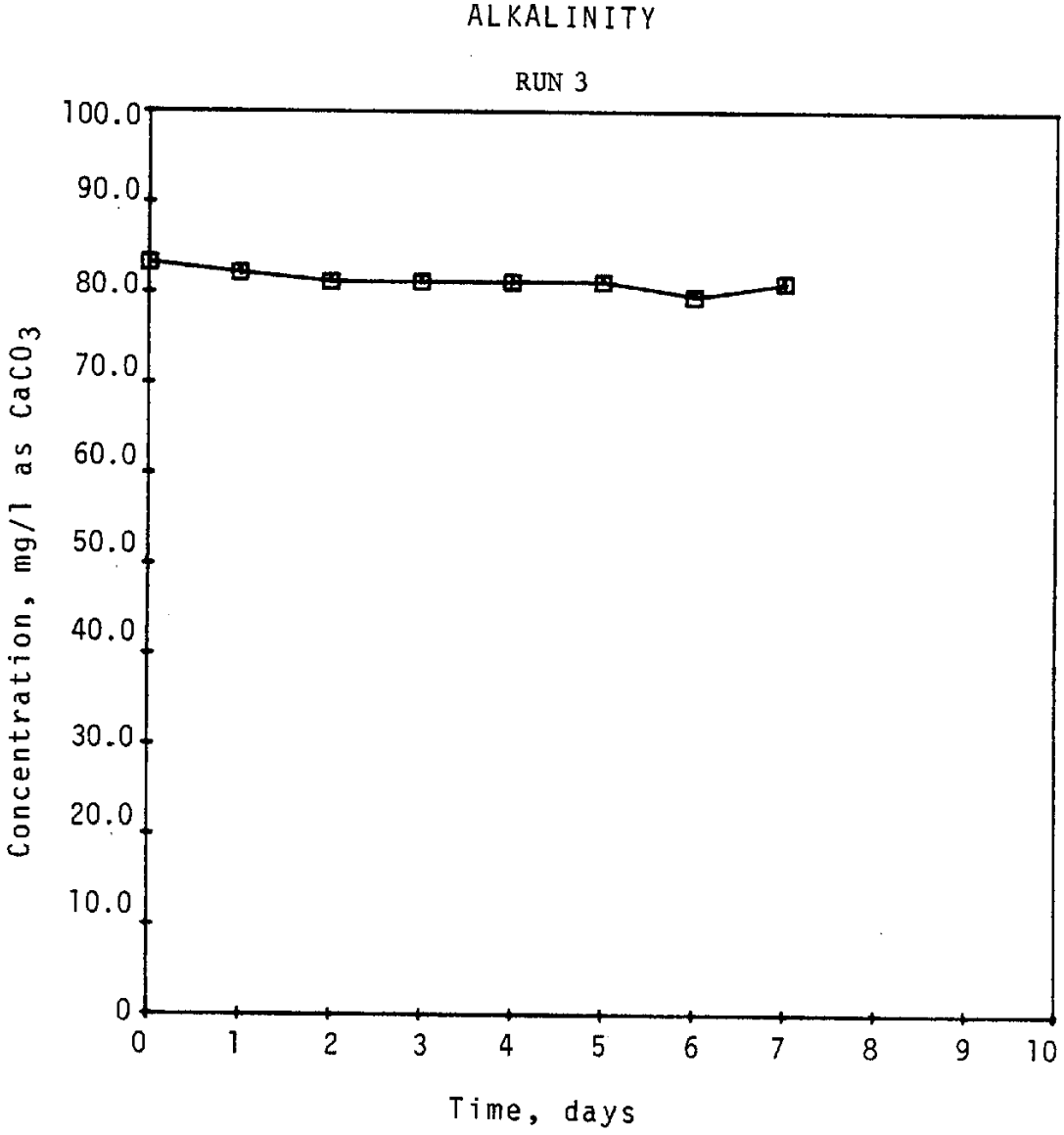
ALKALINITY
RUN 3
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
Concentration, mg/l as $CaCO_3$
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 3d

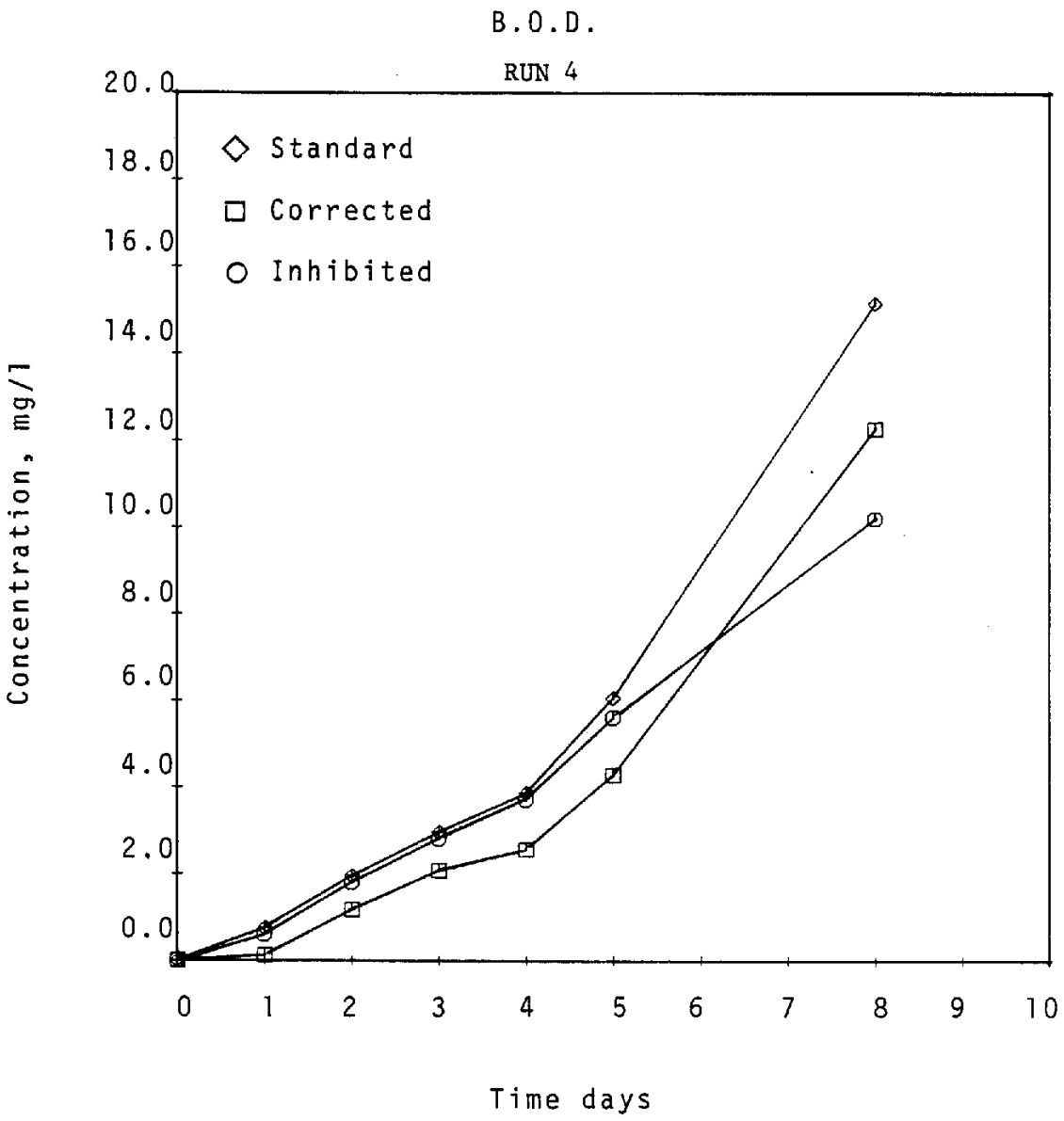
B.O.D.
RUN 4
Standard
Corrected
Inhibited
Concentration, mg/l
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
1
2
3
4
5
6
7
8
9
10
Time days

Figure 4a

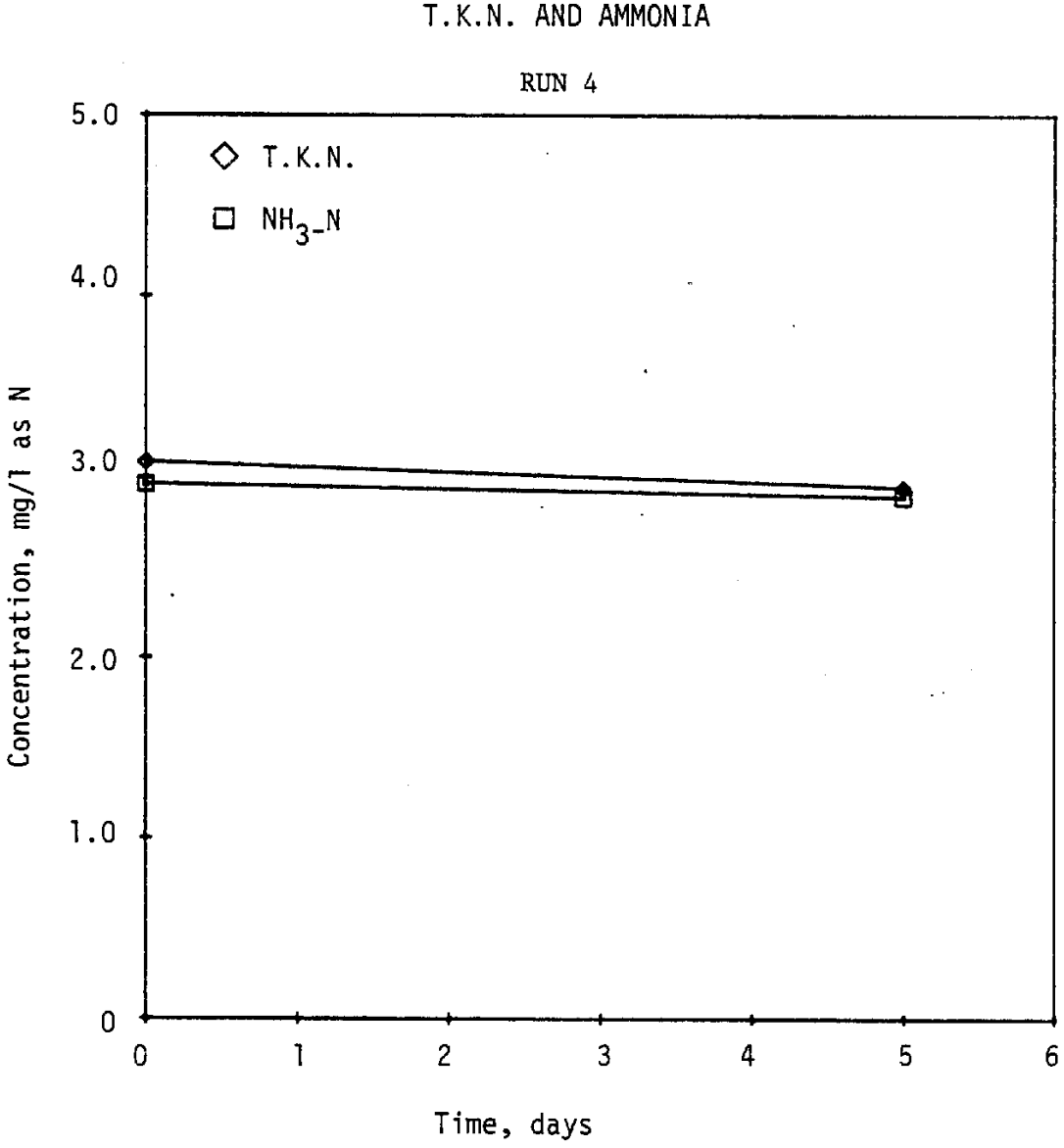
T.K.N. AND AMMONIA
RUN 4
T.K.N.
NH3-N
Concentration, mg/l as N
5.0
4.0
3.0
2.0
1.0
0
0
1
2
3
4
5
6
Time, days

Figure 4b

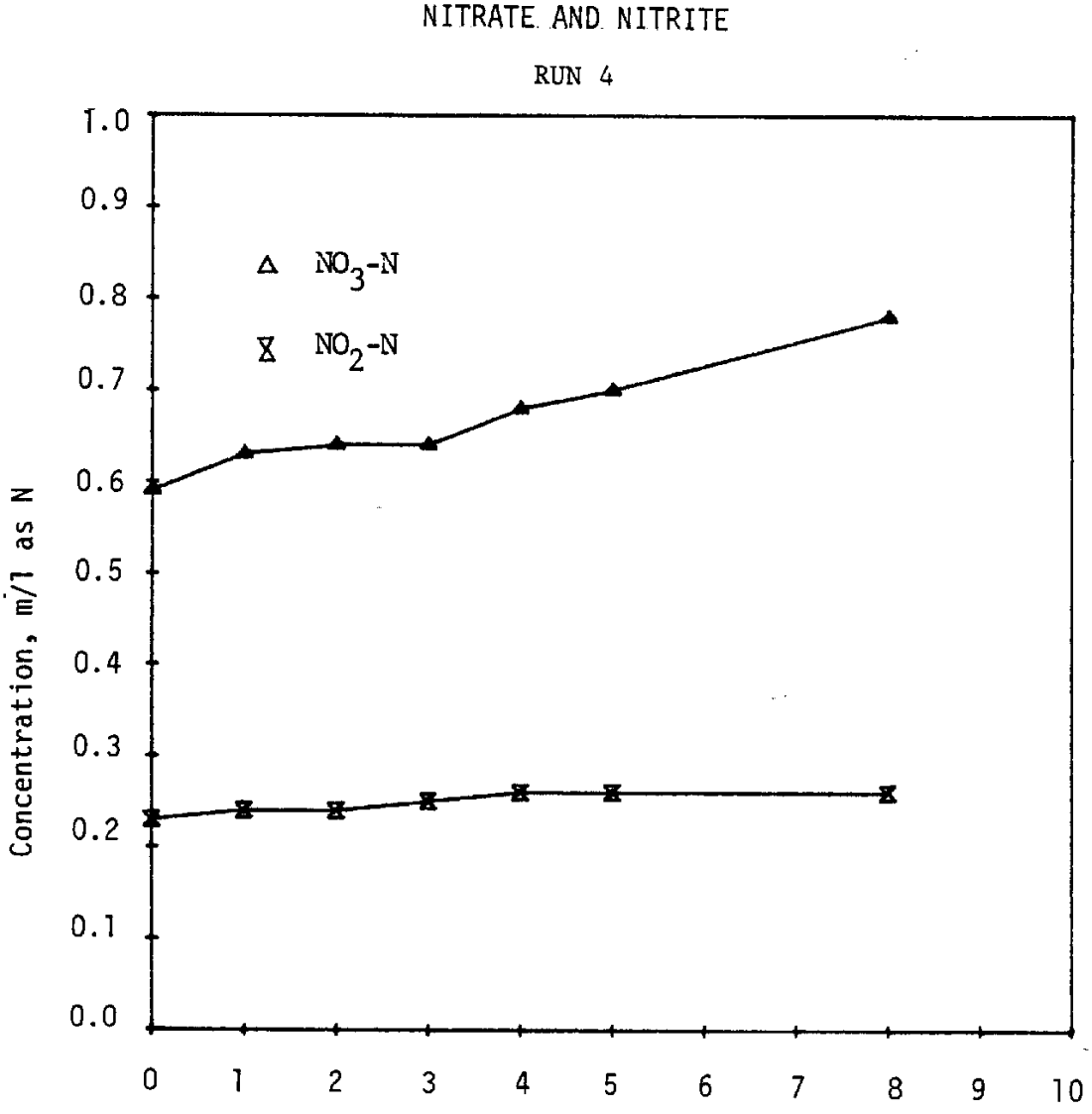
NITRATE AND NITRITE
RUN 4
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Concentration, m/l as N
NO_3-N
NO_2-N
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 4c

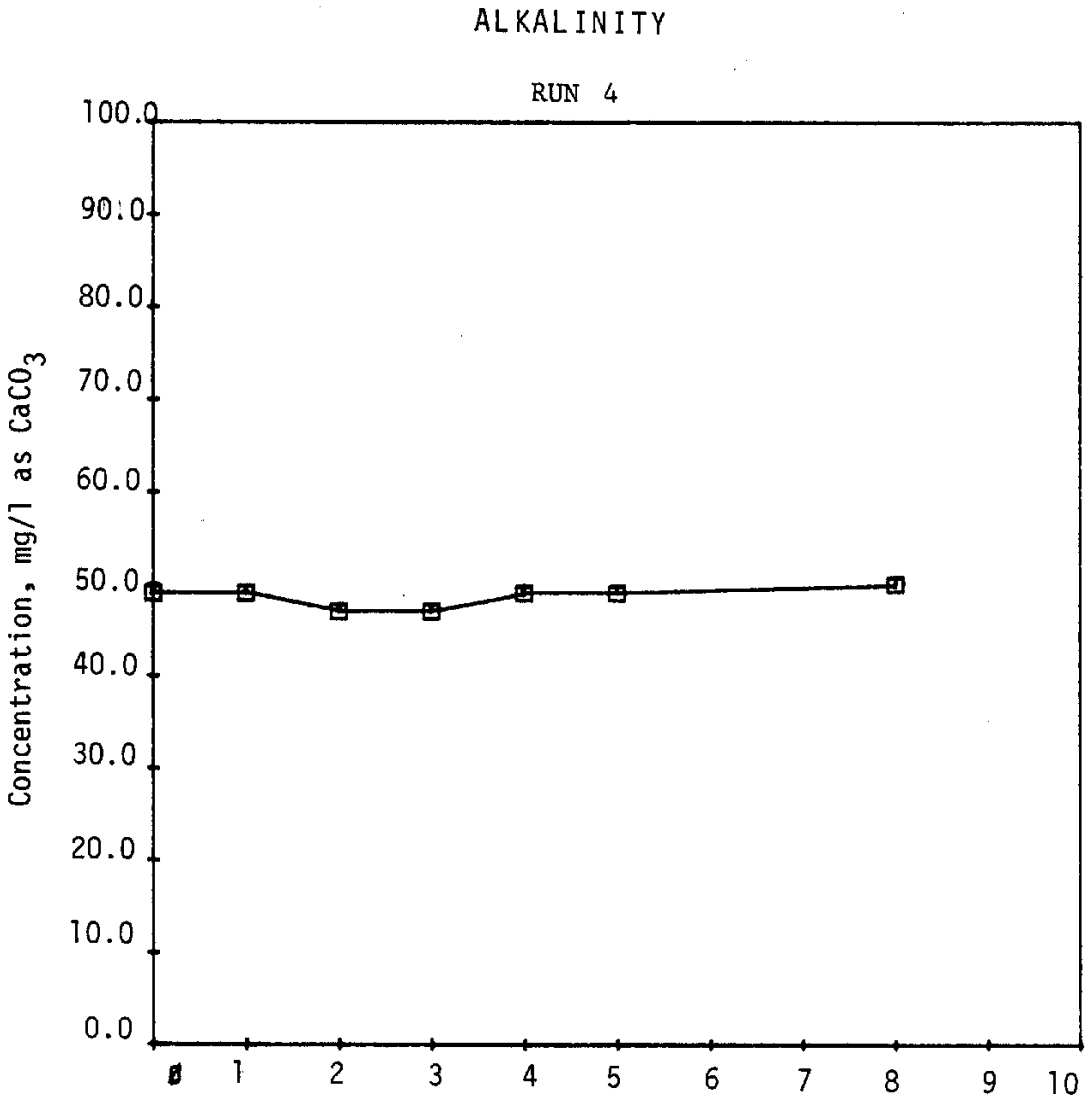
ALKALINITY
RUN 4
Concentration, mg/l as $CaCO_3$
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
1
2
3
4
5
6
7
8
9
10
Time, days

Figure 4d

Correlation Between "Inhibited" BOD and "Corrected" BOD

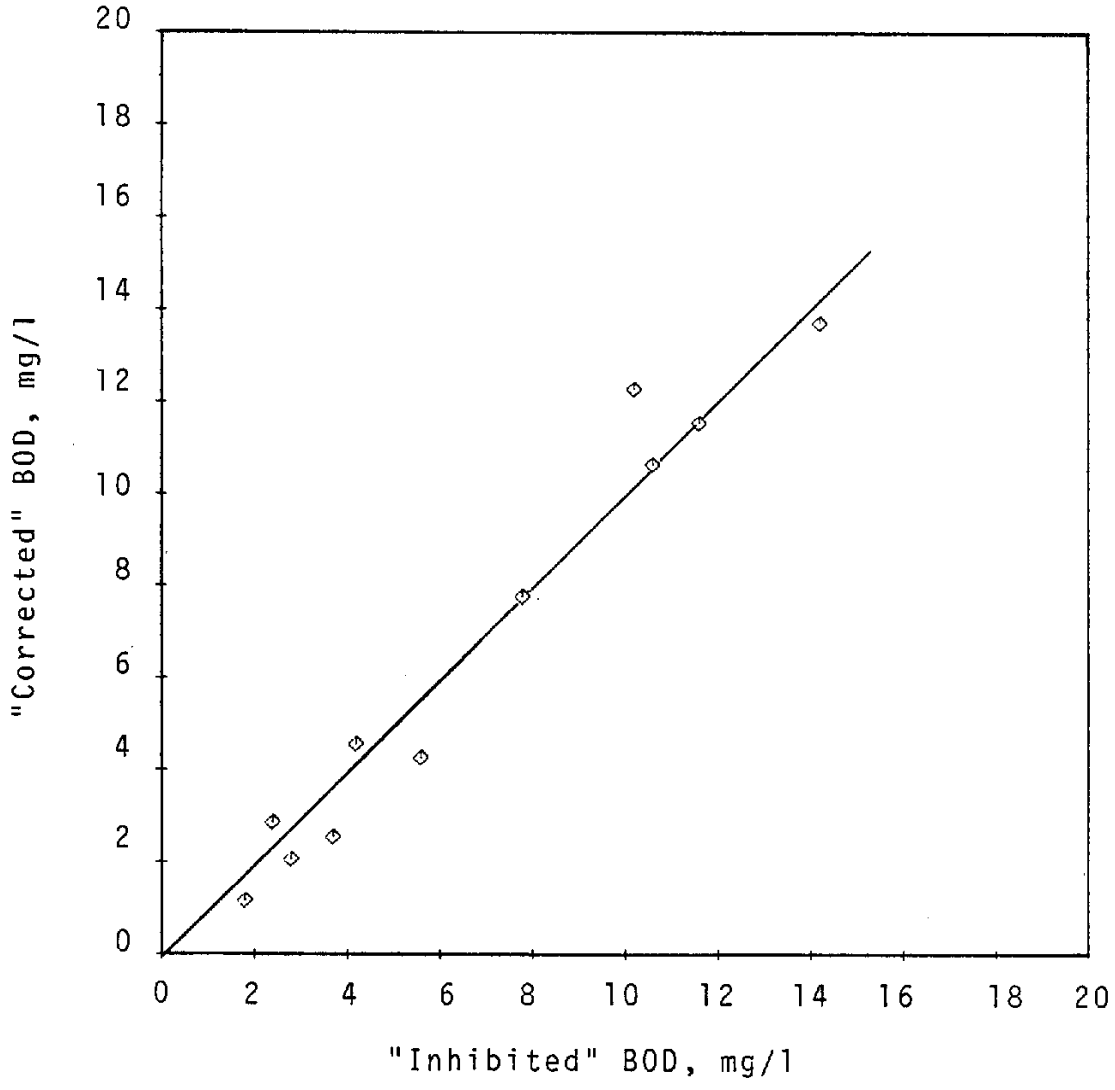

Figure 5

For the sake of completeness we have included, for each run represented here, curves for TKN and NH_3-N (figures lettered "b"), NO_2-N and NO_3-N (figures lettered "c"), and alkalinity (figures lettered "d"). In all cases TKN NH_3-N values remained relatively high during the entire run as compased with NO_2-N and NO_3-N concentrations, proving that nitrification was minor in these tests. This was a rather disappointing situation since a higher degree of nitrification would have been more favorable for the purposes of this study.

Nevertheless, when values of "corrected" BOD were plotted against the corresponding "inhibited" BOD results (figure 5) and the necessary statistical analysis was performed, it was found that a very high correlation exists between both sets of data. The following table and subsequent computations summarize the statistical analysis of the data.

TABLE 1

Statistical Analysis of BOD Data

"Inhibited" BOD	"Corrected" BOD			
X	Y	X^2	XY	Y^2
1.80	1.17	3.24	2.11	1.37
2.80	2.08	7.84	5.82	4.33
3.70	2.55	13.69	9.44	6.50
2.40	2.88	5.76	6.91	8.29
4.20	4.58	17.64	19.24	20.98
5.60	4.22	31.36	23.63	17.81
7.80	7.78	60.84	60.68	60.53
10.60	10.64	112.36	112.78	113.21
11.60	11.54	134.56	133.86	133.17
10.20	12.27	104.04	125.15	150.55
14.20	13.70	201.64	194.54	187.69
Sum: 74.90	73.41	692.97	694.16	704.43

The best straight line that fits the data is obtained as follows:

Finding the slope "a" of the line:

$$a = \frac{N(\Sigma XY)-(\Sigma X)(\Sigma Y)}{N(\Sigma X^2)-(\Sigma X)^2} = \frac{(11)(694.16)-(74.90)(73.41)}{(11)(692.97)-(74.90)^2}$$

(N = 11 points in the table) $a = 1.062$

Finding the Y - intercept "b":

$$b = \frac{(\Sigma X^2)(\Sigma Y)-(\Sigma X)(\Sigma XY)}{N(\Sigma X^2)-(\Sigma X)^2} = \frac{(692.97)(73.41)-(74.90)(694.16)}{(11)(692.97)-(74.90)^2}$$

$$b = 0.557$$

Therefore the equation of the straight line of best fit is:

$$Y = 1.062X - 0.557 \qquad (1)$$

The coefficient of correlation of the plotted points with respect to this line was found to be 0.98 and the variance 0.80.

But, it is hypothesized that the average value of the "inhibited" BOD test for a very large number of samples should be about equal to the average value of the "corrected" BOD test. Therefore, theoretically the line of best fit for a very large number of test points should be a straight line through the origin of the XY coordinate system, with slope equal to 45°. Following this idea, it was decided to determine the line of best fit that passes through the origin of the XY coordinate system. This was done as follows:

$b = 0$ (Y-intercept)

$$a = \frac{\Sigma XY}{\Sigma X^2} = \frac{694.16}{692.97} = 1.0017$$

The equation of the line is, then:

$$Y = 1.0017\ X \qquad (2)$$

and the variance of the test points with respect to this line is 0.89, which is slightly higher than that obtained for equation (1). Nevertheless, equation (2) is considered to be a better fit for the points than equation (1)

because it is the equation of a straight line inclined to the X-axis by a 45.05° angle (45°3') which is very close to the theoretical line, as previously explained. In fact, the slight deviation of the line represented by equation (2) from the theoretical 45° line is well within the expected error of a BOD test.

Equation (1) predicts that for small values of BOD (less than about 9 mg/l), the value of "corrected" BOD will be slightly less than the corresponding value for the "inhibited" BOD. For larger values of BOD, the "corrected" BOD will be slightly higher than the "inhibited" BOD. Equation (2) predicts that both values will be about the same, with a very slight tendency for the "corrected" BOD value to be somewhat larger than the corresponding "inhibited" BOD value, although not significantly so.

Conclusions

From the previously presented results and discussion, the following conclusions may be derived from the performance of this study:

1. It is possible to obtain a valid figure for the carbonaceous BOD in a sample by stoichiometry, through the use of nitrate and nitrite tests run in parallel with the standard BOD test.
2. The result obtained above is comparable to that obtained through the "inhibited" BOD test.
3. A very high correlation exists between the "corrected" BOD of a sample, in which the nitrification effect is taken into account estoichiometrically, and the "inhibited" BOD, although some variation may exist in individual samples. Nevertheless, these variations are well within the expected error of a BOD test.
4. There was no measurable effect of the oxidation of organic sulfur, nor any interference with the BOD test, during the performance of this study.

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