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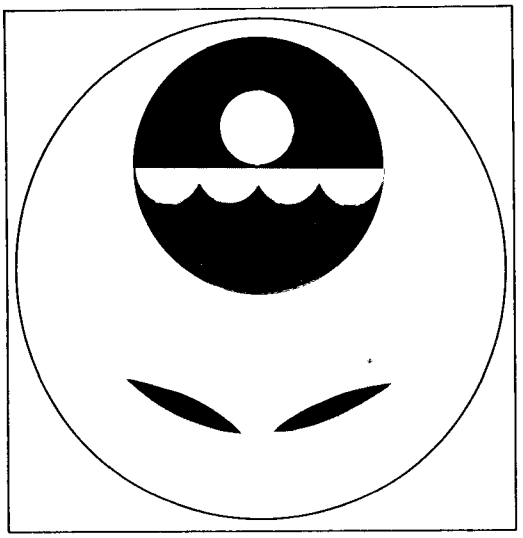

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SIMPLIFIED N.O.D. DETERMINATION*

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Biochemical oxygen demand (BOD) is a bioassay procedure concerned with the utilization of oxygen in the biochemical oxidation (respiration) of organic material. This test is one of the most widely used measures of organic pollution and is applied both to surface and waste waters. The standard method of BOD measurements adopted by APHA[1] is a five day test in which a water sample is maintained at 20°C in the dark and oxygen depletion is monitored. The five day incubation period was selected to maximize the oxygen demand associated with the oxidation of carbon compounds while minimizing the oxygen demand of autotrophic organisms. That portion of the BOD due to the respiration of organic matter by heterotrophic organisms is termed the carbonaceous oxygen demand and that portion involved with nitrification is termed nitrogenous oxygen demand. The desire to separate the NOD and CBOD results not only from the fact that the organisms responsible for these components have different nutrient requirements, but also because they differ in reaction rates, $\Delta O_2/\Delta$time; temperature coefficients; and tolerance to toxic materials. Nitrifying bacteria are in general slower growing[2]; more drastically affected by temperature[3]; and are more

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sensitive to materials as[4]: phenols; cresol; halogenated solvents; heavy metals; and cyanide. The organisms involved in the CBOD and NOD processes would therefore be expected to react differently to the same aquatic environment. The determination of the BOD components would better define the BOD test results and aid in extrapolating these results to the prediction of dissolved oxygen profiles in a body of water.

The purpose of this paper is to demonstrate that a simple procedure involving an inhibitor to nitrification, N-serve, could provide an accurate and precise measurement of nitrification occurring in the BOD test while not affecting the carbonaceous oxygen demand.

Nitrification

Nitrification is the conversion of ammonia to nitrate by biological respiration. This type of respiration is employed by seven genera of autotrophic nitrifyers.[5]

It should be noted that heterotrophic nitrification can also produce NO_2^- and NO_3^- by reactions that do not involve oxidation.[6] However, only Nitrosomonas spp and Nitrobacter spp are regularly reported by in situ nitrification studies.[2] Therefore, the treatment of nitrifying river samples with inhibitors specific to Nitrosomonas and Nitrobacter can be expected to stop all appreciable nitrification.[7]

The reactions involved in nitrification are as follows:

$$NH_4^+ + 1\tfrac{1}{2}\, O_2 \xrightarrow{\text{Nitrosomonas}} 2H^+ + NO_2^- + H_2O \qquad \text{Equation 1}$$

$$NO_2^- + \tfrac{1}{2}\, O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \qquad \text{Equation 2}$$

The stoichiometries of the nitrification reactions dictate that the conversion of 1 gram of nitrogen from ammonium to nitrite utilizes

3.43 grams of oxygen and the conversion of 1 gram of nitrite-nitrogen to nitrate-nitrogen involves the utilization of 1.14 grams of oxygen. However, nitrifying bacteria are autotrophic and as such utilize a portion of the energy derived from nitrogen oxidation to reduce CO_2, their primary source of carbon. The net result is a reduction in the amount of oxygen actually consumed. Short term, zero to five day, laboratory experiments[8,9,10] employing cultures of Nitrosomonas and Nitrobacter have related the depletion of oxygen to the production of nitrite and nitrate with the corresponding oxygen to nitrogen ratios of 3.22 and 1.11. However, in long term experiments, the decay of these organisms would be expected to exert an oxygen demand approximately equivalent to the oxygen originally generated, resulting in an overall relation not significantly different from 4.57.[11]

The equation used to calculate the NOD from the changes in nitrogen states upon incubation was:

$$NOD = 3.43\ (\Delta NO_2\text{-}N + \Delta NO_3\text{-}N) + 1.14\ (\Delta NO_3\text{-}N) \qquad \text{Equation 3}$$

where Δ = final - initial.

The potential NOD was calculated as:

$$\text{potential NOD} = 4.57\ (TKN) \qquad \text{Equation 4}$$

where TKN = (NH_3-N + Norg-N) and NO_2-N was insignificant.

The NOD was also measured by the difference in oxygen depletion in an unaltered sample and in a sample altered by the addition of the nitrification inhibitor, nitrapyrin.

Nitrification Inhibitor

The inhibitor used was formula 2533 Nitrification Inhibitor, a product of the Hach Chemical Company. The product consists of

2-chloro-6-(trichloromethyl) pyridine known as TCMP or nitrapyrin. This compound is plated onto a simple inorganic salt which serves as a carrier and is soluble in water. The DOW Chemical Company, Midland, Michigan, markets this chemical under the name N-Serve as a fertilizer additive.

Studies[12,13,14,15] using nitrapyrin suggest that it acts as a "biostat" at moderate concentrations to delay nitrification and aids in the retention of ammonia or urea fertilizers on crops by retarding conversion to the more highly leachable NO_3^-. TCMP is slowly biodegraded to 6-chloropicolinic acid which leaves the fields in their original state, with no further inhibition to nitrification. The advantage of this is that 20 to 30 day NOD assays may be performed without significant inhibitor contribution to the carbonaceous demand.[11,16]

Because of concern for the potential environmental impact resulting from extensive farm use, studies were performed on the toxicity of this material. These studies have revealed the inhibitor to be very selective and effective at stopping nitrification when used at a concentration of 10 mg TCMP/l.[11,16,17]

Experimental

A. NOD Synthetic Ammonia Experiment

1. 300 ml BOD bottles were weighed before and after the addition of water and found to be reliable to within 1%. They were used as volumetric flasks for all experiments.
2. Two ml of a solution of 0.150g glucose/l plus 0.150g glutamic acid/l were spiked into BOD bottles using a repipet.
3. Stale settled sewage was filtered through Kimwipes[18] and diluted. One ml was dispensed into each BOD bottle.
4. NH_3-N spikes were made using a 44.5 mg NH_4Cl-N/l stock solution.
5. The BOD bottles were then filled with APHA standard dilution water.[1]
6. Ammonia was assayed using a Technicon automated colorimetric phenate method.[19] Nitrate was determined using a Technicon automated cadmium reduction method and nitrite was assayed using a Technicon automated NEDA-diazotizing method.[19]
7. Dissolved oxygen (DO) was monitored using a YSI Model #57 meter and #5720 probe. DO measurements were made before and after incubation which was carried out in the dark at 20°C.
8. The nitrification inhibitor (Hach Chemical Co. #2533) was dispensed, using a powder dispenser, directly into the BOD bottles. This allowed quick and uniform additions of the inhibitor. Two sets of 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.

B. NOD Synthetic Nitrite Experiment

This experiment was identical to the synthetic ammonia experiment except spikes of $NaNO_2$ were substituted for NH_4Cl.

C. Synthetic Glucose Samples-Respiration Experiment

1. BOD bottles were spiked with approximately 3.0 ml of a 3.0g/l stock glucose solution using a repipet. Raw sewage influent was filtered through Kimwipes and diluted with distilled water. One ml of this seed was spiked into each bottle. TCMP was added to one-half of the bottles using the Hach powder dispenser and all bottles were filled with standard BOD dilution water.[1]

2. Oxygen was bubbled through the bottles using a Fisher gas dispersion tube and purified oxygen. The samples were then incubated in the dark at 20°C.

3. Initially and after different periods of incubation, samples were placed in a refrigerator at 4°C to stop bacterial activity. At the conclusion of the experiment bottles were assayed for glucose.[20] The samples were first filtered through a 0.45μ Millipore filter to remove bacteria. Four ml of each filtrate were placed into 125 ml Erlenmyer flasks; which had been chromic acid washed and muffle furnaced for 24 hrs. at 550°C. Repipets were then used to dispense 4 ml of phenol solution (25.0 gms/500 ml deionized water) and 20 ml of acid reagent (2.5 g hydrazine sulfate/500 ml conc. H_2SO_4). The acid reagent was added with swirling and the flasks were placed in a refrigerator at 4°C for 2 hours to cool. The absorbance

was read on a Varian 635 spectrophotometer using 5 cm quartz cells at 490 mμ. A 500 mg/l glucose stock solution was prepared and appropriate volumes were diluted with deionized water to generate standard curve solutions. The resultant standards were filtered and assayed as samples.

Calibration Curve Data

Glucose (mg/l)	Absorbance
0	0
2.5	0.125
5.0	0.252
10.0	0.485
15.0	0.660
20.0	0.832
25.0	1.068
30.0	1.230
35.0	1.469

slope = 0.0402

intercept = 0.0484

correlation coefficient = 0.999

4. Dissolved oxygen was measured directly in the BOD bottles using the YSI 5720 probe and the pH was determined using a Corning 110 research meter and electrode.

D. TCMP and the Measurement of Dissolved Oxygen

1. Electrode and Winkler Methods

a. A 20 liter carboy of deionized water was stirred with a magnetic stirring bar as water was slowly siphoned into 16 sets of four 300 ml BOD bottles and capped. This

procedure was repeated to generate 32 sets of 4 bottles.

b. TCMP was added to two bottles from each set using the Hach powder dispenser.

c. Two bottles (one with TCMP) were analyzed for DO via the Winkler azide modified method[1] using a Fisher Model 41 potentiometric titralyzer. An incubation period of 2 to 3 hours after the addition of the inhibitor and Winkler reagents was allowed prior to titration to enable potential reactions, which may have resulted in interferences, to occur.

d. The remaining two bottles of each set (one with TCMP) were analyzed by a YSI 5720 DO probe and #57 meter. This meter had been previously calibrated against the Winkler method as outlined in Standard Methods.[1]

2. Starch End Point - Azide Modified Winkler DO

a. Fourteen potassium biiodate standards, each with 3 ml of Fisher SO-P-340 stock biiodate solution (0.0250 N), were prepared as outlined in APHA Standard Methods[1] for Winkler Dissolved Oxygen measurements.

b. To seven of these TCMP and starch (Fisher T-138 thyodene) were added.

c. The samples were titrated with sodium thiosulfate solution using a Fisher Model 41 titralyzer in the manual mode and titrating to the disappearance of the blue color.

E. Potomac River Study

1. The BOD test employed was that outlined in Standard Methods APHA 14th edition.[1] The river water samples were stored at

4°C until analysis. Three-hundred ml of each sample was placed in each of two BOD bottles. The bottles were purged for 15 seconds using purified oxygen and a Fisher gas dispersion tube to obtain an initial DO of 10 to 15 mg/l. One bottle of each pair was dosed with the Hach Co. #2533 Nitrification Inhibitor.

2. Dissolved oxygen was measured immediately using a YSI 5720 DO probe and again after 20 days of incubation in the dark at 20°C.
3. TKN was analyzed on the unaltered river samples using a Technicon automated phenate method.[19]

F. Lehigh River Study

1. Samples were prepared in six replicate BOD bottles and two bottles of each set were spiked with TCMP using the Hach powder dispenser.
2. Dissolved oxygen was analyzed immediately and after several periods of incubation in the dark at 20°C using a YSI 5720 DO probe.
3. One bottle was sacrificed after each DO reading and assayed for NO_2^--N and NO_3^--N by the automated methods previously described.
4. Three classes of sample preparation were employed to allow for differences in sample character:
   a. River samples were unaltered.
   b. Industrial effluents with low level NH_3-N were seeded with 1 ml of stale settled sewage per 300 ml BOD bottle and correction blanks were carried through the experiment.

c. Sewage treatment plant effluent samples and industrial effluents with high levels of ammonia were diluted. Samples of October 4 were diluted by a factor of 30 and those of October 5 and 6 were diluted by a factor of 15 with seeded APHA diluted water. Correction blanks were carried through the experiment.

Results and Discussion

NOD Synthetic Ammonia Experiments

Initial experiments were performed on synthetic samples to establish the accuracy of the NOD determinations made using TCMP. The experiment consisted of spiking samples of APHA dilution water[1] with a glucose-glutamic acid solution, bacteria, and ammonia. The concentrations of ammonia, nitrate, and nitrite were then determined before and after incubation. The changes (Δ) in the states of nitrogen were determined and used to calculate the actual NOD wich had occurred (Equation #3).

The dissolved oxygen initially and finally present was determined in all bottles. The oxygen utilized in the inhibited bottles was taken as CBOD where as the depletion in the uninhibited bottles was taken as NOD plus CBOD. This NOD, signified as NOD-TCMP, was determined by the average difference observed between these sets.

The results of these experiments are presented in Table 1. A paired student's t-test of the nitrogenous oxygen demand established ($t=1.41$, $n=32$) at $\alpha=.05$ that there was no significant difference between these two methods of NOD determination. The average difference between the two methods was 0.3 mg/l NOD.

Table 1. NOD of synthetic ammonia samples as determined by analysis of nitrogen conversions and by measurement with TCMP

NH_3-N_i	NO_2-N_i	NO_2-N_f	ΔNO_2-N	NO_3-N_i	NO_3-N_f	ΔNO_3-N	3.43 (ΔNO_2-N +ΔNO_3-N)	1.14 (ΔNO_3-N)	NOD calc.	NOD TCMP
mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
.361	.053	.052	-.001	.023	.060	.037	.12	.04	.2	.2
		.0	-.053		.079	.056	.01	.06	.1	.5
		.052	-.001		.385	.362	1.24	.41	1.7	1.6
		.0	-.053		.079	.056	.01	.06	0.1	0.1
.637	.052	.00	-.052	.023	.676	.653	2.06	.74	2.8	3.1
		.052	.00		.614	.591	2.03	.67	2.7	3.0
		.049	-.003		.638	.615	2.10	.70	2.8	3.2
		.052	.00		.027	.004	.01	.00	0.0	0.0
.938	.049	.00	-.049	.018	.079	.061	.04	.07	0.1	0.4
		.061	.012		.855	.837	2.91	.95	3.9	3.0
		.029	-.020		.876	.858	2.87	.98	3.9	3.0
		.00	-.049		.046	.028	- .02	.03	0.0	0.0
1.460	.050	.00	-.050	.016	1.331	1.315	4.34	1.50	5.8	5.8
		.968	.918		.360	.344	4.33	.39	4.7	5.4
		.00	-.050		1.328	1.312	4.33	1.50	5.8	6.0
		.061	.011		.018	.002	.04	0	0	0
.462	0	0	0	0	.419	.419	1.44	.48	1.9	1.8
		0	0		.419	.419	1.44	.48	1.9	1.8
		.276	.276		.060	.060	1.15	.07	1.2	1.2
.619	0	0	0	0	.550	.550	1.89	.63	2.5	2.2
		0	0		.552	.552	1.89	.63	2.5	2.5
.921	0	.700	.700	0	.008	.008	2.43	.01	2.4	2.9
		0	0		.720	.720	2.47	.82	3.3	4.0
		0	0		0	0	0	0	0	0
		0	0		.823	.823	2.82	.94	3.8	4.1
1.705	0	0	0	0	1.467	1.467	5.03	1.67	6.7	6.8
		0	0		1.450	1.450	4.97	1.65	6.6	6.7
		0	0		1.489	1.489	5.10	1.70	6.8	6.7
		0	0		1.489	1.489	5.11	1.70	6.8	6.6
.240*	0	.187	.187	0	0	0	.64	0	.6	.5
.800	0	0	0	0	.800	.800	2.74	.91	3.7	4.0
1.630	0	.949	.949	0	.382	.382	4.57	.44	5.0	6.7

i = initial reading; initial nitrogen values are the average of three measurements
f = final reading; after 29 days of incubation
Δ = final-initial
* = ammonia ~ one-half that of APHA dilution water

The oxygen depletion was monitored over time for several of the samples and the DO data is presented in Figure 1. This work illustrates the potential use of the inhibitor in establishing deoxygenation constants for NOD separate from CBOD.

The seed source for these experiments was stale sewage. The sporadic growth of the nitrifyers observed during these experiments was largely corrected in later work by filtration and the use of more seed material.

NOD Synthetic Nitrite Experiment

The effect of TCMP upon the growth of nitrifying bacteria was tested using spikes of sodium nitrite into seeded APHA dilution water containing glucose/glutamic acid (Table 2). The calculated nitrogenous oxygen demand based on the measured changes in the states of nitrogen was significantly higher than that predicted by the use of TCMP when compared by a paired t test ($t=7.3$ at $\alpha=0.05$ & $n=15$). The changes in nitrite and nitrate were also measured in the TCMP spiked bottles, which allowed the calculation of the NOD occurring despite the presence of TCMP. This calculated error matched favorably (correlation coefficient = .92) with the average error actually observed between the calculated NOD in the samples and that measured using TCMP. The inhibitor had little inhibitory effect upon Nitrobacter spp, since all of the NO_2^--N in the spike was converted to NO_3^--N after 30 days of incubation.

Although the mechanism of its action is unclear, the inhibitory effect of nitrapyrin is apparently restricted to Nitrosomonas. This selectivity is advantageous in that it stops the process of nitrification at ammonia with little or no effect on urea hydrolysis[21], thus assuring an adequate nitrogen source for the heterotrophic bacteria contributing

Figure 1. Oxygen depletion of synthetic glucose/glutamic acid samples spiked with ammonium chloride

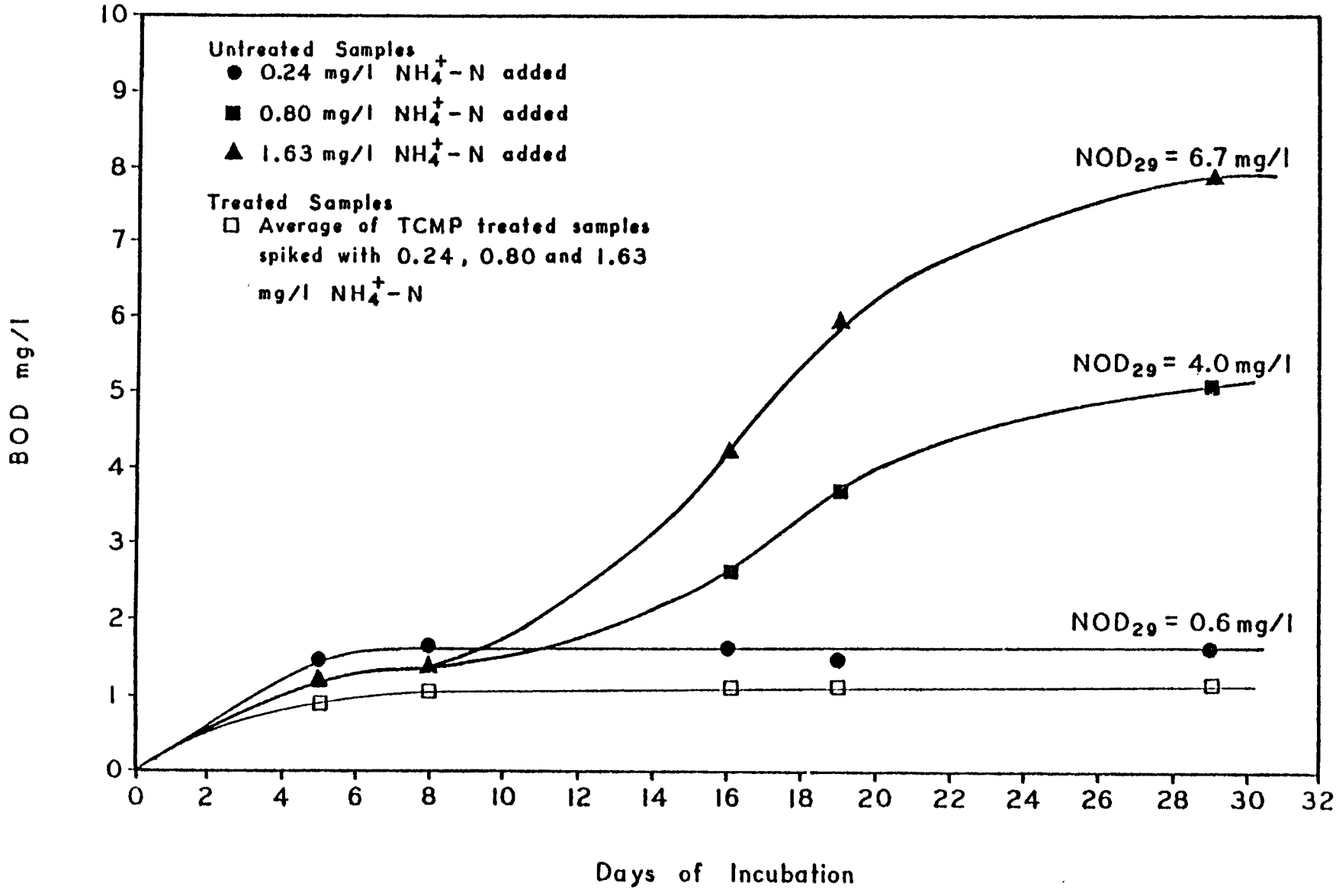


Table 2. NOD of synthetic nitrite samples as determined by analysis of nitrogen conversions and by measurement with TCMP

Uninhibited Samples

NH_3-N_i* mg/l	NO_2-N_i mg/l	NO_2-N_f mg/l	ΔNO_2-N mg/l	NO_3-N_i mg/l	NO_3-N_f mg/l	ΔNO_3-N mg/l	3.43 (ΔNO_2-N +ΔNO_3-N) mg/l	1.14 (ΔNO_3-N) mg/l	NOD calc mg/l	NOD TCMP mg/l	Av ob. err
.436	.456	0	-.456	0	.870	.870	1.42	.99	2.4	1.9	
	.456	0	-.456	0	.880	.880	1.45	1.00	2.5	2.0	
	.456	0	-.456	0	.864	.864	1.40	.98	2.4	1.9	
	.456	0	-.456	0	.880	.880	1.45	1.00	2.5	2.0	.5
	.934	0	-.934	0	1.363	1.363	1.47	1.55	3.0	1.5	
	.934	0	-.934	0	.984	.984	.17	1.12	1.3	0	
	.934	0	-.934	0	1.370	1.370	1.50	1.56	3.1	1.5	
	.934	0	-.934	0	1.401	1.401	1.60	1.60	3.2	1.8	1.
	1.408	0	-1.408	.045	1.828	1.783	1.29	2.03	3.3	1.9	
	1.408	0	-1.408	.045	1.880	1.835	1.46	2.09	3.6	2.1	
	1.408	1.50	.092	.045	0	- .045	.16	- .05	0.1	**(0)	1.4
	1.408	0	-1.408	.045	1.807	1.762	1.21	2.01	3.2	1.9	
	1.769	0	-1.769	.061	2.068	2.007	.82	2.29	3.1	0	
	1.769	0	-1.769	.061	2.101	2.040	.93	2.33	3.3	2.1	1.7
	1.769	0	-1.769	.061	2.117	2.056	.98	2.34	3.3	2.1	
	1.769	0	-1.769	.061	1.835	1.774	- .04	2.02	2.0	0.9	

TCMP Inhibited Samples

								NOD (calc. err.)	Ave. calc. err.
.436	.459	0	- .459	0	.468	.468	.03	.53	.6	
	.459	0	- .459	0	.468	.468	.03	.53	.6	
	.459	0	- .459	0	.468	.468	.03	.53	.6	
	.459	0	- .459	0	.468	.468	.03	.53	.6	.6
	.942	0	- .942	0	.984	.984	.14	1.12	1.3	
	.942	0	- .942	0	.974	.974	.11	1.11	1.2	
	.942	0	- .942	0	.974	.974	.11	1.11	1.2	
	.942	0	- .942	0	.984	.984	.14	1.12	1.3	1.3
	1.419	0	-1.419	.045	1.424	1.379	- .14	1.57	1.4	
	1.419	0	-1.419	.045	1.467	1.422	.01	1.62	1.6	
	1.419	0	-1.419	.045	1.455	1.410	- .03	1.61	1.6	
	1.419	0	-1.419	.045	1.614	1.569	.51	1.79	2.3	1.7
	1.787	0	-1.787	.056	1.835	1.779	- .03	2.03	2.0	
	1.787	0	-1.787	.056	1.835	1.779	- .03	2.03	2.0	
	1.787	0	-1.787	.056	1.829	1.773	- .04	2.02	2.0	2.0

* initial NH_3-N value is an average of 24 values with s.d. = 0.02
** omitted from calculation
i = initial reading; initial nitrogen values are the average of three measurements
f = final reading; after 30 days of incubation
Δ = final-initial

to the CBOD. The disadvantage of this selectivity is that Nitrobacter are not inhibited and NO_2^- will be oxidized to NO_3^-. This limitation generally represents a small error since the concentration of nitrite-nitrogen is generally much smaller than Total Kjeldahl Nitrogen in river water. Further, the demand associated with the NO_2-N initially present is 1.14/4.57 or one-quarter that associated with the TKN-N initially in the sample.

Synthetic Glucose Samples-Respiration Experiment

To directly determine the effect of TCMP on the rate of heterotrophic respiration, synthetic samples of APHA dilution water were spiked with glucose and seed bacteria. Several bottles were immediately assayed for glucose, dissolved oxygen, and pH, while others were incubated and later analyzed for these parameters. The results, compiled in Table 3, indicate that TCMP did not appreciably decrease the rate at which glucose was utilized. The potential problem with this interpretation is that these results may have been at steady state and therefore may not actually represent the rate at which steady state was achieved.

This experiment was again performed with the emphasis placed on determining when steady state occurred in bottles in which growth was observed. Glucose concentration, pH, and dissolved oxygen level were measured initially and periodically during incubation. The final levels determined were similar to those in the previous experiments. The results, compiled in Table 4 and Figure 2, indicate that: the glucose respiration rate was not significantly affected by TCMP; steady state was not established after 4 days of incubation; and suggested that the interpretation of the first experiment was valid.

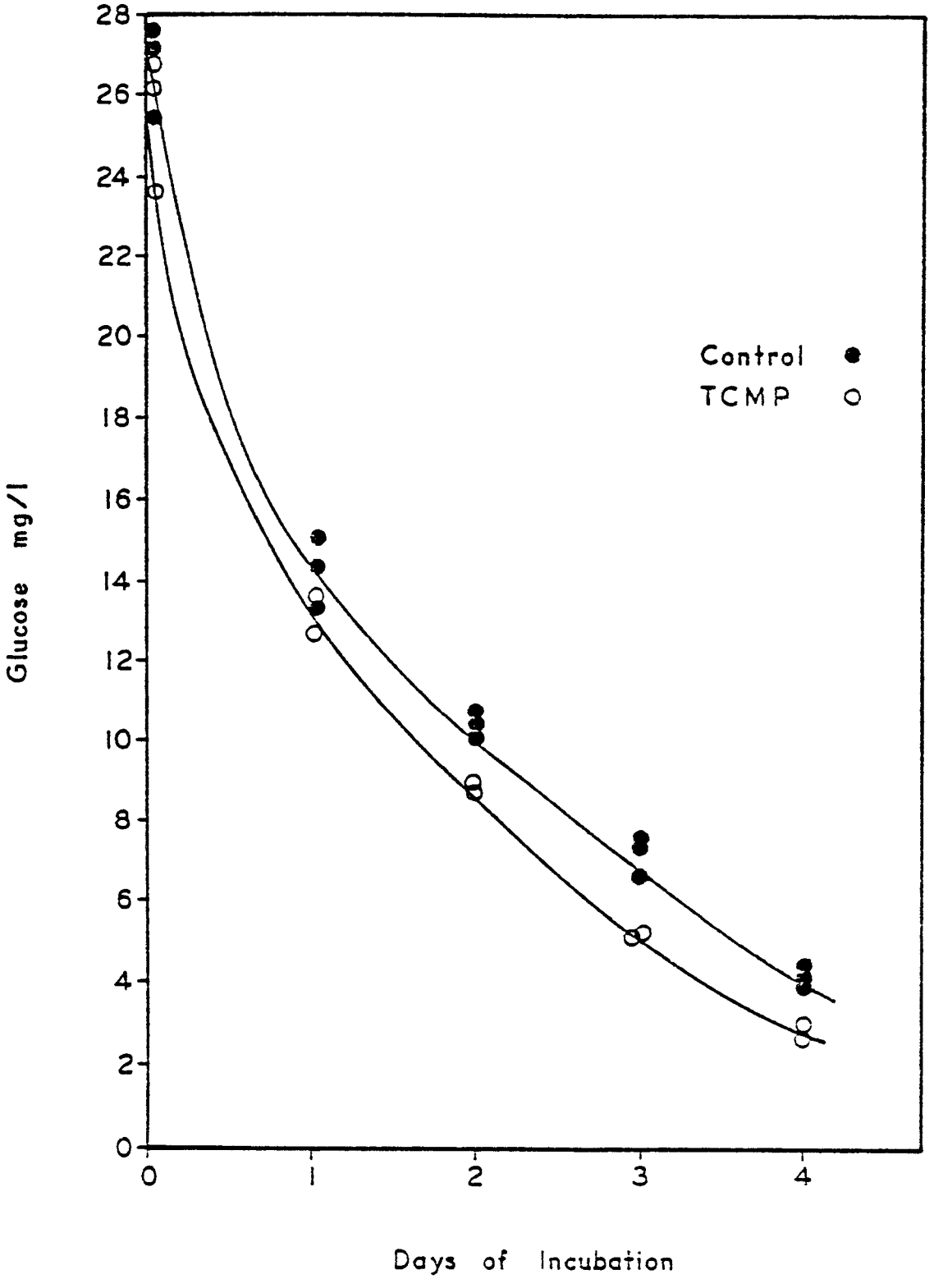
Table 3. Effect of TCMP on the utilization of glucose in synthetic samples

Day 0					Day 2			
TCMP Inhibited Sample	Glucose mg/l	Δ Glucose ave. mg/l	Ave. pH	Ave. D.O. mg/l	Glucose mg/l	Δ Glucose ave. mg/l	Ave. pH	Ave. D.O. mg/l
	27.3	0	6.8	15.5	7.3	20.8	5.9	6.9
	27.7				6.9			
	28.0				7.1			
	29.8				8.7			
					6.8			
Uninhibited Sample	29.6	0	6.7	15.5	8.5	21.9	5.7	6.9
	28.9				6.7			
	29.6				6.7			
	28.6				5.3			
	29.1				9.4			
Day 0					Day 2			
TCMP Inhibited Sample	28.0	0	6.5	13.2	9.9	16.5	6.0	5.4
	26.2				10.9			
	26.9				10.2			
	27.6				12.2			
	26.7				9.5			
	26.9				10.5			
	26.0				10.1			
	27.1				10.2			
	26.6				9.8			
	26.9				10.2			
Uninhibited Sample	28.0	0	6.3	13.2	11.0	16.5	5.8	6.4
	27.2				12.0			
	26.7				13.4			
	27.6				10.4			
	27.5				9.9			
	27.9				9.4			
	27.9				11.0			
	27.7				10.9			
	27.0							
	27.1							

Table 4. Rate of glucose respiration during inhibition of nitrification

	Glucose mg/l	Δ Glucose ave. mg/l	Ave. pH	Ave. D.O. mg/l
Day 0				
TCMP Inhibited Sample	23.6	0	6.7	15.7
	26.2			
	26.7			
Uninhibited Sample	27.1	0	6.8	15.6
	27.6			
	25.6			
Day 1				
TCMP Inhibited Sample	12.8	12.3	6.2	9.2
	13.6			
Uninhibited Sample	15.0	12.6	6.1	9.0
	14.3			
	13.2			
Day 2				
TCMP Inhibited Sample	9.0	16.5	6.1	7.3
	9.0			
Uninhibited Sample	10.4	16.2	6.0	7.3
	10.8			
	10.5			
Day 3				
TCMP Inhibited Sample	5.4	20.2	6.1	6.8
	5.2			
Uninhibited Sample	6.8	19.4	5.9	6.8
	7.7			
	7.6			
Day 4				
TCMP Inhibited Sample	3.0	22.3	5.9	5.7
	3.3			
Uninhibited Sample	4.3	22.4	5.9	6.0
	4.6			
	4.4			

Figure 2. Effect of the inhibitor on the rate of glucose respiration



Assays on TCMP treated samples consistently gave lower glucose values than the control samples. Bottles which were assayed immediately after preparation demonstrated this same pattern and this suggested that a chemical rather than a biological mechanism was involved.

It has been suggested that glucose is toxic to the growth of nitrifying bacteria.[22] It has also been suggested that the lack of nitrate and nitrite formation when glucose was added to actively nitrifying samples indicated a preference for glucose respiration by nitrifying bacteria.[23] The contribution of nitrifying bacteria to the overall glucose utilization measured in this study was probably insignificant since the nitrifyer population present in stale settled sewage collected during freezing weather is relatively sparce and an incubation time of 4 days or less is not sufficient for significant nitrifyer growth from this seed. Further, the acidic pH conditions which occurred during this experiment were not ideal for nitrifyer growth.

TCMP and The Measurement of Dissolved Oxygen

The effect of TCMP on dissolved oxygen measurements made using the azide modified Winkler potentiometric method and the polarographic electrode method was determined using inhibited and uninhibited deionized water samples. A paired t-test for the Winkler assayed bottles ($t=1.24$, $n=31$) revealed no significant affect on the Winkler DO method at a 95% confidence level. The average difference between TCMP treated and untreated bottles was 0.1 mg/l D.O. Similar results were obtained for the electrode method with a paired-t test result of 1.48 with $n=32$ and $\alpha=.05$.

Fourteen identical biiodate standards were also analyzed using the starch end point in the Winkler determination. The average difference in the titrant required for inhibited and uninhibited bottles was 0.03 ml, which indicated that TCMP did not affect the starch end point determination.

Potomac River Study

With the completion of the preliminary experimentation using synthetic samples, the use of TCMP in the determination of nitrogenous oxygen demand was tested using environmental samples. Potomac River samples were assayed for NOD during the summer of 1977. Nitrogen analyses were limited to TKN. The river historically[3] had a pattern of rapid biological activity and long term incubation was expected to yield essentially complete nitrification. The potential NOD was calculated from the TKN originally in the sample as: (TKN) x 4.57 = potential NOD. This compared favorably with the NOD measured using the nitrification inhibitor with an average difference of 0.9 mg/l. The results are compiled in Table 5. It should be emphasized that the potential NOD estimate from the TKN may not occur. However, the coefficient of linear correlation (r=0.88) suggested that after 20 days of incubation nitrification was generally complete and that the method utilizing TCMP gave reasonable NOD results.

Lehigh River Study

The inhibitor TCMP was also employed in an intensive nitrification study undertaken on the Lehigh River during fall 1977. The study included the determination of nitrogen states and dissolved oxygen depletion of unaltered and inhibited samples at several times during a long term incubation interval. The data are presented in Figure 3 and Tables 6 and 7 and reflect the different sample types and preparations involved:

Table 5. Comparison of the potential NOD and the actual NOD measured using TCMP (mg/l)

Potomac River Samples

NOD_{20} (TCMP)	Potential NOD (4.57)(TKN)	NOD_{20} (TCMP)	Potential NOD (4.57)(TKN)	NOD_{20} (TCMP)	Potential NOD (4.57)(TKN)
2.2	3.4	3.3	4.9	2.0	2.1
2.3	3.2	4.4	4.0	2.2	1.9
4.4	3.8	4.0	3.4	4.5	4.8
6.2	9.4	3.8	3.1	8.9	6.5
11.0	11.4	1.8	2.5	11.0	8.4
11.1	10.1	3.0	2.2	---	
4.0	6.2	2.7	2.2	3.6	3.3
3.6	4.9	4.0	4.1	3.0	2.1
3.0	3.9	4.4	6.3	2.5	1.3
2.6	2.8	3.4	5.3	3.0	1.8
1.4	2.1	4.1	5.0		
1.5	1.7	3.5	5.1		
2.6	2.7	6.6	5.8		
5.3	4.5	6.8	6.1		
5.6	5.5	4.2	3.7		
6.8	5.9	1.6	2.2		
5.5	4.1	1.2	1.8		
3.8	3.3	7.1	8.0		
2.4	2.8	4.7	6.4		
3.6	2.3	5.1	5.8		
LA	2.00	4.9	5.0		
1.4	1.6	4.3	4.4		
7.3	6.7	5.2	5.6		
4.8	5.8	4.9	5.5		
5.0	5.9	5.6	3.7		

linear correlation coefficient = 0.88 with n = 58

1. unaltered samples - river stations
2. seeded samples - industrial effluents
3. seeded and diluted samples - sewage treatment plants and industrial effluents

The average difference between the two NOD methods for river samples, with an oxygen demand of less than 10 mg/l, was 0.4 mg/l (n=128 and s.d.=0.349). The seeded effluent samples had an average NOD difference of 0.5 mg/l (n=42 and s.d.=0.463). The increased error and variability of the results reflects the added measurements of the seeded blank made for both nitrogen conversions and oxygen depletion determinations. The average NOD difference for seeded and diluted effluent samples was 5.7 mg/l (n=36 and s.d.= 7.83), which represented an average error of 10% for the NOD. The NOD error for diluted samples was amplified by the dilution factors of 15 and 30 necessary for the BOD analysis. A paired t-test of the nitrogenous oxygen demand over the combined 206 paired data sets established at the 95% confidence level (t=.75) that there was no significant difference in the results of the two NOD methods.

Station 031, an industrial effluent sample from a steel plant slag leachate was unique in that the outfall had an average BOD_{20-31} of 763 mg/l and an average initial TKN of 359 mg/l on the three days it was sampled. However, nitrate and nitrite were not formed after 31 days of incubation. The sample was analyzed for phenol and cyanide and was found to contain 35.9 mg/l total phenol and 50 mg/l cyanide. This suggested that the outfall was toxic to nitrifying bacteria, but not to the heterotrophic species present.

Figure 3. NOD of Lehigh River samples calculated from nitrogen analyses and measured using the inhibitor, TCMP

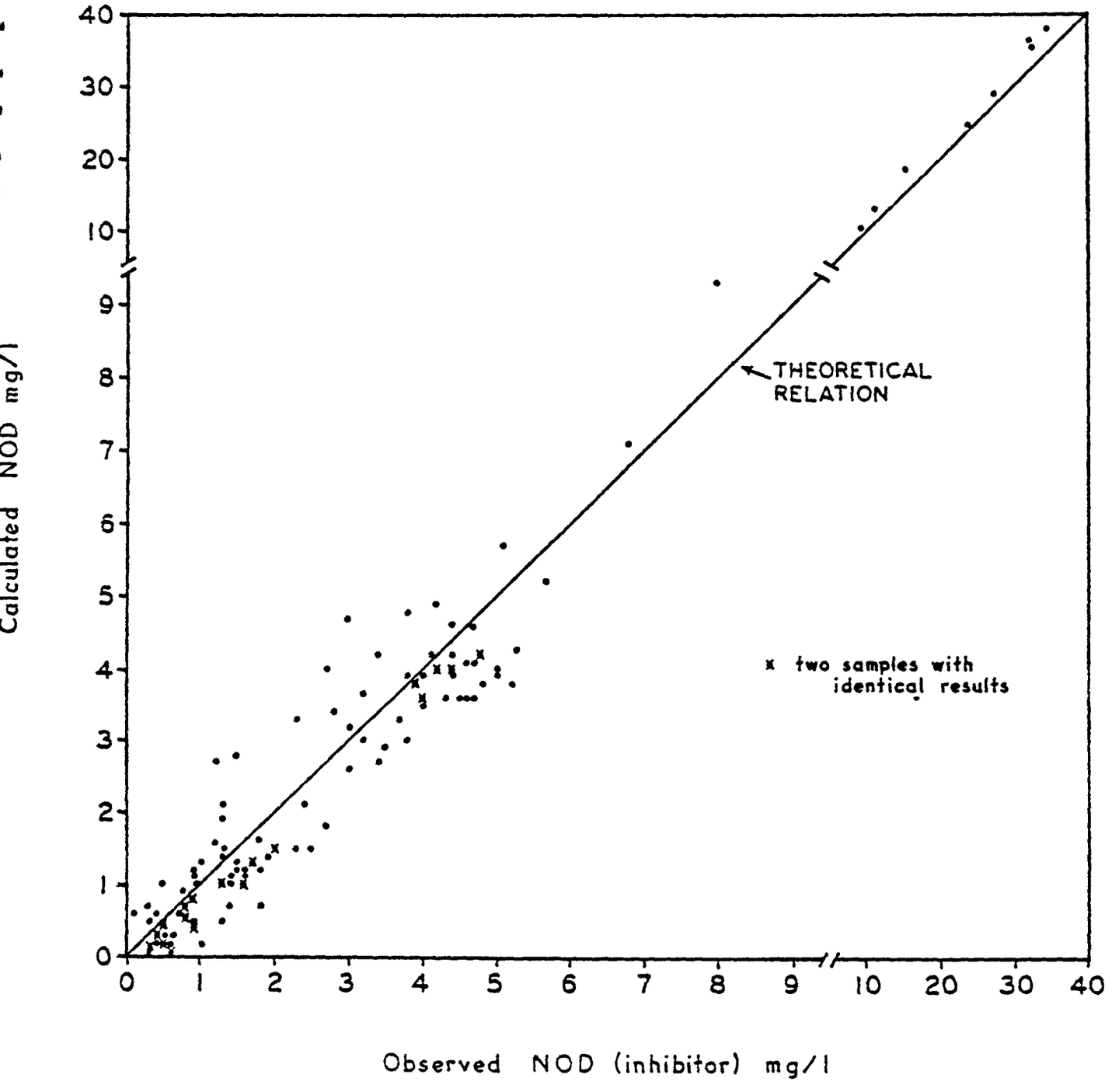


Table 6. NOD of seeded Lehigh industrial effluent samples determined by analysis of nitrogen conversions and by measurement with TCMP

Date-Sta.	Days of Incubation	NO_2-N_f mg/l	NO_2-N_i mg/l	ΔNO_2-N mg/l	NO_3-N_f mg/l	NO_3-N_i mg/l	ΔNO_3-N mg/l	BOD mg/l	3.43 (ΔNO_2-N +ΔNO_3-N) mg/l	1.14 (ΔNO_3-N) mg/l	NOD* calc. mg/l	NOD TCMP mg/l
10/05 005	6	.082	.048	.034	1.538	1.576	- .038	2.4	- .01372	- .04332	0	.7
Blast furnace	12	.710	.048	.662	1.690	1.576	.114	8.0	2.66168	.12996	2.8	4.8
	29	0	.048	- .048	3.04	1.576	1.464	9.8	4.85688	1.66896	6.5	5.7
006	6	.182	.061	.121	1.648	1.673	- .025	2.3	.32928	- .0285	.3	.7
Blast furnace	12	.100	.061	.039	2.040	1.673	.367	4.3	1.39258	.41838	1.8	1.6
cooling	29	0	.061	- .061	2.19	1.673	.517	5.8	1.56408	.58938	2.2	2.2
007	6	.178	.045	.133	1.762	1.749	.013	2.2	.50078	.01482	.5	.7
Blast furnace	12	.075	.045	.030	2.065	1.749	.316	4.4	1.18678	.36024	1.5	1.6
cooling	29	0	.045	- .045	2.23	1.749	.481	5.7	1.49548	.54834	2.0	2.0
008	6	.132	.045	.087	2.068	2.039	.029	2.1	.39788	.03306	.4	.3
Blast furnace	12	0	.045	- .045	2.240	2.039	.201	3.8	.53508	.22914	.8	1.3
cooling	29	0	.045	- .045	2.340	2.039	.301	5.4	.87808	.34314	1.2	1.8
010	6	.149	.050	.099	1.661	1.684	- .023	1.8	.26068	- .02622	.2	.9
Heat treatment	12	.057	.050	.007	1.793	1.684	.109	3.6	.39788	.12426	.5	1.4
cooling	29	0	.050	- .050	1.980	1.684	.296	5.5	.84378	.33744	1.2	2.3
012	6	.082	0	.082	1.598	1.584	.014	1.6	.32928	.01596	.3	.5
Scale pit	12	.294	0	.294	1.646	1.584	.062	3.6	1.22108	.07068	1.3	1.5
	29	0	0	0	1.940	1.584	.356	6.1	1.22108	.40584	1.6	2.8
014	6	.113	.041	.072	1.607	1.593	.014	1.9	.29498	.01596	.3	.4
Saw house	12	.143	.041	.102	1.737	1.593	.144	3.3	.84378	.16416	1.0	.6
cooling	29	0	.041	- .041	1.880	1.593	.287	5.9	.84378	.32718	1.2	1.7
10/06 005	6	.177	.054	.123	1.613	1.656	- .043	3.6	.27	- .05	.2	1.0
Blast furnace	12	.764	.054	.710	2.176	1.656	.520	8.2	4.22	.59	4.8	4.8
	31	0	.054	- .054	2.940	1.656	1.284	11.2	4.22	1.46	5.7	6.1

Table 6. (con't) NOD of seeded Lehigh industrial effluent samples determined by analysis of nitrogen conversions and by measurement with TCMP

Date-Sta.	Days of Incubation	NO_2-N_f mg/l	NO_2-N_i mg/l	ΔNO_2-N mg/l	NO_3-N_f mg/l	NO_3-N_i mg/l	ΔNO_3-N mg/l	BOD mg/l	3.43 (ΔNO_2-N + ΔNO_3-N) mg/l	1.14 (ΔNO_3-N) mg/l	NOD* calc. mg/l	NOD TCMP mg/l
10/06 006	6	.285	.065	.220	1.715	1.775	- .060	3.2	.55	- .07	.5	1.0
Blast furnace	12	.045	.065	- .020	2.115	1.775	.340	4.8	1.10	.40	1.5	1.3
cooling	31	0	.065	- .065	2.160	1.775	.385	6.4	1.10	.40	1.5	2.2
007	6	.224	.044	.180	1.796	1.756	.040	3.6	.75	.05	.8	1.5
Blast furnace	12	0	.044	- .044	2.120	1.756	.364	5.0	1.10	.41	1.5	1.3
cooling	31	0	.044	- .044	2.100	1.756	.344	6.6	1.03	.39	1.4	2.4
008	6	.153	.054	.099	2.067	2.026	.041	3.5	.43	.05	.5	.4
Blast furnace	12	0	.054	- .054	2.190	2.026	.164	5.3	.40	.19	.6	0.6
cooling	31	0	.054	- .054	2.190	2.026	.164	7.5	.40	.19	.6	2.0
010	6	.206	.058	.148	1.724	1.782	- .058	3.4	.30	- .07	.2	.9
Heat treatment	12	0	.058	- .058	1.970	1.782	.188	4.9	.50	.20	.7	0.8
cooling	31	0	.058	- .058	1.970	1.782	.188	7.0	.50	.20	.7	2.0
012	6	.217	0	.217	1.683	1.660	.023	3.1	.80	.03	.8	.8
Scale pit	12	0	0	0	1.970	1.660	.310	5.4	1.06	.35	1.4	1.4
	31	0	0	0	2.000	1.660	.340	8.0	1.17	.40	1.6	2.8
014	6	.172	.047	.125	1.678	1.703	- .030	3.4	.33	- .03	.3	.5
Saw house	12	0	.047	- .047	2.000	1.703	.297	5.1	.86	.34	1.2	1.5
cooling	31	0	.047	- .047	1.980	1.703	.277	7.0	.79	.32	1.1	1.6

*NOD = 3.43 (ΔNO_2 + ΔNO_3) + 1.14 (ΔNO_3)
where Δ=final - initial

Table 7. NOD of seeded and diluted STP and industrial effluent samples determined by analysis of nitrogen conversions and by measurement with TCMP

Date-Sta.	Days of Incubation	NO_2-N_f mg/l	NO_2-N_i mg/l	ΔNO_2-N mg/l	NO_3-N_f mg/l	NO_3-N_i mg/l	ΔNO_3-N mg/l	BOD mg/l	3.43 (ΔNO_2-N +ΔNO_3-N) mg/l	1.14 (ΔNO_3-N) mg/l	NOD* calc. mg/l	NOD TCMP mg/l
10/04	6	8.4	.27	8.13	4.89	4.44	.45	54	29.4294	.513	29.9	33
Allentown	12	13.74	.27	13.47	7.26	4.44	2.82	93	55.8747	3.2148	59.1	63
STP	29	0	.27	- .27	21.0	4.44	16.56	120	55.8747	18.8784	74.8	75
015	6	9.93	.45	9.48	2.10	1.41	.69	57	34.8831	.7866	35.7	46.5
Coke works	12	55.53	.45	55.08	2.67	1.41	1.26	204	193.2462	1.4364	194.7	189
	29	0	.45	- .45	60.6	1.41	59.19	264	201.4782	67.4766	269.0	249
031	6	0	.12	- .12	0	0	0	241.5	- .4116	0	0	0
Slag	12	0	.12	- .12	0	0	0	417.0	- .4116	0	0	0
leachate	29	0	.12	- .12	0	0	0	1203.0	- .4116	0	0	0
Bethlehem	6	12.72	.33	12.39	2.49	0	2.49	99	51.0384	2.8386	53.9	60
STP	12	21.33	.33	21.0	4.77	0	4.77	120	88.3911	5.4378	93.8	102
	29	0	.33	- .33	26.1	0	26.1	189	88.3911	29.754	118.1	115
10/05	6	1.395	.045	1.35	3.015	2.355	.66	21	6.8943	.7524	7.6	10.5
Allentown	12	11.94	.045	11.895	4.05	2.355	+1.695	64.5	46.6137	1.9323	48.5	48
STP	29	0	.045	- .045	15.99	2.355	13.635	90	46.6137	15.5439	62	52
015	6	1.125	.195	.93	.825	.825	0	4.5	3.1899	0	3.2	3.0
Coke works	12	26.4	.195	26.205	2.19	.825	1.365	64.5	94.5651	1.5561	96.1	64.5
	29	14.835	.195	14.64	13.665	.825	12.84	103.5	94.2564	14.6376	108.9	103.5
031	6	0	0	0	0	0	0	123	0	0	0	0
Slag	12	0	0	0	0	0	0	244.5	0	0	0	0
leachate	29	0	0	0	0	0	0	576	0	0	0	0
Bethlehem	6	1.515	.045	1.47	.330	.330	0	36	5.0421	0	5.0	19.5
STP	12	17.7	.045	17.655	2.79	.330	2.46	105	68.9945	2.8044	71.8	78
	29	0	.045	- .04	20.85	.330	20.52	129	70.2464	23.3928	93.6	81.0

Table 7. (cont.) NOD of seeded and diluted STP and industrial effluent samples determined by analysis of nitrogen conversions and by measurement with TOMP

Date-Sta.	Days of Incubation	NO_2-N_f mg/l	NO_2-N_i mg/l	ΔNO_2-N mg/l	NO_3-N_f mg/l	NO_3-N_i mg/l	ΔNO_3-N mg/l	BOD mg/l	3.43 (ΔNO_2-N +ΔNO_3-N) mg/l	1.14 (ΔNO_3-N) mg/l	NOD* calc. mg/l	NOD TOMP mg/l
10/06	6	7.77	.03	7.74	3.405	2.52	.885	47.0	29.58	1.01	30.6	32.0
Allentown	12	3.375	.03	3.345	12.195	2.52	9.675	79.5	44.66	11.03	55.7	55.5
STP	31	0	.03	- .03	15.57	2.52	13.05	106.5	44.66	14.88	59.5	60.0
015	6	7.515	.285	7.23	1.185	.810	.375	25.5	26.09	.4	26.5	25.5
Coke works	12	24.135	.285	23.85	2.685	.810	1.875	88.5	88.24	2.14	90.4	81.8
	31	0	.285	- .285	27.285	.810	26.475	121.5	90.81	30.18	121.0	115.5
031	6	0	0	0	0	0	0	121.5	0	0	0	0
Slag	12	0	0	0	0	0	0	256.5	0	0	0	0
leachate	31	0	0	0	0	0	0	510.0	0	0	0	0
Bethlehem	6	7.8	0	7.8	.57	.135	.435	64.5	28.25	.50	28.3	39.0
STP	12	10.335	0	10.335	9.585	.135	9.45	117.0	67.86	10.77	78.6	81.0
	31	0	0	0	19.92	.135	19.79	159.0	67.88	22.56	90.4	99.0

*NOD = 3.43 (ΔNO_2 + ΔNO_3) + 1.14 (ΔNO_3)
where Δ= final - initial

**LA = laboratory accident

Conclusions

The results of this study on synthetic, river, sewage treatment plant and industrial effluent samples suggested that:

1) TCMP was an effective inhibitor to nitrification. The inhibitor stopped the nitrification of ammonia by inhibiting the formation of nitrite.
2) TCMP did not inhibit the conversion of nitrite to nitrate.
3) TCMP did not inhibit the respiration of glucose.
4) TCMP did not significantly contribute to the CBOD even after 31 days of incubation at 20°C.
5) The determination of NOD using the difference in oxygen depletion in inhibited and uninhibited BOD bottles was quick and easy. This method did not involve the expensive equipment, nor time associated with the chemical analysis of nitrogen states to determine the NOD.
6) The inhibitor did not interfere with the determination of oxygen by the azide modified Winkler or electrode methods.
7) The inhibitor method yielded reliably accurate NOD determinations.

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