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U.S. ENVIRONMENTAL PROTECTION AGENCY

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EPA 903/9-79-005

BIOCHEMICAL STUDIES

OF THE

POTOMAC ESTUARY--SUMMER 1978

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May 1979

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U.S. Environmental Protection Agency

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I. Introduction

During the summer of 1978 an intensive survey of the middle reach of the Potomac River was undertaken by the A.F.O. (Table 1, Figure 1). As part of this work biochemical assays were performed to:

(1) determine the carbonaceous and nitrogenous oxygen demand rate constants for river and STP effluent samples;

(2) establish the relative contributions to the BOD_5 of algal respiration and the oxygen utilized in algal decay; and

(3) characterize the elemental composition of the phytoplankton present and establish the relative digestion efficiencies of several methods of algal TKN determinations.

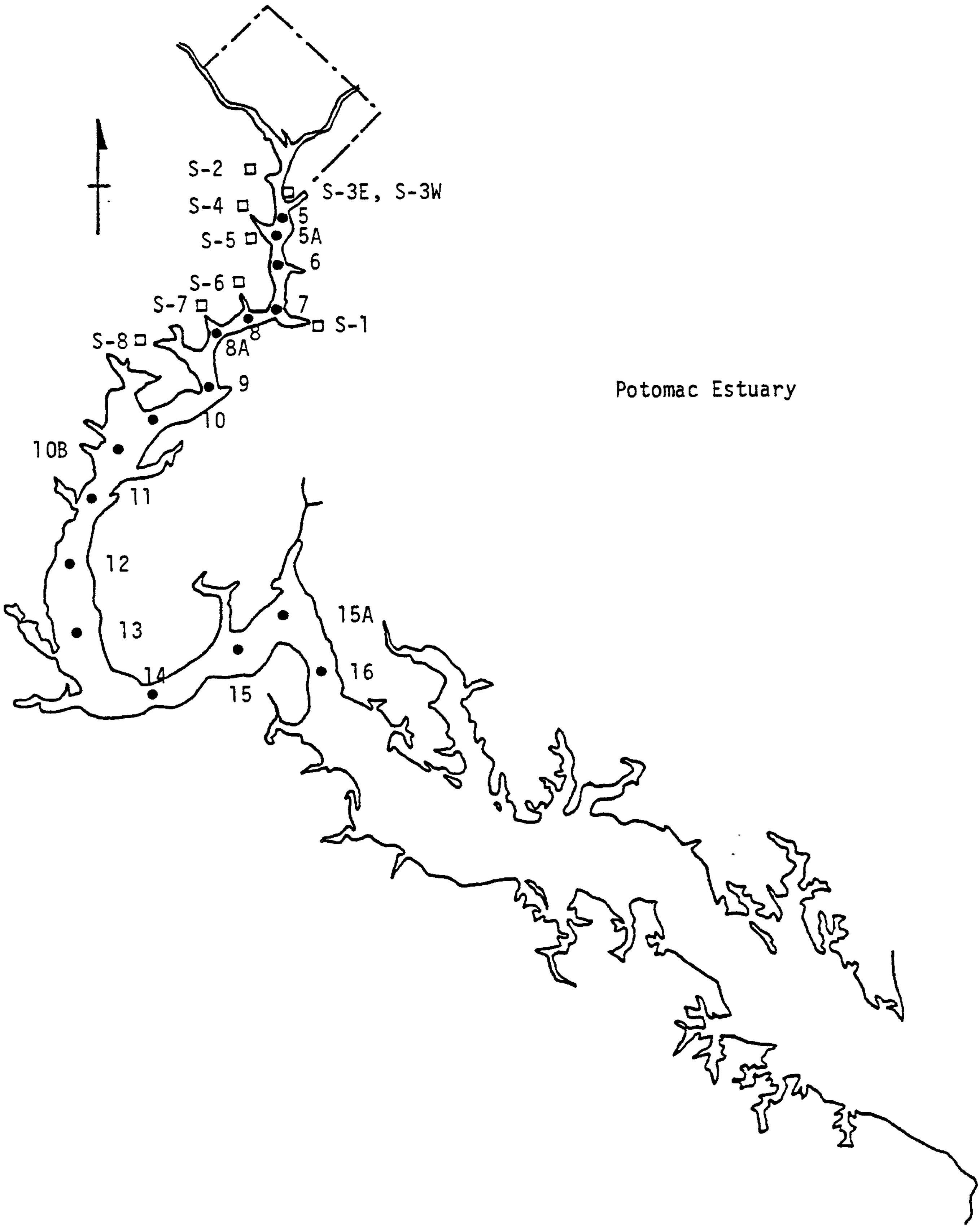
The mention of trade names or commercial products in this report is for illustration purposes and does not constitute endorsement or recommendation by the U.S. Environmental Protection Agency.

Table 1. Station Locations

Station Number	Station Name	RMI	Buoy Reference
P-8	Chain Bridge	0.0	
P-4	Windy Run	1.9	
1	Key Bridge	3.4	
1-A	Memorial Bridge	4.9	
2	14th Street Bridge	5.9	
3	Hains Point	7.6	C "1"
4	Bellevue	10.0	FLR-23' Bell
5	Woodrow Wilson Bridge	12.1	
5-A	Rosier Bluff	13.6	C "87"
6	Broad Creek	15.2	N "86"
7	Ft. Washington	18.4	FL "77"
8	Dogue Creek	22.3	FL "67"
8-A	Gunston Cove	24.3	R "64"
9	Chapman Point	26.9	FL "59"
10	Indian Head	30.6	N "54"
10-B	Deep Point	34.0	
11	Possum Point	38.0	R "44"
12	Sandy Point	42.5	N "40"
13	Smith Point	45.8	N "30"
14	Maryland Point	52.4	G "21"
15	Nanjemoy Creek	58.6	N "10"
15-A	Mathias Point	62.8	C "3"
16	Rt. 301 Bridge	67.4	

Station Number	Treatment Plant Name
S-1	Piscataway STP
S-2	Arlington STP
S-3	Blue Plains STP East & West
S-4	Alexandria STP
S-5	Westgate STP
S-6	Hunting Creek STP
S-7	Dogue Creek STP
S-8	Pohick Creek STP

Figure 1. Study Area



II. Conclusions

(1) The carbonaceous oxygen demand of the Potomac River samples followed first order kinetics with an average deoxygenation constant k_e = 0.12 day^{-1} and standard deviation = 0.03 day^{-1} (k_{10} = 0.051 day^{-1}).

(2) The growth kinetics of river nitrification were more erratic but in general were first order with an average k_e = 0.10 day^{-1} and standard deviation of 0.06.

(3) The $CBOD_5$ on the average was 58% of the BOD_5 for river samples and therefore estimates of $CBOD_5$ from BOD_5 values are prone to error unless a nitrification inhibitor is employed.

(4) The CBOD of the Potomac STP effluent samples followed first order kinetics with an average k_e = 0.16 day^{-1} and standard deviation of 0.05.

(5) The NOD for the STP effluent samples had a significant lag time resulting in poor correlation coefficients for first order fit. This lag time was probably an artifact of the APHA dilution method, since nitrification in the receiving waters was immediate.

(6) The NOD_{20} observed for river samples did not significantly differ from the product of 4.57 and the TKN concentration (4.57 x TKN).

(7) In concentrated algal samples the average algal contribution to the BOD_5 was 0.027 mg BOD_5/μg chlorophyll _a_. The predominant species present was the filamentous blue green algae _Pseudanabaena_.

(8) Phytoplankton decay represented 70% of the algal BOD_5 and algal respiration accounted for the remaining 30% of the five day oxygen depletion.

(9) The average composition of the phytoplankton present in the study area was (mg/μg):
Org C/Chlor _a_ = .021; PO_4/Chlor _a_ = .002; TKN/Chlor _a_ = .005

(10) Relative to manual digestion the Technicon continuous digestor and Technicon block digestor recovered respectively an average of 58% and 83% of the algal TKN.

III. Procedures

Biochemical Oxygen Demand: The BOD test is outlined in Standard Methods APHA, 14th edition[1]. All dissolved oxygen measurements were made with a YSI BOD probe #5720 and a YSI model 57 meter. The BOD of river water was determined on unaltered samples. STP effluent samples were altered by: the addition of 1 ml of stale settled sewage (seed); sufficient sodium sulfite (Na_2SO_3) to dechlorinate the samples; and dilution with APHA dilution water.

Nitrification: Formula 2533 nitrification inhibitor (Hach Chemical Co.) was dispensed directly into the BOD bottles. Two bottles were filled with each sample---one received the inhibitor and represented CBOD and the uninhibited bottle expressed total BOD. The NOD was determined by difference[2].

Algal BOD Measurements: The algae in 4 to 10 liters of sample were concentrated by continuous centrifugation (Sharples Continuous Centrifuge Model T-1 at 12,000 rpm and 1.5-2 liters/min). The pellet was resuspended in 500 ml of collected supernatant. The resultant suspension was diluted in a 300 ml BOD bottle as follows:

a. 50 ml suspension + 250 ml supernatant
b. 50 ml suspension (freeze dried) + 250 ml supernatant
c. 50 ml deionized water + 250 ml supernatant

a^1. 50 ml suspension + 249 ml supernatant + 1 ml seed/bottle
b^1. 50 ml suspension (freeze dried) + 249 ml supernatant + 1 ml seed/bottle
c^1. 50 ml deionized water + 249 ml supernatant + 1 ml seed/bottle

The sample composite on September 6 consisted of approximately 2 gallons each from stations: 8; 8A; 9; 10; and 10B.

The composite of September 14 consisted of about 1/2 gallon each from stations: 8; 8A; 9; and 10. Twenty ml volumes were used instead of the 50 ml volumes indicated above for this composite.

Freeze Drying: Samples were freeze-dried in a Virtis model 10-100 Unitrap freeze-drier. The suspension was spread as a thin sheet and slowly frozen to avoid foaming and to shorten drying time. Samples required 4 to 6 hours to reach the manufacturer's specified end point.

The freeze-dried samples were washed into BOD bottles with supernatant from centrifugation.

Elemental Analysis:

1. Sample Preparation: Samples were stored on ice and returned to the laboratory where 4 to 8 liters were immediately concentrated using a Sharples T-1 Continuous Centrifuge at 12,000 rpm and 1.5-2.0 liters/min. Microscopic examination revealed no

apparent morphological damage to the predominant phytoplankton species present. The pellet was resuspended in 250 ml of clear supernatant, which had been collected during centrifugation. Aliquots of the suspension and the supernatant were chemically analyzed. The supernatant values were used for blank corrections.

2. Chlorophyll a: The photosynthetic pigment from 5-20 ml of algal suspension was retained on a 0.45μ Millipore filter and extracted into 90% acetone with grinding. The extracted solution was centrifuged and measured spectrophotometrically[3].

3. Total Organic Carbon (TOC): 10 ml of algal suspension was diluted to 100 ml in a volumetric flask using deionized water. A blank was run using 10 ml of supernatant river water diluted to 100 ml in deionized water. The samples and calibration standards were then acidified by the addition of 1 ml of 6% phosphoric acid to 25 ml and purged free of inorganic carbon with oxygen. The total organic carbon was then determined on a Beckman 915 TOC analyzer[4].

4. Total Phosphate: 5 ml of sample and blank were diluted to 25 ml with deionized water. The sample and blank were placed in aluminum foil covered pyrex test tubes to which ammonium persulfate and sulfuric acid were added and autoclaved at 15 psi for 30 minutes. The digests were then analyzed for total phosphate by the Technicon automated ascorbic acid reduction method[4].

5. Algal Nitrogen: 5 ml of sample and blank were diluted to 25 ml with deionized water. The prepared solutions were then analyzed for TKN by the following methods:

   A. Helix: Samples and blanks were digested by a Technicon Continuous Digestor (Helix) and analyzed by the automated colorimetric phenolate method[4].

   B. Manual: Samples and blanks were manually digested with 10 ml aliquots placed in reflux tubes and 8.0 ml of H_2SO_4/K_2SO_4 digestion solution added. The tubes were placed over flame until boiling and reflux stopped. The contents of the tubes were washed into a graduated cylinder with deionized water and brought to 50 ml. The resultant digests were analyzed using a Technicon Continuous Digestor (Helix) and the Technicon automated colorimetric phenolate method[4].

   C. Block: Samples and blanks were analyzed by a Technicon Block Digestor BD-40 and analyzed by the salicylate/nitroprusside method[5].

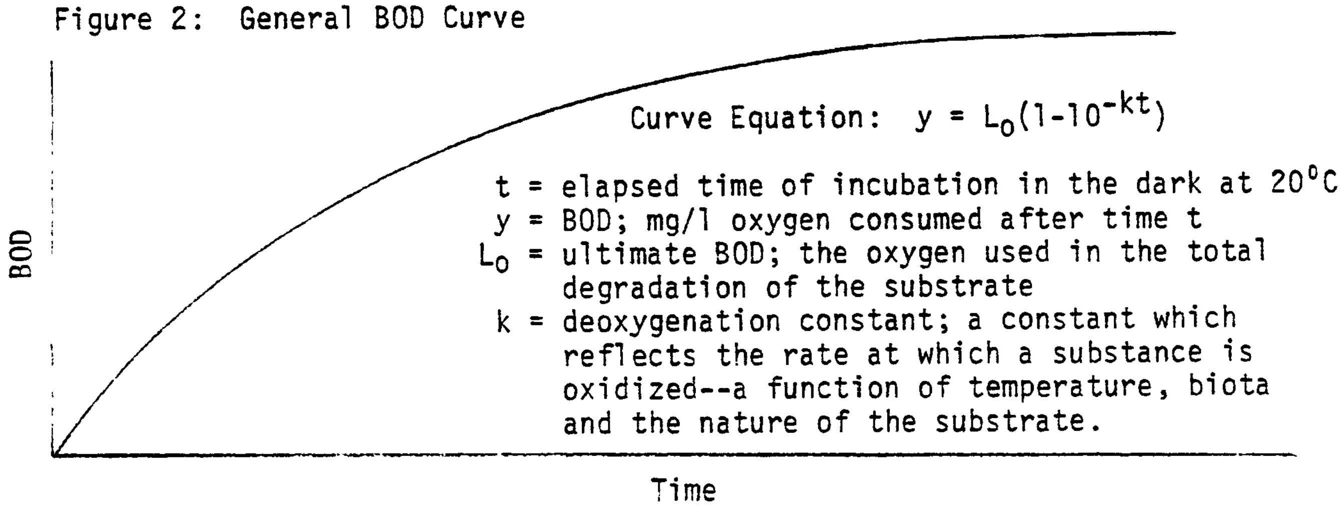
The blank carried throughout these methods was used to correct for non-algal nitrogen.

IV. CBOD and NOD Kinetics in the Potomac Estuary

Biochemical oxygen demand (BOD) is a bioassay in which the oxygen utilization of a complex and changing population of micro-organisms is measured as they respire in a changing mixture of nutrients. That portion of the BOD due to the respiration of organic matter by heterotrophic organisms is termed the carbonaceous oxygen demand and that portion resulting from autotrophic nitrification is termed nitrogenous oxygen demand. Nitrification is the conversion of ammonium to nitrate by biological respiration. These BOD components were delineated using an inhibitor to nitrification. The inhibitor, formula 2533 of the Hach Chemical Company, has been shown to effectively stop the growth of Nitrosomonas[2,6,7]. The product consists of 2-chloro-6 (trichloromethyl) pyridine, known as nitrapyrin, plated onto an inorganic salt. The salt serves as a carrier because it is soluble in water. The organic component is not biodegradable, even after 30 days of BOD incubation, and therefore does not contribute to the measured carbonaceous oxygen demand[2].

The shape of the oxygen depletion curves (Figures 2, 3, and 4) were such that the slope of the curves decreased with increased time of incubation.

Figure 2: General BOD Curve



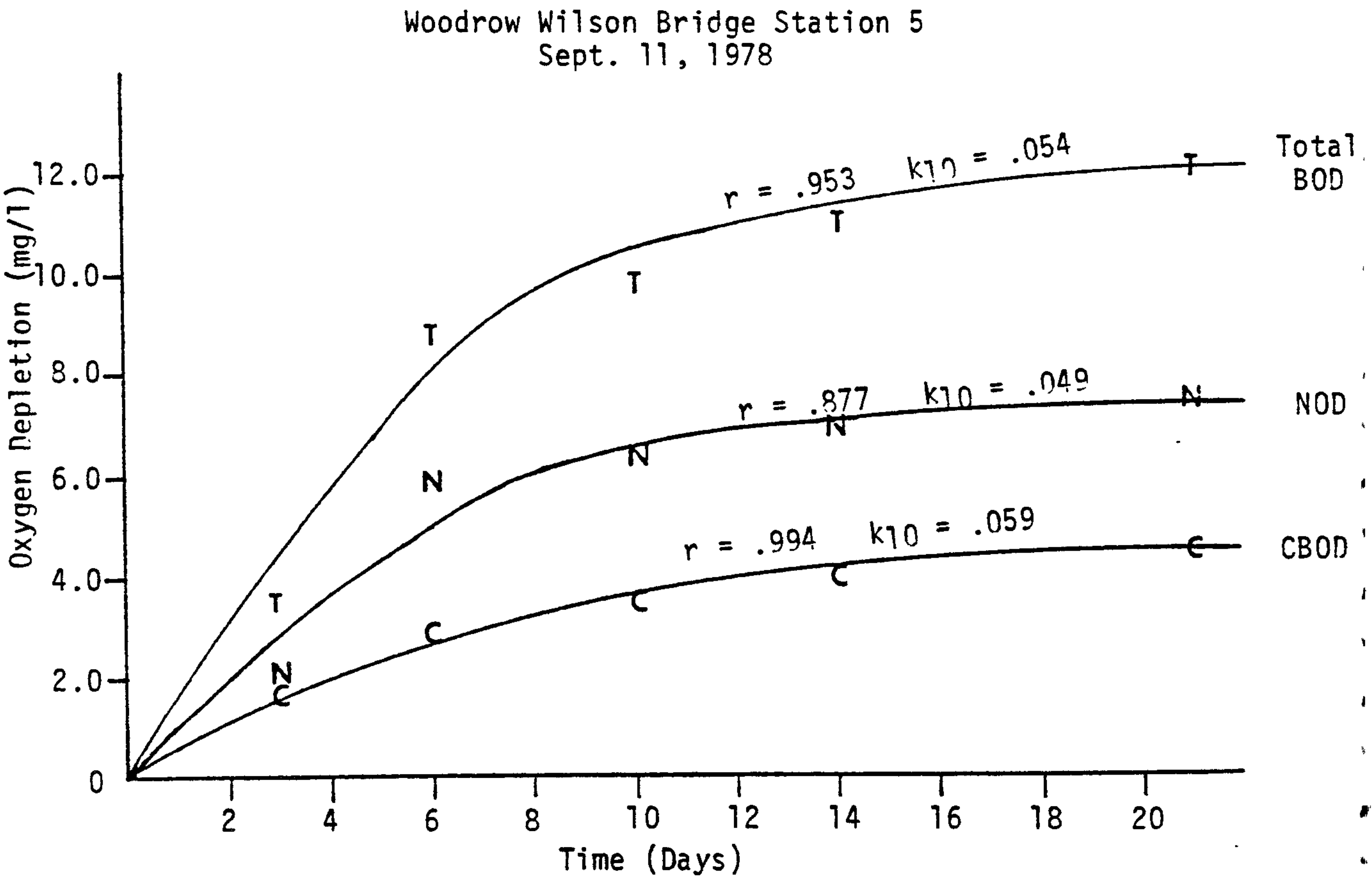
The rate of reaction associated with oxidation-respiration ($\Delta y/\Delta t$) was initially rapid corresponding to an initial relatively large substrate concentration. This rate decreased with time as the oxidizable substrate was depleted. Other nutrients are provided in excess and do not effect the rate of oxygen consumption in the standard BOD test. The quantity and nature of the organic material in the sample will limit oxygen consumption and determine the rate of depletion. This type of reaction, in which the rate is proportional to the amount of the reactant remaining at any time is referred to as a "first order" reaction. In general, the first order reaction pattern was observed for both the carbonaceous oxygen demand and the nitrogenous oxygen demand BOD components of Potomac River samples.

Long-term BOD incubation data were used to give the best available estimate of k_{10} and L_0 using the Thomas Graphical Determination[8,9,10] in which a plot of $(t/y)^{1/3}$ vs. t yielded a linear relation where k_{10} = 2.61 x (slope/intercept) and $L_o = (2.3 \times (\text{intercept})^3 \times k_{10})^{-1}$. The correlation coefficient of the linearized data was taken as a measure of goodness of fit to first order reaction kinetics.

The CBOD results for river samples were compiled in Table 2. The average (n=23) k_{10} value for river CBOD's was 0.051 day^{-1} or k_e = 0.12 day^{-1} with an average correlation coefficient = 0.98 and standard deviation = 0.03 (base e). The value of k_e obtained in a 1977 Potomac study[8] was 0.14 day^{-1}, with n = 43 and a standard deviation of 0.02. The ratio of $CBOD_5$ to BOD_5 was found to be 0.58 in the 1978 study.

The NOD of the river samples (Table 3) followed first order kinetics with a correlation coefficient of 0.86 (n=22) and an average k_e of 0.10 day^{-1}. The standard deviation of k_e was 0.06.

Figure 3: River Samples-Oxygen Depletion Curves



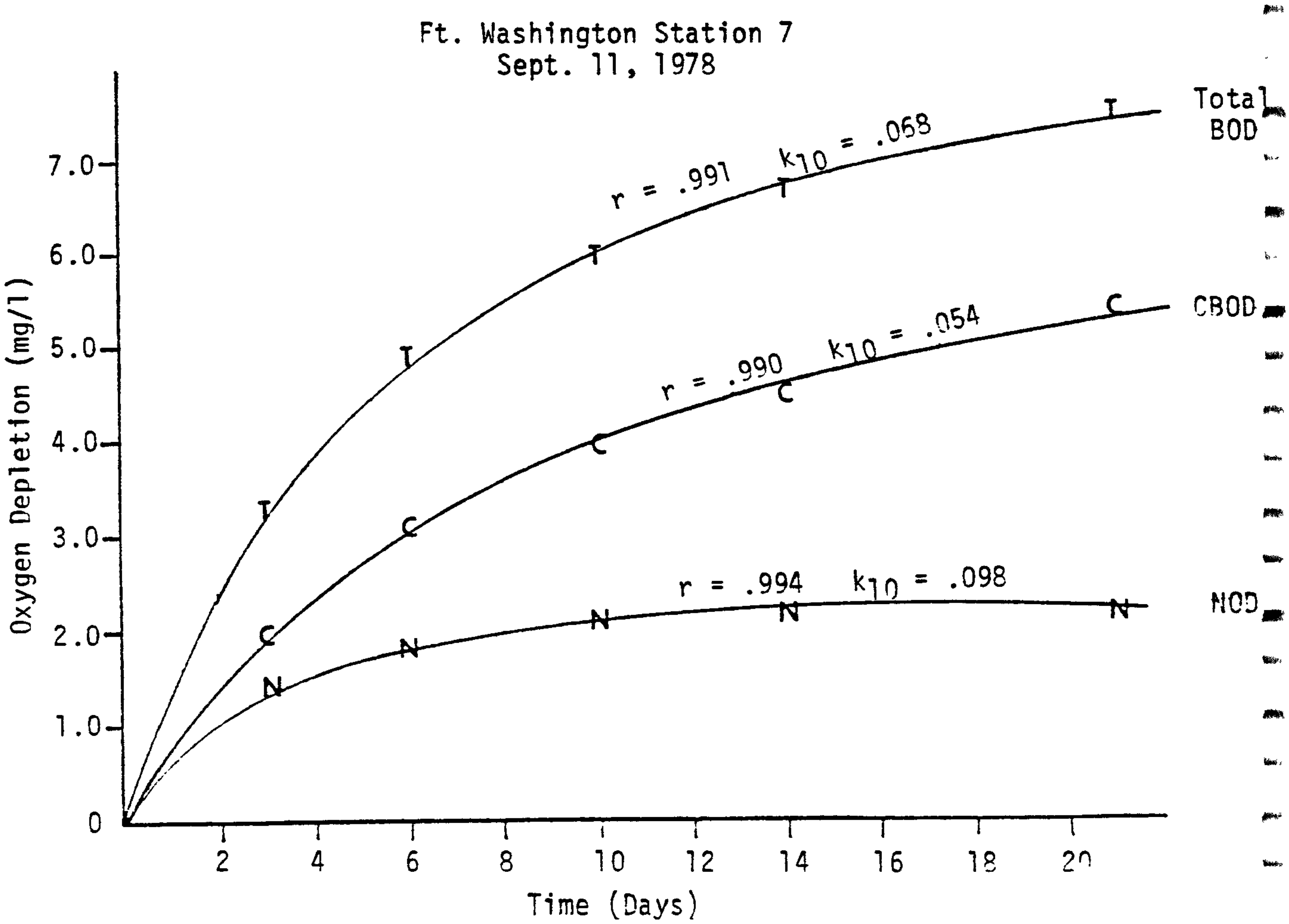
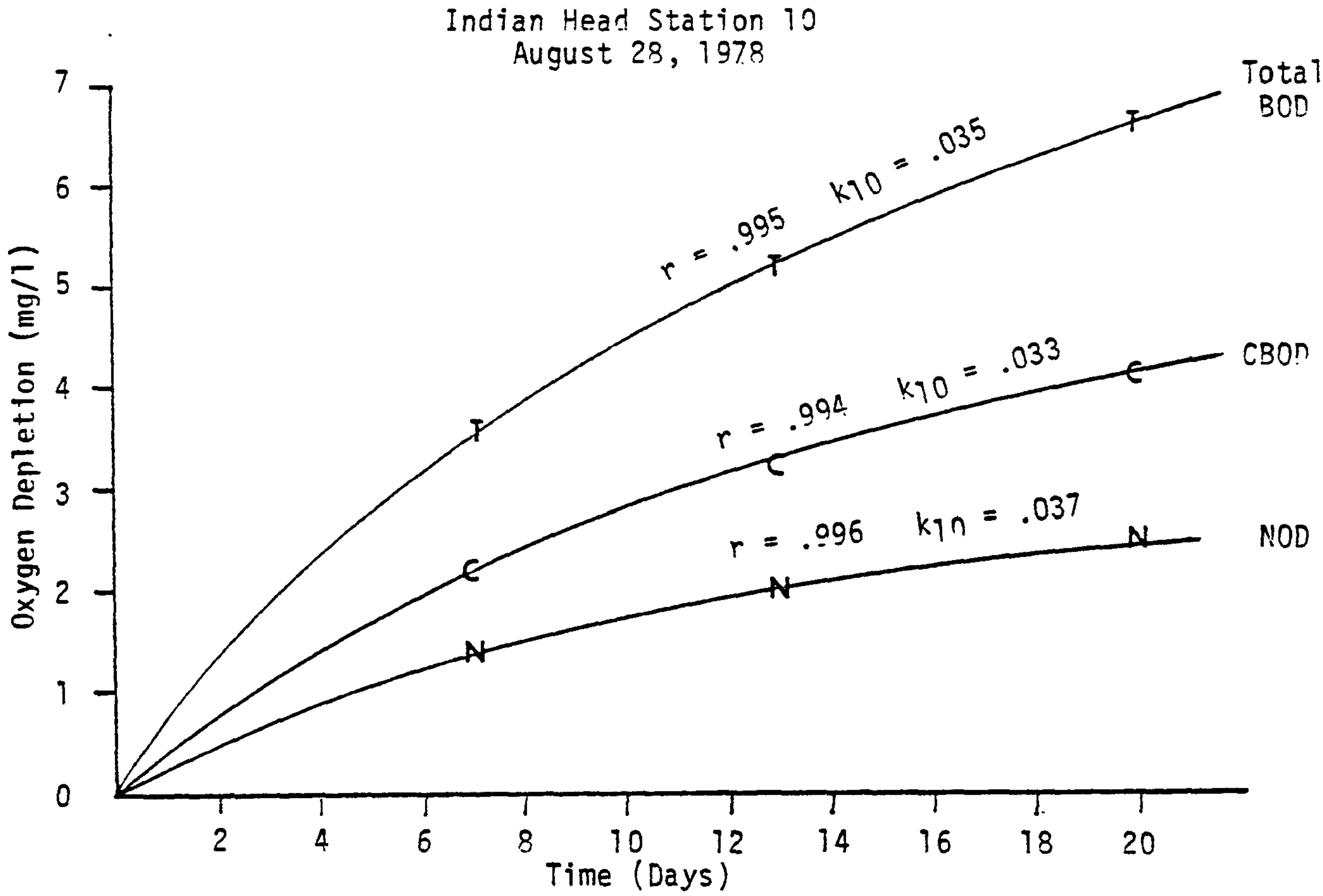


Figure 4 : River Samples-Oxygen Depletion Curves



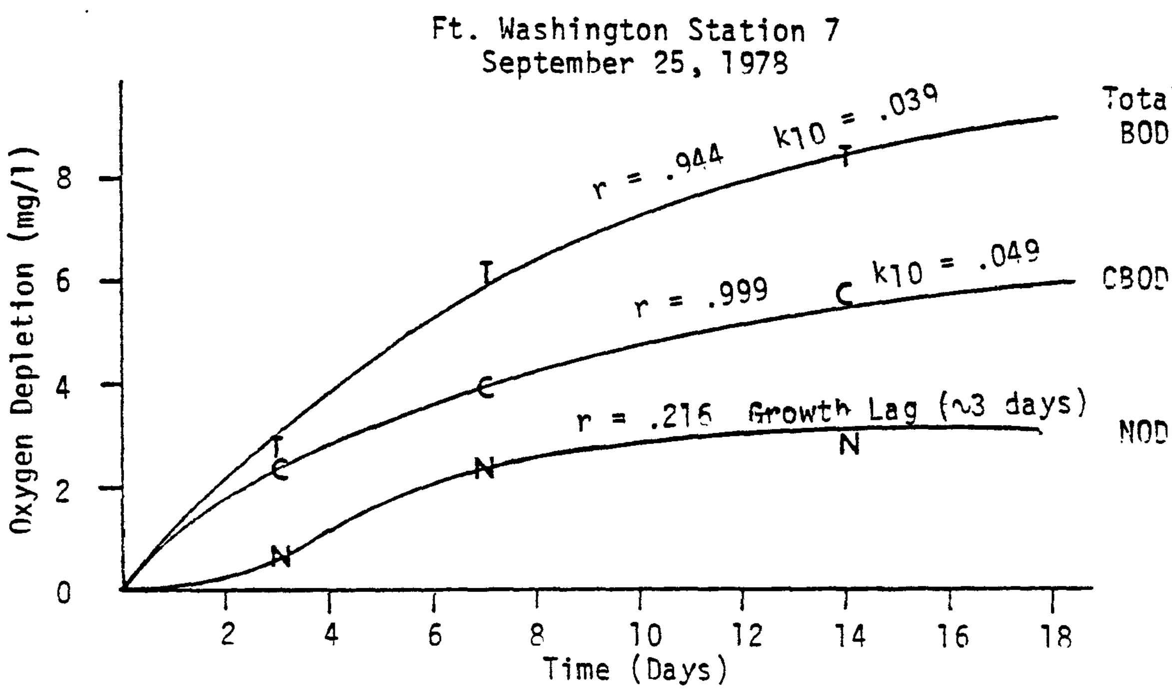


Table 2: Thomas Graphical Determinations of k_{10}, L_o, and r for River CBOD's

Date - Sta	r	k_{10} (day^{-1})	L_o (mg/l)	Calc.* $CBOD_5$ (mg/l)	Calc. $CBOD_{20}$ (mg/l)	$CBOD_5/BOD_5$	Calc. BOD_5 (mg/l)
Aug. 14							
5	.931	.045	2.5	1.0	2.2	.50	2.0
7	.951	.059	2.0	1.0	1.9	.42	2.4
8A	.966	.038	5.3	1.9	4.4	.50	3.8
10	.968	.057	4.8	2.3	4.4	.70	3.3
11	.991	.067	5.5	2.9	5.2	.74	3.9
14	.984	.062	4.2	2.2	4.0	.73	3.0
16	.985	.089	2.1	1.4	2.1	---	---
Aug. 28							
5	.993	.046	4.5	1.8	3.9	.43	4.2
7	.996	.040	5.7	2.1	4.7	.43	4.9
8A	.992	.039	6.5	2.4	5.4	.71	3.4
10	.994	.033	5.2	1.7	4.1	.61	2.8
11	1.000	.029	6.7	1.9	5.0	.60	3.2
14	.990	.027	3.4	0.9	2.4	.38	2.4
16	.996	.056	5.8	2.8	5.4	.93	3.0
Sept. 11							
5	.994	.059	5.0	2.5	4.7	.39	6.4
7	.990	.054	5.9	2.7	5.4	.61	4.4
8A	.987	.044	7.9	3.1	6.8	.70	4.4
10	.989	.044	6.7	2.7	5.9	.69	3.9
11	.940	.041	5.1	1.9	4.3	.49	3.6
14	.981	.054	3.5	1.6	3.2	---	---
16	.997	.069	5.5	3.0	5.3	---	---
Sept. 25							
5	.999	.079	5.4	3.2	5.3	.41	7.9
7	.996	.049	7.2	3.1	6.5		
8A	(.931)	(.020)	(15.7)	(3.2)	(9.5)		
10	(.231)	Lag					
11	(-.231)						
14	(.126)						
16	(.557)						

r: (correlation coefficient)
n = 23
Average = .98
Std. deviation = .02 (base 10)

k_{10}:
n = 23
Average = .051 day^{-1} or k_e = .12 day^{-1}
Std. deviation = .015 day^{-1} (base 10)

$CBOD_5/BOD_5$:
n = 19
Average = .58
Std. deviation = .15

* calc. = Calculated value based upon Thomas Graphical determinatio

Table 3: Thomas Graphical Determinations of k_{10}, L_o, and r for River NOD's

Date - Sta	r	k_{10} (day^{-1})	L_o (mg/l)	Calc.* NOD_5 (mg/l)	Calc. NOD_{20} (mg/l)	Potential** NOD (mg/l)
Aug. 14						
5	.957	.077	1.7	1.0	1.7	2.5
7	.780	.032	4.7	1.4	3.6	2.9
8A	.939	.037	5.5	1.9	4.5	2.8
10	.600	.019	5.3	1.0	3.0	1.9
11	.949	.037	3.0	1.0	2.4	2.3
14	.802	.024	3.6	.8	2.4	1.3
16	-.441	Lag				(.9)
Aug. 28						
5	.600	.017	13.8	2.4	7.4	7.2
7	.995	.067	5.2	2.8	5.0	5.1
8A	.978	.039	2.9	1.0	2.4	2.5
10	.996	.037	3.1	1.1	2.5	2.4
11	.989	.048	3.1	1.3	2.7	2.3
14	.876	.049	1.9	1.5	1.6	1.5
16	.877	.030	0.8	0.2	0.6	1.4
Sept. 11						
5	.877	.049	9.1	3.9	8.1	7.0
7	.994	.098	2.6	1.7	2.5	2.9
8A	.628	.028	4.8	1.3	3.4	2.9
10	.755	.023	5.0	1.2	3.3	3.1
11	.937	.039	4.7	1.7	3.9	2.3
14	-.619	Lag				(1.4)
16	-.381	Lag				(1.4)
Sept. 25						
5	.974	.104	6.7	4.7	6.7	8.3
7	.216	Lag				(5.0)
8A	-.276	Lag				(4.3)
10	.668	.022	4.0	.9	2.5	3.4
11	.727	.023	5.2	1.2	3.4	3.7
14	-.735	Lag				(3.5)
16	.995	.088	1.1	0.7	1.1	3.3

r: (correlation coefficient)
n = 22
Average = .86
Std. deviation = .14 (base 10)

---

k_{10}:
n = 22
Average = .045 day^{-1} or k_e = .104 day^{-1}
Std. deviation = .026 day^{-1} (base 10)

---

* calc. = calculated
** Potential NOD = 4.57 x TKN

The NOD results agreed with previous Potomac demand studies[8] in which the average NOD k_e was 0.14 day^{-1} with a standard deviation of 0.05.

The larger standard deviation observed for the NOD reflects both the more fragile nature of nitrification[11] and the method by which it was determined--uninhibited depletion minus inhibited depletion. The NOD_{20} was found not to be significantly different from the potential NOD expressed as 4.57 x TKN (Figure 5). The critical value of the paired t-test at a 95% confidence level was 2.08 and the calculated value was 0.37. The 4.57 constant is the stoichiometric conversion factor for the milligrams of oxygen consumed by the oxidation of ammonia to nitrate.

The CBOD kinetics observed for the sewage treatment plant effluents were first order with an average k_e of 0.16 day^{-1} (n = 36 and standard deviation of 0.05). The average correlation coefficient was 0.985 (Table 4, Figure 6).

The NOD kinetics observed for the sewage treatment plants were characterized by a lag period (Figure 6) which resulted in poor correlation to first order reaction kinetics (Table 5). This lag time was probably an artifact of the APHA dilution method, since nitrification in the receiving waters was immediate. Because the Potomac waste treatment effluents are characterized by high ammonia levels[8], the initial lack of nitrification is probably the result of an insignificant number of nitrifying bacteria in the samples and/or in the seed innoculum. The long term BOD oxygen depletion data is included in Section VII.

Figure 5: NOD_{20} (Inhibitor) vs NOD (TKN x 4.57) River Water Samples

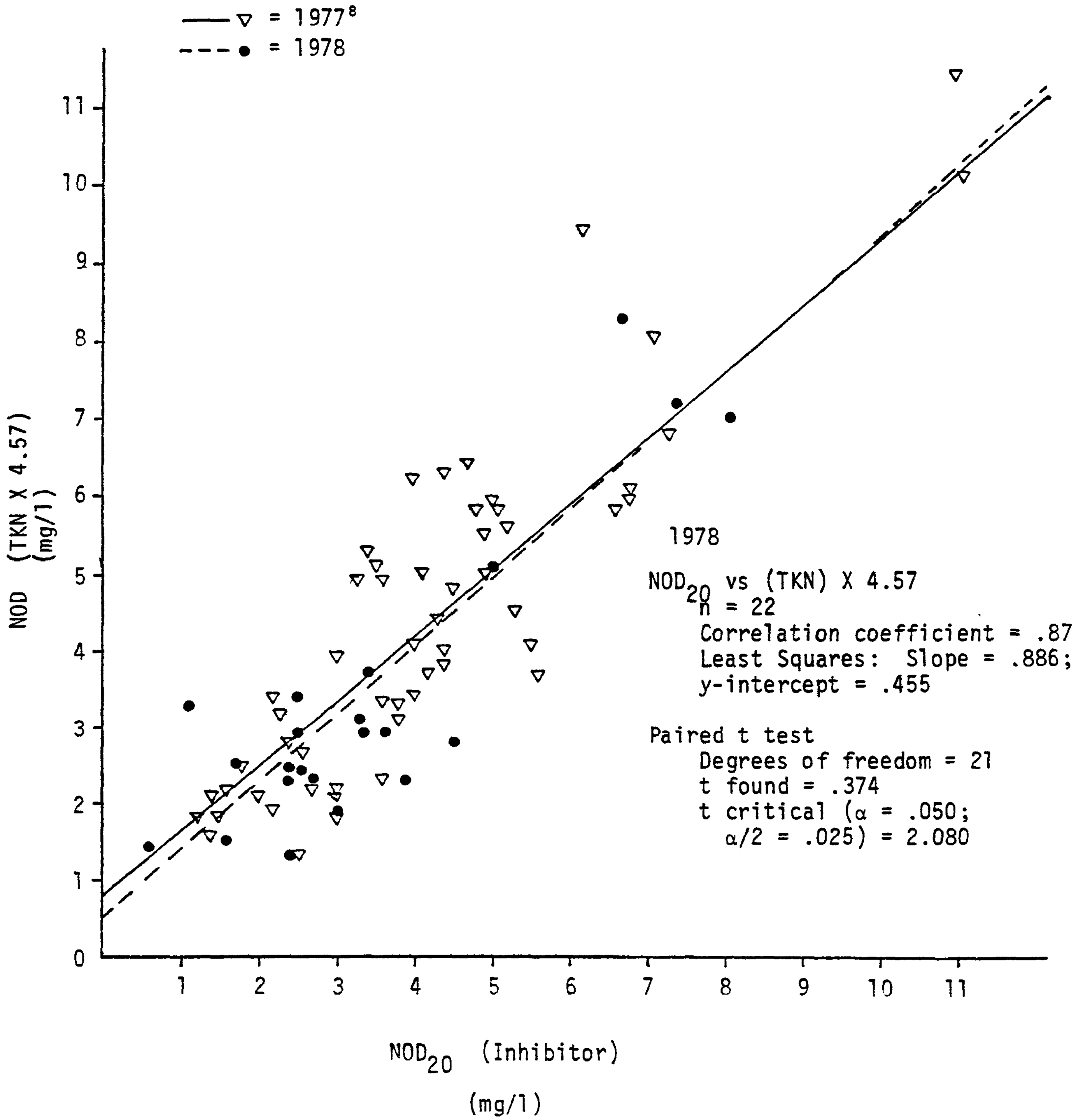


Table 4: Thomas Graphical Determinations of k_{10}, L_o, and r for STP CBOD's

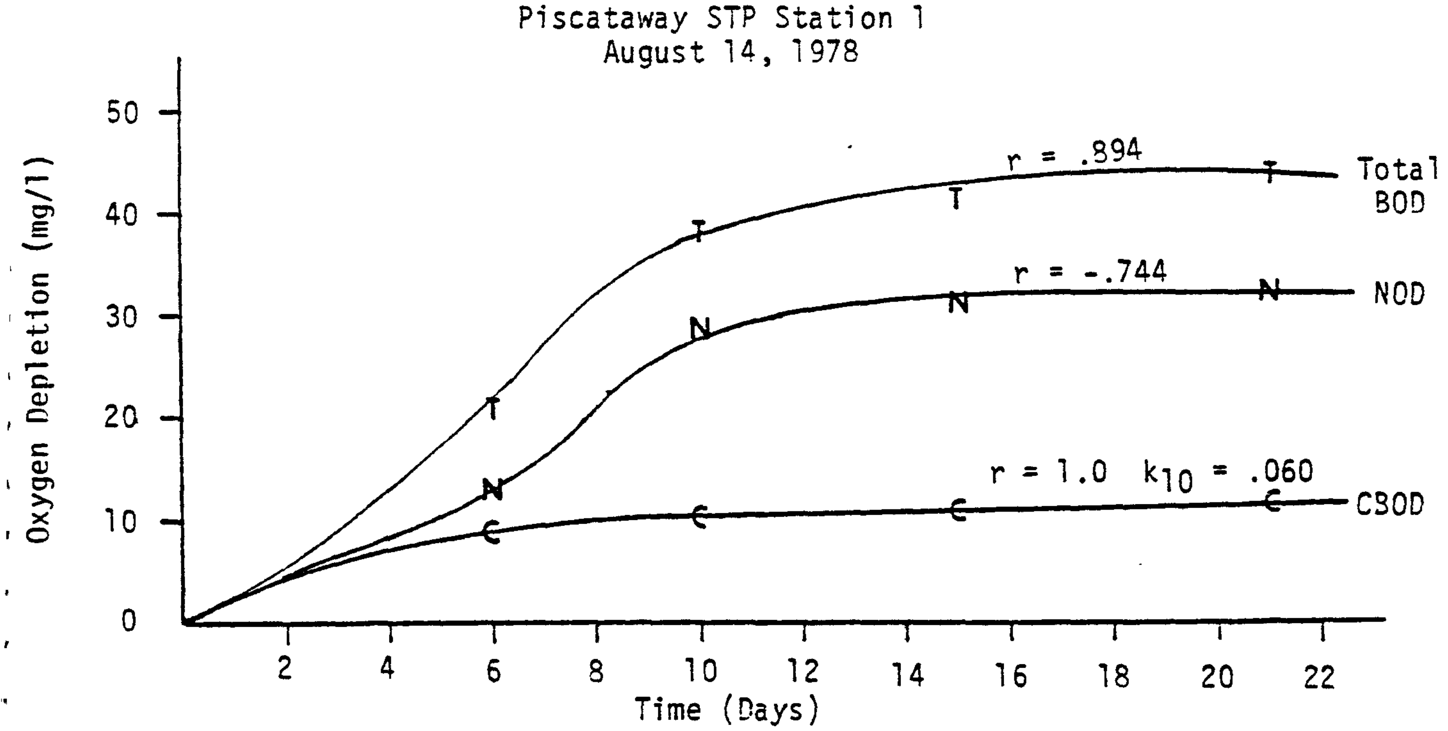
Date - Sta	Name	r	k_{10} (day^{-1})	L_o (mg/l)	Calc.* $CBOD_5$ (mg/l)	Calc. $CBOD_{20}$ (mg/l)
Aug. 14						
S-1	Piscataway	1.000	.060	12.8	6.4	12.0
S-2	Arlington	.997	.032	17.3	5.3	13.2
S-3 E	Blue Plains East	.999	.081	109.4	66.3	106.7
S-3 W	Blue Plains West	.997	.054	21.1	9.7	19.3
S-4	Alexandria	.999	.080	45.9	27.7	44.8
S-5	Westgate	.995	.053	18.3	8.3	16.7
S-6	Hunting Creek	1.000	.050	29.3	12.9	26.4
S-7	Dogue Creek	.993	.064	24.7	12.9	23.4
S-8	Pohick Creek	1.000	.024	31.4	7.44	20.8
Aug. 28						
S-1	Piscataway	1.000	.067	11.7	6.3	11.2
S-2	Arlington	.997	.092	9.90	6.5	9.8
S-3 E	Blue Plains East	.999	.067	41.8	22.4	39.9
S-3 W	Blue Plains West	1.000	.067	32.0	17.2	30.6
S-4	Alexandria	.998	.071	47.7	26.8	45.9
S-5	Westgate	.993	.069	12.9	7.1	12.4
S-6	Hunting Creek	1.000	.053	22.9	10.4	20.8
S-7	Dogue Creek	1.000	.060	24.4	12.2	22.9
S-8	Pohick Creek	.998	.032	26.6	8.20	20.5
Sept. 11						
S-1	Piscataway	.975	.079	15.9	9.5	15.5
S-2	Arlington	.969	.094	11.0	7.3	10.9
S-3 E	Blue Plains East	.982	.077	30.1	17.7	29.2
S-3 W	Blue Plains West	.994	.082	26.4	16.1	25.8
S-4	Alexandria	.987	.087	33.8	21.4	33.2
S-5	Westgate	.994	.078	20.4	12.0	19.8
S-6	Hunting Creek	.988	.077	22.5	13.2	21.8
S-7	Dogue Creek	.977	.060	23.9	11.9	22.4
S-8	Pohick Creek	.950	.049	23.0	9.9	20.5
Sept. 25						
S-1	Piscataway	.885	.059	18.4	9.1	17.2
S-2	Arlington	.933	.062	17.1	8.8	16.2
S-3 E	Blue Plains East	1.00	.090	42.0	27.1	41.4
S-3 W	Blue Plains West	.999	.071	68.5	38.2	65.9
S-4	Alexandria	.991	.113	41.6	30.3	41.4
S-5	Westgate	.987	.115	15.3	11.2	15.2
S-6	Hunting Creek	.954	.071	32.5	18.1	31.2
S-7	Dogue Creek	.992	.095	22.4	15.0	22.2
S-8	Pohick Creek	.964	.103	16.8	11.6	16.6

k_{10}:
n = 36
Average = .071 day^{-1} or k_e = .16 day^{-1}
Std deviation = .021 day^{-1} (base 10)

r: (correlation coefficient for first-order kinetics)
n = 36
Average = .986
Std Deviation = .024

* calc. = calculated value based upon Thomas Graphical determination

Figure 6: STP Effluent Samples - Oxygen Depletion Curves



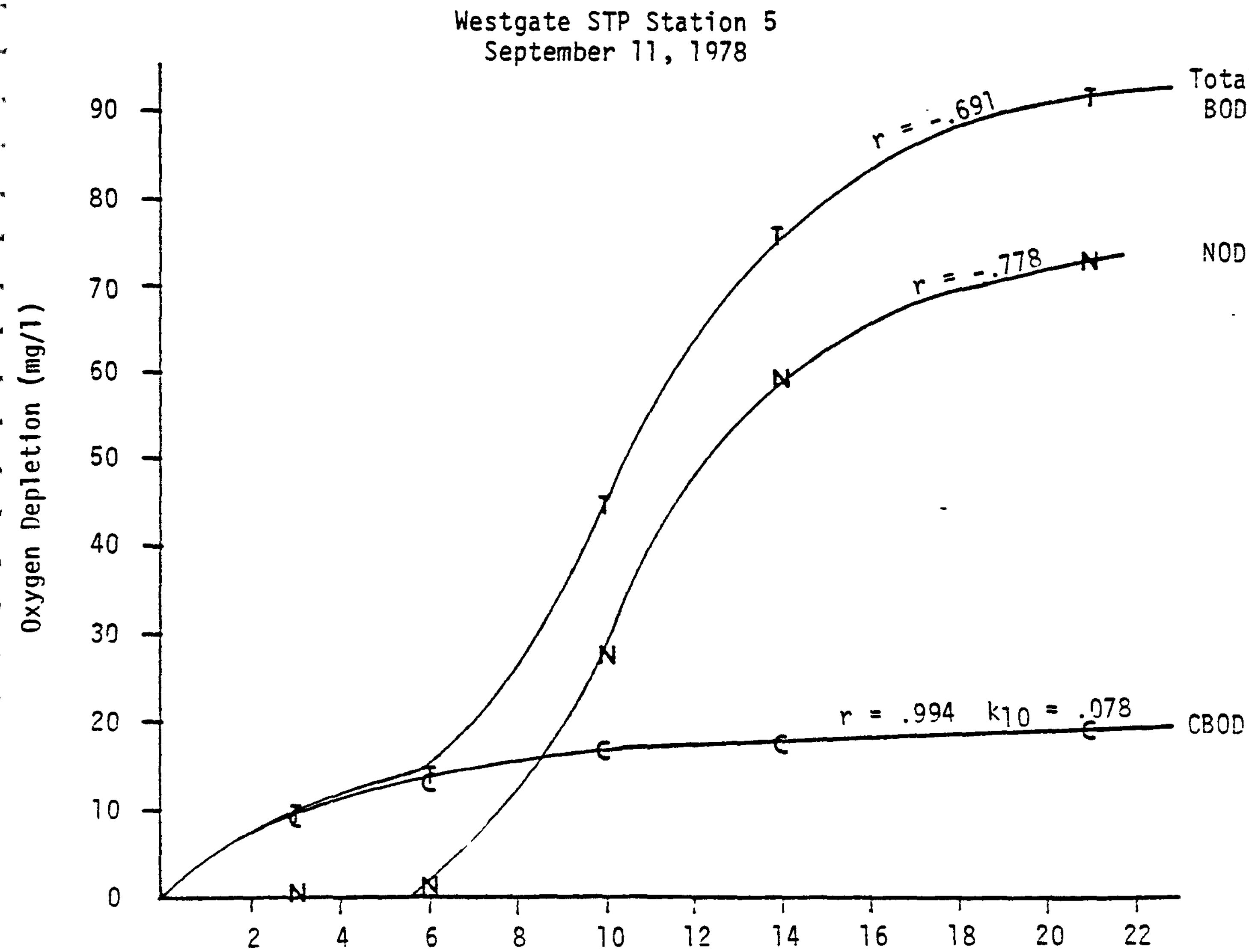


Table 5: First Order Correlation Coefficients for STP NOD's

Sta.	Name	Aug 14 r*	Aug 28 r	Sept 11 r	Sept 25 r
S-1	Piscataway	-.744	-.863	-.629	-.210
S-2	Arlington	.060	-.995	.351	.987
S-3	Blue Plains East	-.574	-.886	-.642	-.816
S-3	Blue Plains West	-.335	-.892	.972	-.833
S-4	Alexandria	-.597	-.905	-.994	-.872
S-5	Westgate	-.691	-.902	-.778	-.619
S-6	Hunting Creek	-.538	-.582	-.594	-.816
S-7	Dogue Creek	.957	-.993	-.778	-.829
S-8	Pohick Creek	-.722	-.982	-.709	-.619

r = correlation coefficient

V. Oxygen Demand of Algal Respiration and Algal Decay

Potomac BOD_5 samples containing algae historically[8,12] expressed significantly high oxygen demand. The oxygen demand of such samples was the result of: algal respiration; the decay of phytoplankton; and the carbonaceous and nitrogenous demand of other non-algal sample constituents. To resolve the BOD fractions of the sample, it was assumed that algae represented the only significant particulate contribution to the BOD of the sample. The non-algal BOD of the sample was assumed to be associated with the soluble organic and ammonium/nitrite fractions of the sample. The non-algal or background BOD was measured in the supernatant which had been obtained from the centrifugation of the algae containing samples. It was further assumed that the BOD of freeze-dried algae corrected for seed addition and the BOD of the dilution water (river water supernatant) represented the biochemical oxygen demand of algal decay. Freeze-drying has been shown to effectively kill phytoplankton without significantly altering their physical structure[13] thus providing a method of separating algal respiration and algal decay measurements in a BOD analysis.

The results of these experiments are presented in Figures 7,8,and 9 and Tables 6 and 7. Algal decay was found to be the major contribution to algal BOD_5 with an average mg algal BOD_5 per μg chlorophyll _a_ of 0.019. Algal respiration represented about 30% of the algal BOD_5 contribution with an average of 0.008 mg algal BOD_5 per μg chlorophyll _a_. The predominent species present in the Potomac during this study was the

Figure 7: Oxygen Depletion Curves of Algal Respiration and Decay
September 14, 1978

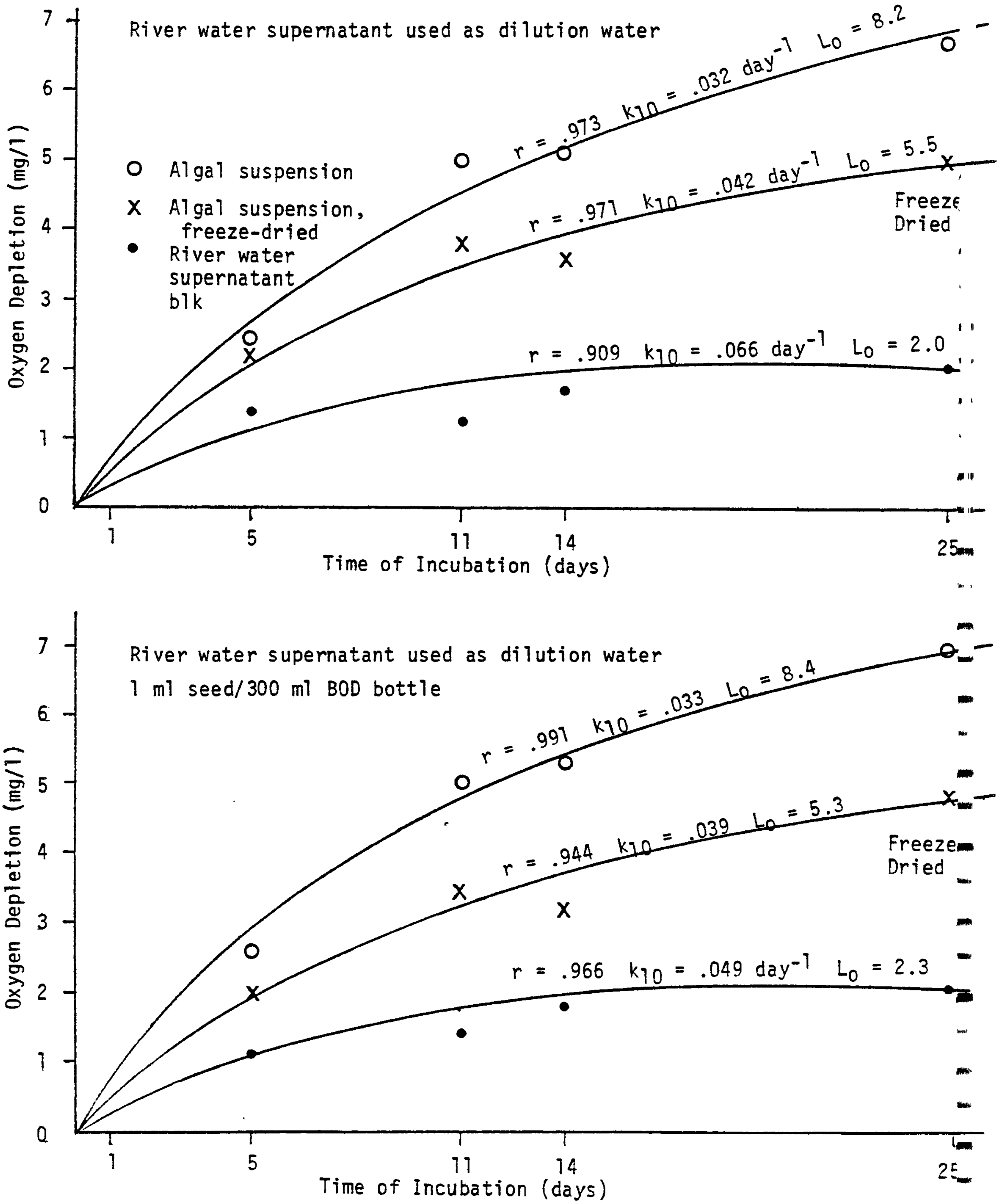


Figure 8: Oxygen Depletion Curves of Algal Respiration and Decay
September 6, 1978

1 ml Seed/BOD Bottle

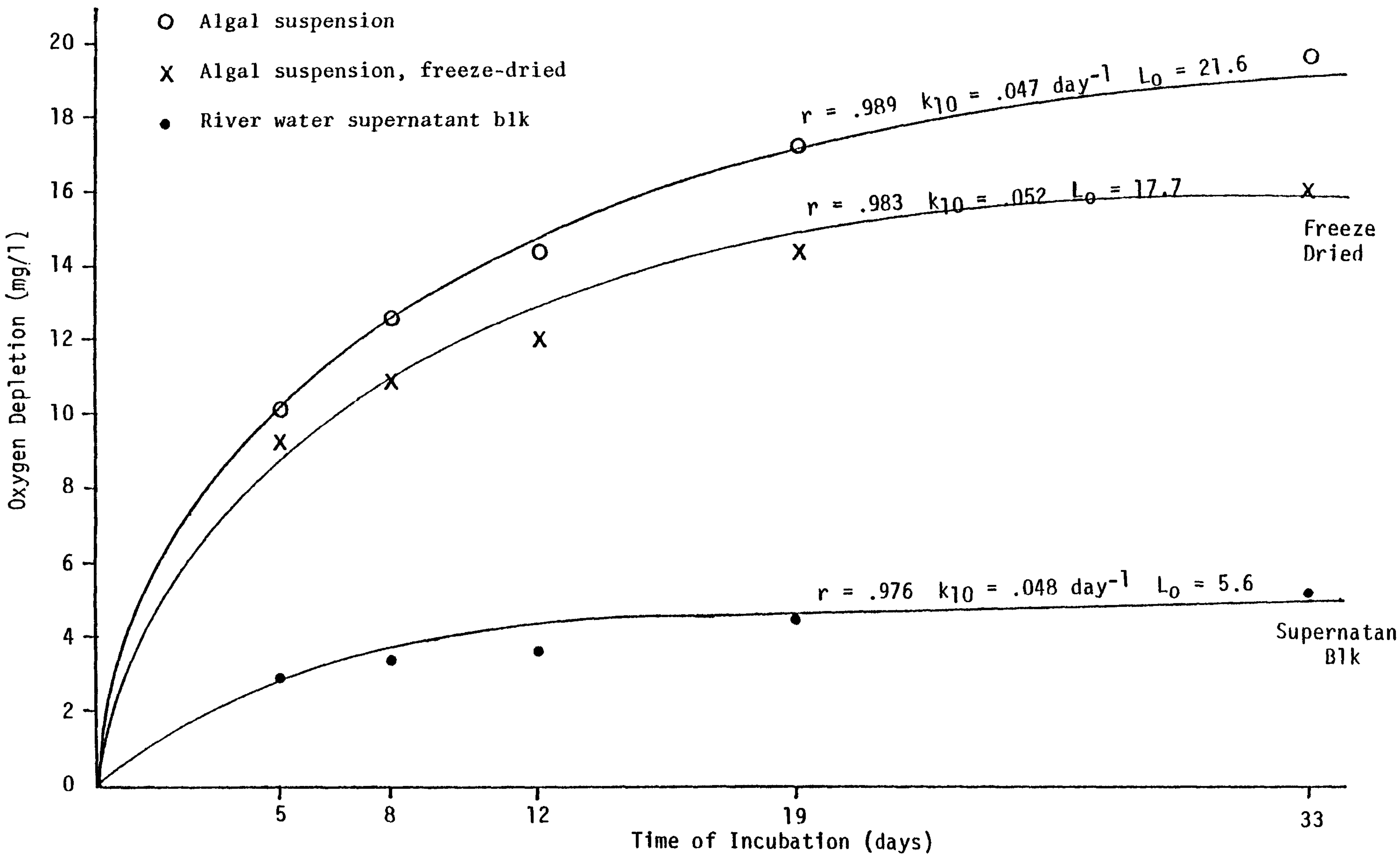


Figure . Oxygen Depletion Curves of Algal Respiration and Decay
September 6, 1978

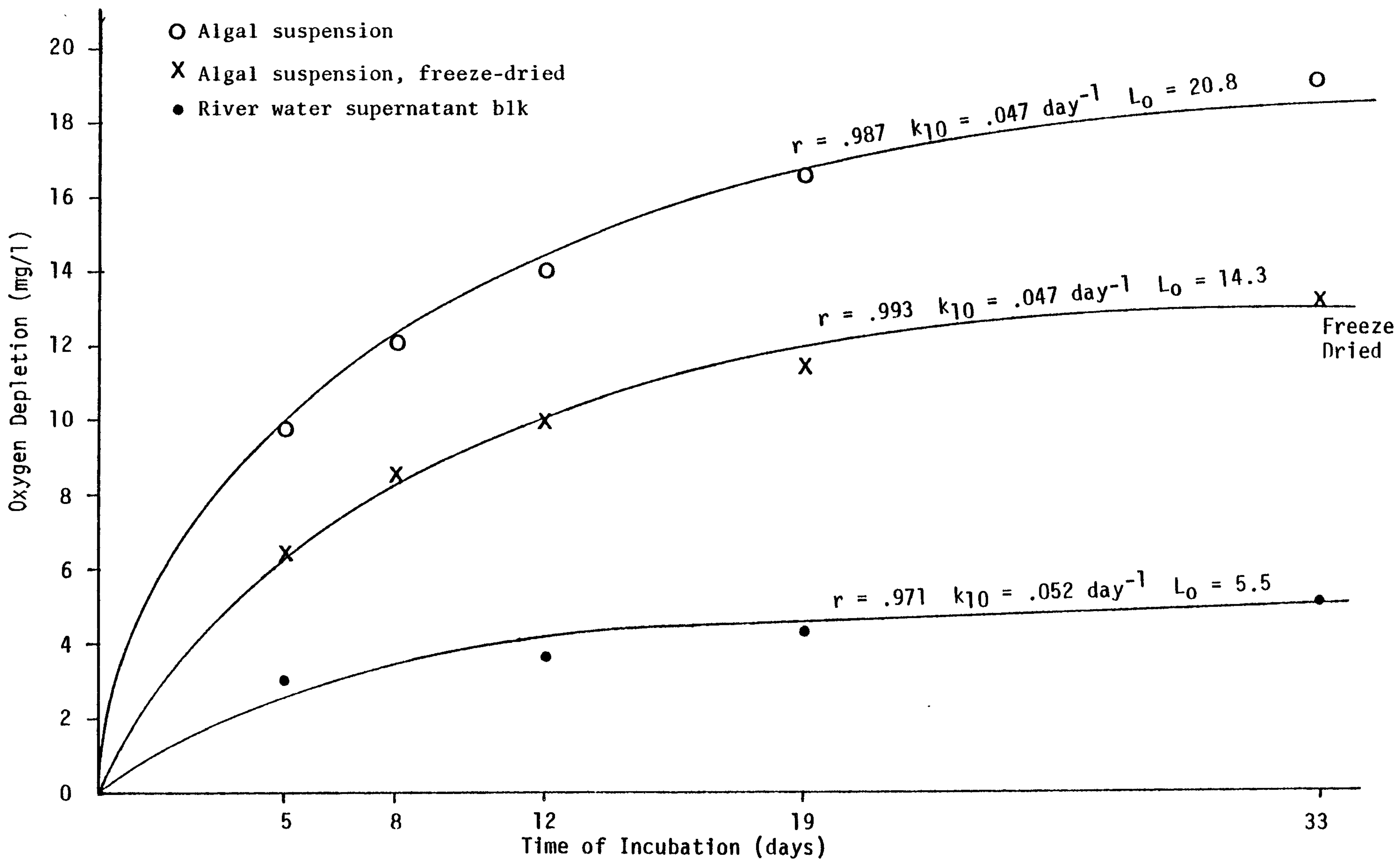


Table 6: Phytoplankton Oxygen Depletion

Date/Sample	Days of Incubation				
Sept. 6, 1978	5	8	12	19	33
Algal Suspension	9.8	12.0	13.8	16.6	19.1
Algal Suspension Freeze-Dried	6.4	8.5	9.8	11.4	13.1
River Water Supernatant Blk	3.0	3.4	3.6	9.3	5.1
Seeded Algal Suspension	10.0	12.6	14.4	17.2	19.8
Seeded Algal Suspension Freeze-Dried	9.3	10.8	12.0	14.3	16.1
Seeded River Water	2.8	3.3	3.6	4.4	5.2
Sept. 14, 1978	5	11	14	25	
Algal Suspension	2.4	5.0	5.1	6.7	
Algal Suspension Freeze-Dried	2.2	3.8	3.6	5.0	
River Water Supernatant Blk	1.4	1.2	1.7	1.9	
Seeded Algal Suspension	2.6	5.0	5.3	7.0	
Seeded Algal Suspension Freeze-Dried	2.0	3.5	3.2	4.8	
Seeded River Water	1.1	1.4	1.8	2.1	

Table 7: BOD_5 Requirements for Algal Decay and Respiration

Decay

$$\left(\left(BOD_5\ (\text{freeze-dried algal suspension}) - \text{Background } BOD_5\right) \times \text{Dilution factor}\right) \div \text{chloro } a = \text{5-Day Algal Decay}$$

Date	BOD_5 (freeze-dried algal suspension) mg/l	Background BOD_5 mg/l	Dilution factor	chloro a µg/l	5-Day Algal Decay mg O_2 depletion / µg chlor a
Sept. 6	6.4	3.0	6.0	1386	.0147
Sept. 14	2.2	1.4	15.0	810	.0148
Sept. 6	9.3	2.8	6.0	1386	.0281
Sept. 14	2.0	1.1	15.0	810	.0167
				average	.019

Respiration

$$\left(\left(BOD_5\ \text{algal suspension} - BOD_5\ (\text{freeze-dried algal suspension})\right) \times \text{Dilution factor}\right) \div \text{chloro } a = \text{5-Day Algal Respiration}$$

Date	BOD_5 algal suspension mg/l	BOD_5 (freeze-dried algal suspension) mg/l	Dilution factor	chloro a µg/l	5-Day Algal Respiration mg O_2 depletion / µg chlor a
Sept. 6	9.8	6.4	6.0	1386	.0147
Sept. 14	2.4	2.2	15.0	810	.0037
Sept. 6	10.0	9.3	6.0	1386	.0030
Sept. 14	2.6	2.0	15.0	810	.0111
				average	.008

filamentous blue-green algae *Psuedanabaena*. Figures 7,8,and9 also revealed that seeding of the samples with 1 ml per bottle of stale settled sewage[1] had little effect upon the amount and rate of oxygen depletion. This suggested that the supernatant contained sufficient microorganisms for algal decay.

VI. Phytoplankton Elemental Analysis and Methods of TKN Digestion of Algal Samples

The algae bloom of *Psuedanabaena* occurred in mid to late September with a chlorophyll *a* peak of 159 µg/l on September 27. The elemental composition of the phytoplankton is compiled in Table 8. The average elemental ratios to chlorophyll *a* were: .021 mg C/µg chlorophyll *a*; .0054 mg N/µg chlorophyll *a*; and .0020 mg PO_4/µg chlorophyll *a*. It should be emphasized that the results are based on the overall phytoplankton standing corp. The nitrogen values reported for elemental analysis were obtained by the automated colorimetric phenolate procedure employing the continuous (helix) digestor with preliminary manual digestion. Neither the Technicon block digestor nor the Technicon continuous digestor alone provided satisfactory digestion of algal TKN. The data from side-by-side algal digestions are compiled in Table 9. The average recovery relative to preliminary manual digestion for the Technicon continuous digestor and block digestor were 58% and 83% respectively. This suggested that 42% of algal nitrogen was refractory to the Technicon continuous digestor. This agreed with a 50% TKN recovery estimate suggested in a previous study.[14]

Table 8: Phytoplankton Elemental Analysis

Date	Station	mg TOC / µg chloro a	mg TOC / mg TSS	mg TKN / µg chloro a	mg TKN / mg TSS	mg PO_4 / µg chloro a	mg PO_4 / mg TSS	mg TSS / µg chloro a
Sept. 7	5-A	.017	.147	.0057	.050	.0021	.018	.115
	8-A	.019	.147	.0052	.040	.0020	.015	.130
	9	.015	.093	.0052	.033	.0021	.013	.158
	10	.027	.171	.0054	.035	.0020	.013	.156
	10-B	.027	.124	.0065	.030	.0029	.013	.220
Sept. 11	8-A	.024	.130	.0054	.029	.0023	.012	
	9					.0024	.013	.185
	10-B	.023	.130	.0052	.028	.0023	.012	
	11					.0023	.012	
Sept. 26	8-A	.019	.096	.0058	.029	.0021	.011	.201
						.0021	.010	
	9	.026	.119	.0086	.039	.0026	.012	.218
						.0028	.013	
	10	.018	.127	.0060	.042	.0017	.012	.142
						.0016	.012	
	10-B	.020	.134	.0060	.040	.0018	.012	.148
						.0018	.012	
	11	.021	.112	.0060	.035	.0020	.011	.189
						.0022	.012	
Sept. 28	7	.018		.0042		.0012		
	9	.018		.0043		.0012		
	10	.019		.0035		.0010		
	10-B	.020		.0039		.0010		
	11	.022		.0044		.0014		
	average	.021	.13	.0054	.036	.0020	.013	.169
	std. deviation	.004	.022	.0012	.007	.0005	.002	.035

Table 9: Results From Three TKN Digestion Methods

Date	Station	Manual mg/l TKN	Block mg/l TKN	Helix mg/l TKN	Helix / Manual	Block / Manual	Helix / Block
Sept. 7	5-A	14.52	11.10	9.15	.63	.76	.82
	8-A	15.14	14.50	9.52	.63	.96	.66
		15.14	13.03	9.27	.61	.86	.71
	9	14.89	14.47	9.52	.64	.97	.66
		15.89	14.09	9.27	.58	.89	.66
	10	15.89	13.63	9.21	.58	.86	.68
		15.89	14.06	8.81	.55	.88	.63
	10-B	14.52	13.09	8.24	.57	.90	.63
		15.14	14.36	8.06	.53	.95	.56
Sept. 11	8-A	29.27	19.49	12.92	.44	.67	.66
	9	28.28	20.00	12.61	.45	.71	.63
	10-B						
	11						
Sept. 11	8	23.32	---	11.83	.51	---	---
	8-A	29.05	---	11.83	.41	---	---
	9						
	10						
Sept. 26	8-A	21.73	16.58	13.66	.63	.76	.82
			17.74			.82	.77
	9	25.17	20.63	16.86	.67	.82	.82
			19.46			.77	.87
	10	34.66	30.88	22.36	.65	.89	.72
			28.02			.81	.80
	10-B	31.95	24.00	22.84	.71	.75	.95
			26.30			.82	.87
	11	26.74	20.60	18.53	.69	.77	.90
			20.32			.76	.91
	average				.58	.83	
	std. deviation				.09	.08	

VII. Potomac River Long-Term BOD Survey Data - Summer 1978

Date 8/14/78

Station		Days of Incubation 6	10	15	21
5	T*	2.4	3.0	3.4	3.8
	C*	1.3	1.4	2.1	2.2
	N*	1.1	1.6	1.3	1.6
7	T	2.7	4.4	4.9	5.3
	C	1.3	1.3	1.7	1.9
	N	1.4	3.1	3.2	3.4
8-A	T	4.3	6.3	8.0	8.7
	C	2.3	2.8	3.9	4.4
	N	2.0	3.5	4.1	4.3
10	T	3.9	5.3	6.8	7.2
	C	2.9	3.1	4.0	4.4
	N	1.0	2.2	2.8	2.8
11	T	4.6	5.8	7.0	7.3
	C	3.5	4.0	4.7	5.0
	N	1.1	1.8	2.3	2.3
14	T	3.5	4.7	5.6	6.2
	C	2.6	2.9	3.7	3.8
	N	0.9	1.8	1.9	2.4
16	T	1.8	2.0	2.4	2.9
	C	1.6	1.6	2.0	1.8
	N	0.2	0.4	0.4	1.1

Date 8/28/78

Station		Days of Incubation 7	13	20
5	T	4.3	9.3	10.8
	C	2.4	3.2	3.9
	N	1.9	6.1	6.9
7	T	6.2	8.0	9.4
	C	2.7	3.8	4.7
	N	3.5	4.2	4.7
8-A	T	4.4	6.4	7.7
	C	3.1	4.3	5.4
	N	1.3	2.1	2.3

*T-BOD (mg/1)
*C-CBOD (mg/1)
*N-NOD (mg/1)

VII. Potomac River Long-Term BOD Survey Data - Summer 1978 (con't)

Date 8/28/78 (con't)

Station		Days of Incubation 7	13	20
10	T	3.6	5.2	6.6
	C	2.2	3.2	4.1
	N	1.4	2.0	2.5
11	T	4.2	6.1	7.6
	C	2.5	3.9	4.9
	N	1.7	2.2	2.7
14	T	1.4	2.7	3.9
	C	1.2	1.8	2.4
	N	0.2	0.9	1.5
16	T	3.8	4.9	5.8
	C	3.5	4.5	5.2
	N	0.3	0.4	0.6

Date 9/11/78

Station		Days of Incubation 3	6	10	14	21
5	T	3.7	8.9	9.8	11.0	12.2
	C	1.7	2.9	3.5	4.0	4.6
	N	2.0	6.0	6.3	7.0	7.6
7	T	3.3	4.9	6.0	6.7	7.6
	C	1.9	3.1	3.9	4.5	5.4
	N	1.4	1.8	2.1	2.2	2.2
8-A	T	2.1	4.8	7.7	9.1	9.9
	C	2.1	3.6	4.6	6.2	6.7
	N	---	1.2	3.1	2.9	3.2
10	T	2.5	4.4	6.6	7.8	8.9
	C	1.9	2.9	4.2	5.0	5.9
	N	0.6	1.5	2.4	2.8	3.0
11	T		3.9	6.3	7.1	8.0
	C	---	2.0	3.2	4.1	4.0
	N	---	1.9	3.1	3.0	4.0
14	T	1.2	2.0	2.8	3.8	4.5
	C	1.2	1.7	2.3	2.7	3.2
	N	0	0.3	0.5	1.1	1.3
16	T	2.2	3.5	4.3	5.0	5.8
	C	2.1	3.5	4.2	4.6	5.0
	N	0.1	0	0.1	0.4	0.8

VII. Potomac River Long-Term BOD Survey Data - Summer 1978 (con't)

Date 9/25/78

Station		Days of Incubation 3	7	14
5	T	6.1	8.5	11.0
	C	2.3	3.8	4.8
	N	3.8	4.7	6.2
7	T	2.7	6.2	8.4
	C	2.1	3.8	5.7
	N	0.6	2.4	2.7
8-A	T	2.5	7.1	10.5
	C	2.1	4.1	7.6
	N	0.4	3.0	2.9
10	T	2.5	7.6	11.0
	C	2.0	6.2	9.1
	N	0.5	1.4	1.9
11	T	2.3	5.7	11.2
	C	1.6	3.8	8.6
	N	0.7	1.9	2.6
14	T	0.8	2.0	4.5
	C	0.7	1.1	2.9
	N	0.1	0.9	1.6
16	T	1.1	1.6	2.7
	C	0.6	0.7	1.7
	N	0.5	0.9	1.0

Date 8/14/78

Station		Days of Incubation 6	10	15	21
S-1	T	20.1	38.7	41.6	43.5
	C	7.2	9.6	10.8	11.4
	N	12.9	29.1	30.8	32.1
S-2	T	21.0	22.8	41.1	55.5
	C	6.0	9.0	11.6	13.2
	N	15.0	13.8	29.5	42.3
S-3 (E)	T	81.0	157.5	174	181.5
	C	75.0	88.5	96.0	96.0
	N	6.0	69.0	78.0	85.5

Date 8/14/78 (con't)

Station		Days of Incubation 6	10	15	21
S-3 (W)	T	21.6	60.0	73.8	77.4
	C	10.8	15.0	18.0	18.3
	N	10.8	45.0	55.8	59.1
S-4	T	36.0	72.0	87.0	92.3
	C	31.5	36.8	40.5	40.3
	N	4.5	35.2	46.5	52.0
S-5	T	14.1	41.7	59.4	72.6
	C	9.6	12.8	14.4	16.5
	N	4.5	28.9	45.0	56.1
S-6	T	18.6	39.9	51.3	55.8
	C	14.7	20.0	23.6	25.8
	N	3.9	19.9	27.7	30.0
S-7	T	30.6	44.4	43.5	46.8
	C	15.2	18.0	20.7	22.5
	N	15.4	26.4	22.8	24.3
S-8	T	10.2	38.7	56.4	75.5
	C	8.7	13.1	17.4	21.2
	N	1.5	25.6	39.0	54.3

Date 8/28/78

Station		Days of Incubation 7	13	20
S-1	T	9.6	43.7	71.7
	C	7.8	9.8	10.5
	N	1.8	33.9	61.2
S-2	T	12.3	22.8	46.8
	C	7.8	8.4	8.6
	N	4.5	14.4	38.2
S-3 (E)	T	28.5	79.5	148.5
	C	27.0	36.0	36.8
	N	1.5	43.5	111.7
S-3 (W)	T	24.0	67.5	117.8
	C	21.0	27.0	28.5
	N	3.0	40.5	89.3

VII. Potomac STP Long-Term BOD Survey Data - Summer 1978 (con't)

Date 8/28/78 (con't)

Station		Days of Incubation 7	13	20
S-4	T	42.0	87.0	132.0
	C	33.0	39.8	42.8
	N	9.0	47.2	89.2
S-5	T	9.5	22.8	47.7
	C	8.9	10.4	11.7
	N	0.6	12.4	36.0
S-6	T	19.4	42.0	47.9
	C	13.1	17.7	20.1
	N	6.3	24.3	27.8
S-7	T	25.2	41.4	53.6
	C	15.0	20.1	21.6
	N	10.2	21.3	36.0
S-8	T	11.7	22.4	52.4
	C	10.8	16.1	20.4
	N	0.9	6.3	32.0

Date 9/11/78

Station		Days of Incubation 3	6	10	14	21
S-1	T	11.4	39.0	52.8	62.4	63.0
	C	7.8	10.2	11.4	13.2	15.0
	N	3.6	28.8	41.4	49.2	48.0
S-2	T	28.8	50.4	68.4	70.8	87.0
	C	6.0	8.4	8.4	8.4	10.4
	N	22.8	42.0	60.0	62.4	76.6
S-3 (E)	T	13.5	20.3	34.5	69.0	79.5
	C	13.5	20.3	22.5	24.0	28.5
	N	0	0	12.0	45.0	51.0
S-3 (W)	T	13.5	22.5	49.5	78.0	90.0
	C	12.0	18.0	21.0	22.0	24.0
	N	1.5	4.5	28.5	56.0	66.0
S-4	T	1.8	27.0	46.5	76.5	99.0
	C	16.5	24.0	27.0	27.0	31.0
	N	1.5	3.0	19.5	49.5	68.0

VII. Potomac STP Long-Term BOD Survey Data - Summer 1978 (con't)

Date 9/11/78

Station		Days of Incubation 3	6	10	14	21
S-5	T	9.0	14.4	44.4	76.2	91.2
	C	9.0	13.2	16.2	16.8	18.6
	N	0	1.2	28.2	59.4	72.6
S-6	T	9.9	15.0	32.4	51.6	55.2
	C	9.9	15.0	17.4	18.0	21.0
	N	0	0	15.0	33.6	34.2
S-7	T	9.6	14.4	31.8	55.8	64.2
	C	9.0	13.2	16.2	18.6	22.8
	N	0.6	1.2	15.6	37.2	41.4
S-8	T	7.8	12.0	42.6	69.0	79.8
	C	7.8	10.2	14.4	16.8	21.6
	N	0	1.8	28.2	52.2	58.2

Date 9/25/78

Station		Days of Incubation 3	7	14
S-1	T	7.8	40.2	49.2
	C	5.4	13.8	14.4
	N	2.4	26.4	34.8
S-2	T	22.8	60.0	91.8
	C	5.4	12.6	13.8
	N	17.4	47.4	78.0
S-3 (E)	T	31.5	69.0	108
	C	19.5	31.5	37.5
	N	12.0	37.5	70.5
S-3 (W)	T	63.0	123.0	163.5
	C	27.0	45.0	60.0
	N	36.0	78.0	103.5
S-4	T	30.0	52.5	111.0
	C	24.0	31.5	37.5
	N	6.0	21.0	73.5
S-5	T	9.0	15.6	59.4
	C	9.0	11.4	13.8
	N	0	4.2	45.6

VII. Potomac STP Long-Term BOD Survey Data - Summer 1978 (con't)

Date 9/25/78 (con't)		Days of Incubation		
		3	7	14
Station				
S-7	T	11.4	21.0	42.0
	C	11.4	16.2	20.4
	N	0	4.8	21.6
S-8	T	14.4	60.0	94.8
	C	9.6	11.4	15.6
	N	4.8	48.6	79.2

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