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STATE OF WASHINGTON
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MEMORANDUM
May 15, 1979

From: Bill Yake and Mike Morhous

To: John Glynn

Subject: Nitrification as a BOD Interference at Burlington STP

Introduction:

A significant issue pertaining to nitrification during the BOD test has been raised several times in Washington State. Most recently, this concern has been expressed by operating personnel at the Burlington Sewage Treatment Plant.

Fundamentally the issue is this: Should permit holders be responsible for nitrogenous oxygen demand (NOD) as measured by the traditional five-day BOD test? The question has numerous philosophical and practical ramifications.

Dave Lyons (Chief of Compliance, EPA), when contacted by this office, indicated that EPA is not presently considering a general ruling on the acceptability of nitrification-inhibited BOD_5 tests in meeting NPDES permit limitations. Their approach is that BOD_5 is what the standard BOD_5 test measures; and, in turn, that for which the permit holder is responsible. Problems with specific plants should be analyzed and "decisions made on a case-by-case basis".

This approach does not enjoy universal concurrence. Stu McKinsey (USGS, Portland) has done extensive work on BOD_5, NOD, and dissolved oxygen modeling on the Willamette River (Hines, *et al.*, 1977). His position is that for permit limitations and effluent (DMR) values to be useful for waste load allocation and stream modeling, they should be equivalent. Because nitrification in five-day BOD test is not controlled, results are not equivalent. He views nitrification as an "interference" in the five-day BOD test.

In addition, Dr. David Jenkins (Chairman, WPCF Standard Methods Committee) was contacted. The committee is slated to include a nitrification-inhibited BOD procedure in the next edition of Standard Methods. Dr. Jenkins' expressed opinion is that permit limitations should be based on the sum of nitrification-suppressed BOD_5 concentrations and the stoichiometric oxygen consumption of ammonia in the plant effluent. Thus the effects of both oxygen demands on the receiving waters would be considered.

Based on our own experience, we feel that the present approach has substantial deficiencies. Our most serious objection is that the present regulatory approach has the effect of penalizing plants which are achieving partial nitrification and discouraging plants from attempting to operate in a nitrifying mode. It is our belief that nitrification should generally be encouraged and that it is difficult enough to achieve even partial nitrification in most plants without discouraging nitrification through the regulatory process.

Nitrification minimizes effluent ammonia. This is desirable for two reasons. First, unionized ammonia is toxic to aquatic fauna. This toxicity is aggravated by high temperatures, low flows, and high pH's. These conditions are typical of receiving waters in the state (particularly Eastern Washington) during summer low flow. Effluent ammonia from the Pullman STP in southeastern Washington was found to contribute substantially to toxic conditions in the South Fork of Palouse River during low flow conditions (Bernhardt and Yake, 1979). Summer conditions are generally best for nitrification in treatment plants as in-plant temperatures are higher and decreased infiltration and inflow lead to longer in-plant retention times. The second reason to encourage the in-plant nitrification of ammonia is to minimize NOD in the receiving water. Although the rate of in-stream nitrification of ammonia varies dramatically depending on stream size and morphology, stream temperature, and the presence of nitrifying populations, the presence of a substantial dissolved oxygen sag in Weaver Creek was attributed almost entirely to nitrification of ammonia contributed by effluent from Battleground STP (Moore and Anderson, 1978). As more efficient organic removal is required and achieved, ammonia's role as an oxygen demanding substance will become proportionally greater. Hines, *et al.*, 1977, in an extensive study of the Willamette River system in Oregon concluded "These results indicated that point-source biochemical oxygen demand was no longer the primary source of dissolved oxygen depletion. Instead, the major causes of deoxygenation were nitrification in a shallow 'surface active' reach below Salem and an anomalous oxygen demand... in Portland Harbor."

It appears to us, therefore, that the issue presently raised by Burlington is one which, potentially, could have much broader implications than those pertaining to this specific plant. The issues of ammonia toxicity, in-stream NOD, permit limitations on BOD_5 and ammonia, waste load allocation, and the federal and state stances with respect to these issues are inter-related. Ultimately, these issues have a bearing on how we respond to Burlington's concerns and similar situations (i.e., Renton STP, Pullman STP [Bernhardt and Yake, 1979], Battleground STP [Moore and Anderson, 1978], etc.).

Background:

The Burlington Sewage Treatment Plant (STP) is an activated sludge plant of standard design. Jake VanPatton (head operator) believes that the plant experiences nitrification on a regular basis. This conclusion appears to be based on the discrepancy between his normal and inhibited BOD results. These results, in and of themselves, are probably insufficient to reach this conclusion. Without regular monitoring of influent and effluent inorganic nitrogen forms, it is difficult to determine the extent to which a facility is nitrifying its wastewaters. Sludge age at the plant is monitored and has varied from a high of about 20 days to the present age of about 9 days.

The plant has one unusual characteristic which may have a bearing on nitrification in the plant. Approximately 20 percent of the plant's 0.5 MGD flow is from the Whatcom Sewer District #5 (Lake Sammamish) lagoon system. This effluent travels about 15 miles to the Burlington plant. It is conceivable that nitrifying populations could either develop in this main if sufficient dissolved oxygen concentrations were maintained or originate in the lagoon and constantly reseed the Burlington STP.

Historically, the Burlington STP has experienced difficulty in meeting 85 percent removal and 30 mg/l limitations on effluent BOD_5. It has been the contention of operating personnel at Burlington that this difficulty is primarily due to the expression of nitrogenous oxygen demand (NOD) in the five-day BOD test. Mr. VanPatton has been instrumental in pursuing the question and has performed numerous duplicate BOD_5 determinations using both normal and nitrification-inhibited tests using Hach Nitrification Inhibitor 2533-TM.

In conjunction with a Class II facility inspection carried out on January 1, 1979, composite wastewater samples were collected and analyzed for a range of parameters. The results of these analyses are given in Table 1.

In addition, these effluent samples (specifically, chlorinated and unchlorinated effluent samples from DOE's compositor samplers and unchlorinated effluent from the treatment plant's composite sampler) were analyzed for five-day biochemical oxygen demand (BOD_5) using both the standard method and nitrification inhibition. Inorganic nitrogen concentrations (NH_3-N, NO_2-N, and NO_3-N) were also determined in both the initial sample dilutions and at the conclusion of the five-day incubation.

Table 1

Results of Class II Inspection (1/9/79) - Burlington STP

	DOE Sample Influent	DOE Sample Unchlorinated Effluent	STP Sample Unchlorinated Effluent	DOE Sample Chlorinated Effluent
COD (mg/l)	400	83	83	53
BOD_5 normal (mg/l)	95	44	52	30
BOD_5 inhibited (mg/l)	--	6	24	12
NH_3-N (mg/l)	21	8.9	7.6	8.2
NO_2-N (mg/l)	0.2	0.5	0.3	0.5
NO_3-N (mg/l)	0.1	2.8	3.5	2.8
T-Kjd-N (mg/l)	38	14	15	11
O-PO_4-P (mg/l)	5.8	7.4	7.1	7.4
Tot. Phosphorus (mg/l)	8.8	7.9	7.7	7.6
Tot. Alkalinity (mg/l)	140	--	--	95

Results and Discussion:

Based on the uninhibited (standard) BOD_5 test, the Burlington STP failed to meet both the 30 mg BOD/l and the 85 percent BOD removal limitations specified in its NPDES permit (see Table 1). Nitrification-inhibited BOD_5 tests were conducted using methyline blue as an inhibitor. The choice of methyline blue as an inhibitor was probably an error. We later learned that at low dissolved oxygen concentrations, methyline blue can act as an electron acceptor. This, however, probably did not introduce a significant error because the lowest five-day dissolved oxygen (DO) concentration used in the inhibited BOD calculations was 3.7 mg/l. All other five-day DO concentrations were greater than 6 mg/l. Based on the inhibited BOD test results, the 30 mg BOD/l limitation was being met for all three effluent samples. The unchlorinated effluent sample collected with the Burlington sampler showed only 75 percent BOD removal, while DOE samples indicated compliance with the 85 percent removal requirement.

Reviewing Table 1, it is also clear that the plant was partially nitrifying influent ammonia. Effluent concentrations were approximately 8 mg NH_3-N/l, 0.5 mg NO_2-N/l, and 3 mg NO_3-N/l.

The stoichiometric equations for the conversion of ammonia first to nitrite and ultimately to nitrate are:

		grams O_2 required for grams N oxidized	
1.	Nitrosification:		
	$NH_3 + 3/2\ O_2 \rightarrow NO_2^- + H_2O + H^+$	3.43	Formula 1
2.	Nitrification:		
	$NO_2^- + 1/2\ O_2 \rightarrow NO_3^-$	1.14	Formula 2
	Overall Reaction		
	$NH_3 + 2O_2 \rightarrow NO_3^- + H_2O + H^+$	4.57	Formula 3

Because nitrification produces hydrogen ions, alkalinity is destroyed. Idealized, the reaction is:

3. $CaCO_3 + 2H^+ \rightarrow H_2CO_3 + Ca^{++}$ Formula 4

Thus approximately 35 grams of $CaCO_3$ alkalinity is destroyed per gram of H^+ neutralized. Because 1 gram of H^+ is produced per 14 grams of NH_3-N, approximately (1/14)(35/1) = 2.50 grams of $CaCO_3$ alkalinity is destroyed per gram NH_3-N nitrified. A substantial drop in alkalinity through a treatment plant is, therefore, symptomatic of nitrification. This drop can be noted in Table 1.

Four normal versus nitrification-inhibited five-day BOD tests were run, as follows:

1. Seeded blank: Dilution water plus 1 ml of seed solution per liter of dilution water.

2. Unchlorinated effluent, DOE composite sample: Seeded. 100 ml sample per liter of sample dilution.

3. Unchlorinated effluent, STP composite sample: Seeded. 100 ml sample per liter of sample dilution.

4. Chlorinated effluent, DOE composite sample: Dechlorinated. Seeded. 100 ml sample per liter of sample dilution.

In addition, the BOD_5 of the seed was determined using both normal and nitrification-inhibited BOD_5 tests. This seed was settled raw influent from the Olympia STP. Results with and without methyline blue added were 1100 and 1140 mg/l, respectively. The effect of methyline blue on a normal (non-nitrifying) sample was therefore minimal.

Concentrations of NH_3-N, NO_2-N, and NO_3-N were determined on the sample dilution immediately after setup (zero-day) and after incubation (five-day). The results of these tests are presented in Tables 2 through 5.

Review of the results in Tables 3 through 5 clearly indicate that nitrification was taking place in uninhibited tests of both chlorinated and unchlorinated Burlington STP effluent. Methyline blue appears to have effectively inhibited nitrification.

Additionally, it can be noted that in all cases except the inhibited seeded blank, total inorganic nitrogen decreased. Because five-day dissolved oxygen concentrations remained above 2.0 mg/l, it is unlikely that denitrification occurred. We surmise that inorganic nitrogen was assimilated by the actively growing bacterial population, and thus converted to organic nitrogen.

This assumption appears to be reasonable. It is generally accepted that approximately 0.50 to 0.55 grams of biomass (bacteria) are generated per gram of BOD_5 consumed. This is often referred to as sludge yield. Additionally, nitrogen makes up approximately 14 percent of the dry weight of bacteria. Thus one would expect that the incorporation of inorganic nitrogen could be estimated by Equations 1 or 2.

Equation 1

$$\text{grams N incorporated} = (\text{grams } BOD_5 \text{ consumed}) \times \left(.50 \frac{\text{grams biomass produced}}{\text{grams } BOD_5 \text{ consumed}}\right) \times \left(.14 \frac{\text{grams organic N}}{\text{grams biomass}}\right);$$

$$\text{or grams N incorporated} = (.070) \times (\text{grams } BOD_5 \text{ consumed})$$

Equation 2

$$\text{grams N incorporated} = (\text{grams } BOD_5 \text{ consumed}) \times \left(.55 \frac{\text{grams biomass produced}}{\text{grams } BOD_5 \text{ consumed}}\right) \times \left(.14 \frac{\text{grams organic N}}{\text{grams biomass}}\right);$$

$$\text{or grams N incorporated} = (.077) \times (\text{grams } BOD_5 \text{ consumed})$$

Figure 1 presents a test of this hypothesis. The amount of inorganic nitrogen which disappeared in each test is plotted against the dissolved oxygen depletion over five days. In these tests, 5-day DO depletion is equivalent to the "BOD_5 consumed" in equations 1 and 2. The best linear relationship for these points was determined using least squares. The equation of this line is:

Equation 3

$$\text{grams N incorporated} = (.101) \times (\text{grams } BOD_5 \text{ consumed}) - .076$$

Table 2 - Seeded Blank Results

Parameter	Normal*		With M.B.**	
	0-day	5-day	0-day	5-day
NH_3-N mg/l	0.59	0.57	0.56	0.80
NO_2-N mg/l	<0.01	<0.01	<0.01	<0.01
NO_3-N mg/l	<0.01	<0.01	<0.01	<0.01
Tot. In.-N^+ mg/l	~0.60	~0.58	~0.57	~0.81
BOD_5 mg/l		0.1		0.1

Table 3 - DOE Unchlorinated Effluent Results

Parameter	Normal*		With M.B.**	
	0-day	5-day	0-day	5-day
NH_3-N mg/l	1.40	0.10	1.40	1.20
NO_2-N mg/l	0.10	0.30	0.20	0.01
NO_3-N mg/l	0.10	0.78	0.40	0.27
Tot. In.-N^+ mg/l	1.60	1.18	2.00	1.48
BOD_5 mg/l		44		6

Table 4 - Burlington STP Unchlorinated Results

Parameter	Normal*		With M.B.**	
	0-day	5-day	0-day	5-day
NH_3-N mg/l	1.50	0.05	1.30	1.10
NO_2-N mg/l	0.20	0.26	0.10	0.01
NO_3-N mg/l	0.15	0.80	0.20	0.34
Tot. In.-N^+ mg/l	1.85	1.11	1.60	1.45
BOD_5 mg/l		52		24

Table 5 - DOE Chlorinated Effluent Results

Parameter	Normal*		With M.B.**	
	0-day	5-day	0-day	5-day
NH_3-N mg/l	1.70	0.31	1.40	1.30
NO_2-N mg/l	0.10	0.69	0.10	0.07
NO_3-N mg/l	0.15	0.52	0.15	0.18
Tot. In.-N^+ mg/l	1.75	1.52	1.65	1.55
BOD_5 mg/l		30		12

*Unsuppressed BOD_5 test.

**Nitrification-suppressed with Methyline Blue.

+Total Inorganic Nitrogen (NH_3-N + NO_2-N + NO_3-N).

Based on Figure 1, it appears reasonable to assume that most, if not all, of the loss in inorganic nitrogen can be accounted for by conversion to organic nitrogen by the bacterial growth in the incubating BOD test solutions.

If we assume that this nitrogen was assimilated as ammonia, we can calculate the theoretical NOD exerted in the uninhibited tests. This value is generated by determining the mg O_2/l required to fully nitrify the 0-day mix and subtracting the mg O_2/l required to nitrify the 5-day mix. The 0-day mix is corrected to account for microbial conversion of NH_3-N using the best fit line generated in Figure 1 (Equation 3).

The difference in dissolved oxygen required to nitrify the 0- and 5-day mixes is then multiplied by the dilution factor (in this case, 10). Results are given in Table 6. These theoretical results are compared to the actual differences between normal and nitrification-inhibited tests.

Table 6 Theoretical versus Experimental Nitrogenous Oxygen Demand in Burlington STP Effluent

Sample	Theoretical NOD	Experimental NOD
DOE Unchlor. Eff.	33.8	38 mg/l
Burlington Unchlor. Eff.	39.1	28 mg/l
DOE Chlor. Eff.	40.1	18 mg/l

Agreement between calculated and experimentally determined NOD's is fair. Some of the disagreement can be accounted for by the fact that 0-day and 5-day nutrient values were determined from replicate dilutions.

The results of these tests appear to confirm contentions that nitrification was largely responsible for the plant's inability to meet NPDES permit limitations. In reviewing the results, it is apparent that a significant portion of the ammonia in the BOD test solution is contributed by the ammonium chloride (NH_4Cl) added to the dilution water as a nutrient source. The addition of NH_4Cl is specified by both Standard Methods and DOE's "Laboratory Test Procedure for Biochemical Oxygen Demand of Water and Wastewater." Dilution water is prepared to contain 0.445 mg NH_3-N/l. Table 7 presents the maximum error which could be attributable to nitrification of this added ammonia at various dilutions.

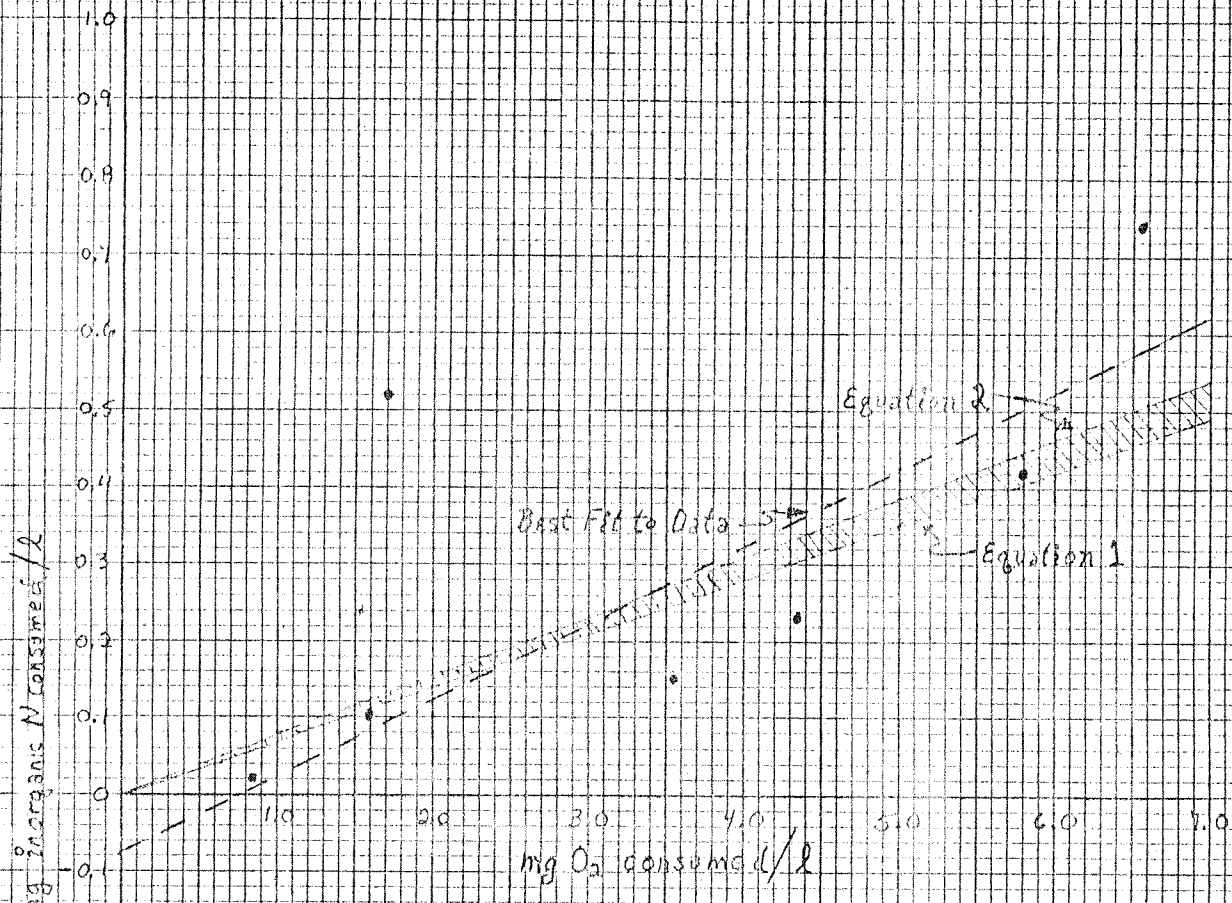

FIGURE 1. INORGANIC N. CONSUMPTION VS. O_2 CONSUMPTION

Table 7 Maximum BOD Test Error
Attributable to Nitrification of Added Ammonia

Sample Dilution (DF) ml sample: ml sample dilution	NH_3-N from Added NH_4Cl mg/l	NOD[1] if Fully Nitrified mg/l	BOD[2] Attributable to NOD of NH_4Cl mg/l
1:1	0	0	0
1:2	.223	1.02	2.04
1:5	.356	1.63	8.14
1:10	.401	1.83	18.3
1:20	.423	1.93	38.6

1) 4.57 mg O_2 consumed/mg NH_3-N oxidized.

2) (NOD)·(DF), where DF is the dilution factor in BOD calculations.

From Table 7 it is apparent that at higher dilutions, the interference caused by the nitrification of ammonia added to the dilution water could induce a substantial error.

Conclusions:

1. The results of these tests indicate that at the time of sampling, nitrification during the 5-day BOD test was preventing the Burlington STP from meeting NPDES permit limitations.

2. Nitrification occurred in both unchlorinated and chlorinated effluent samples. It appears, therefore, that chlorination adequate to meet fecal coliform limitations is not sufficient to eliminate nitrifying organisms.

3. Nitrification occurred in effluent samples collected with both DOE samplers and the Burlington STP samplers. DOE samplers and collection hoses are well cleaned before each use. Nitrifying populations were, therefore, probably contributed primarily by the effluent itself. It is, nonetheless, good practice to clean all sampling lines, collection equipment, and sampling jugs frequently to remove side wall growth and contamination.

4. Inorganic nitrogen (NH_3-N + NO_2-N + NO_3-N) disappeared during the 5-day BOD tests. This disappearance was attributed to the conversion of inorganic to organic nitrogen by micro-organisms incubated on the test solution. This conversion was generally proportional to oxygen consumption during the 5-day test.

5. At high dilution factors, the ammonia added as NH_4Cl to the dilution water could result in substantial increases in calculated BOD's if the ammonia is nitrified during the 5-day test.

Possible Solutions (Options):

1. The frequent monitoring of effluent inorganic nitrogen forms would permit determination of how persistent partial in-plant nitrification is at Burlington. This, in conjunction with normal and nitrification-inhibited effluent BOD tests, would allow better definition of the scope of the problem.

2. The frequent cleaning of sampling lines and equipment has already been instituted and should control one possible source of nitrifying populations. It appears, however, that during the sampling period addressed in this memo, the effluent contributed nitrifying populations sufficient to cause interference in the 5-day BOD test.

3. The questions raised in this memo regarding the responsiblity of permit holders for effluent NOD could be addressed either on a "case-by-case basis" or on a statewide basis. Several possible options are immediately apparent:

   a. Status quo. Require permit holders to be responsible for all effluent oxygen demand as measured by the 5-day BOD test. This penalizes plants which achieve partial nitrification, and leads to inequivalent results as reported on DMR's or when used for stream-load allocations or dissolved oxygen stream modeling.

   b. Allow plants which document nitrification in BOD tests to eliminate NH_4Cl from the dilution water if it can be shown or reasonably ascertained that sufficient inorganic nitrogen is available in the effluent. This would at least eliminate the problem of making the dischargers responsible for ammonia added to dilution water which in no way reflects the oxygen demand of their effluents.

c. Allow plants which document nitrification in BOD tests to report nitrification-inhibited BOD_5 results. It appears that this test will be included in the upcoming 15th Edition of Standard Methods. At the present time, it appears that 2-chloro-6-trichloro-methylperidine (and possibly allothiourea) will be specified as approved nitrification inhibitors. Plants determining nitrification-inhibited BOD_5 results should use one of these two chemicals. Reporting inhibited results would make BOD_5 results more consistent, as typical (non-nitrifying) effluents display no nitrification in the BOD test until well after the 5th day.

d. Change oxygen demand permit requirements for all plants to those similar to Dr. Jenkins' suggestions (i.e., report sum of nitrification-inhibited BOD's plus stoichiometric oxygen demand of effluent ammonia). This makes all plants responsible for both major types of oxygen-demanding substances and would probably require raising the current 30 mg/l requirement on oxygen-demanding substances in effluents. To implement this suggestion might require extended negotiations with USEPA. Nonetheless, it appears to be the most theoretically sound approach short of conducting detailed waste-load allocations on all plants and receiving waters. This option would also require permit holders to monitor effluent ammonia.

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