EFFECT OF TRICHLOROMETHYL PYRIDINE ON CARBONACEOUS BIOCHEMICAL OXYGEN DEMAND IN WASTEWATER

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ABSTRACT

A series of tests was conducted to investigate the effect of the nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine (TCMP), on carbonaceous biochemical oxygen demand (CBOD) measurements. Tests were performed using pure TCMP, HACH Formula 2533 (TCMP + sodium sulfate), and sodium sulfate alone. The study was conducted using the standard dilution BOD procedure and an aerobic respirometric system. In some cases, nitrogen species, TKN, NH_3-N, NO_2^--N, and NO_3^--N, were measured to verify that nitrification had occurred. The study uncovered essentially no evidence that TCMP or HACH Formula 2533 inhibited the carbonaceous biochemical oxygen demand reaction in either dilution or respirometric methods. When the BOD with TCMP was less than the BOD without TCMP, the differences could be attributed to nitrification. Also, the tests revealed that sulfate alone caused a delay in the onset of nitrification but did not adversely affect the CBOD. Suspended solids in the wastewater did not affect the impact of TCMP on CBOD or NOD reactions.

KEY WORDS

Biochemical oxygen demand, BOD, CBOD, nitrification, nitrification inhibition, carbonaceous BOD

INTRODUCTION AND OBJECTIVES

Background: The Biochemical Oxygen Demand (BOD) test is the most commonly used method for measuring the amount of oxygen-demanding material in wastewater. This bioassay uses microorganisms to indirectly measure the amount of biodegradable organic and reduced nitrogenous material, each of which exerts an oxygen demand. Specifically, the oxygen consumption associated with organic material is referred to as carbonaceous BOD (CBOD) and the oxygen consumption associated with the oxidation of reduced nitrogen species is defined as nitrogenous oxygen demand (NOD).

Historically, the BOD test has been conducted with the knowledge that nitrification can occur within the standard five-day test period when measuring BOD of secondary effluents. As a result of this interference, the 15th edition of Standard Methods (1981) recommended the use of a nitrification inhibitor – specifically 2-chloro-6 (trichloromethyl) pyridine (TCMP) – in BOD tests of secondary effluents and stream samples (Young et al., 1981). EPA subsequently allowed the use of nitrification inhibition, and this practice has been adopted by a number of states when writing discharge permits and evaluating the performance of biological treatment processes. Some states also require nitrification inhibition in BOD tests with raw and primary effluents and industrial wastes when inhibitors are used to eliminate nitrification in BOD tests of final effluents. The reasoning generally has been based on the assumption that if nitrification occurs in the BOD tests of raw wastewaters and primary effluents, it must be inhibited for appropriate calculation of treatment plant efficiency.

In spite of the many advantages of using TCMP as a nitrification inhibitor, there have been claims that it also may inhibit the carbonaceous reaction in the dilution BOD test. Specifically, Albertson (1993, 1995) claimed that TCMP adversely affects $CBOD_5$ measurements when used in BOD tests with raw and primary effluents. His claims were based on observations in operating treatment plants that $CBOD_5$/COD ratios were substantially lower than historical ratios of conventional BOD_5/COD ratios. The

Aleem, 1965). Because hydroxylamine is not a critical intermediate in carbonaceous metabolic reactions, low concentrations of TCMP were not expected to interfere with the carbonaceous BOD reaction. This specificity was the basis for selecting TCMP for inhibiting nitrification in the BOD test. This lack of toxicity to carbonaceous reactions was verified in tests conducted by Young (1973) and Slayton and Trovato (1979) for TCMP concentrations up to 20 mg/L. Additional tests have shown no adverse impact on the carbonaceous reaction when using three forms of TCMP to inhibit nitrification in primary effluent samples (Young, 1983) (Figure 2).

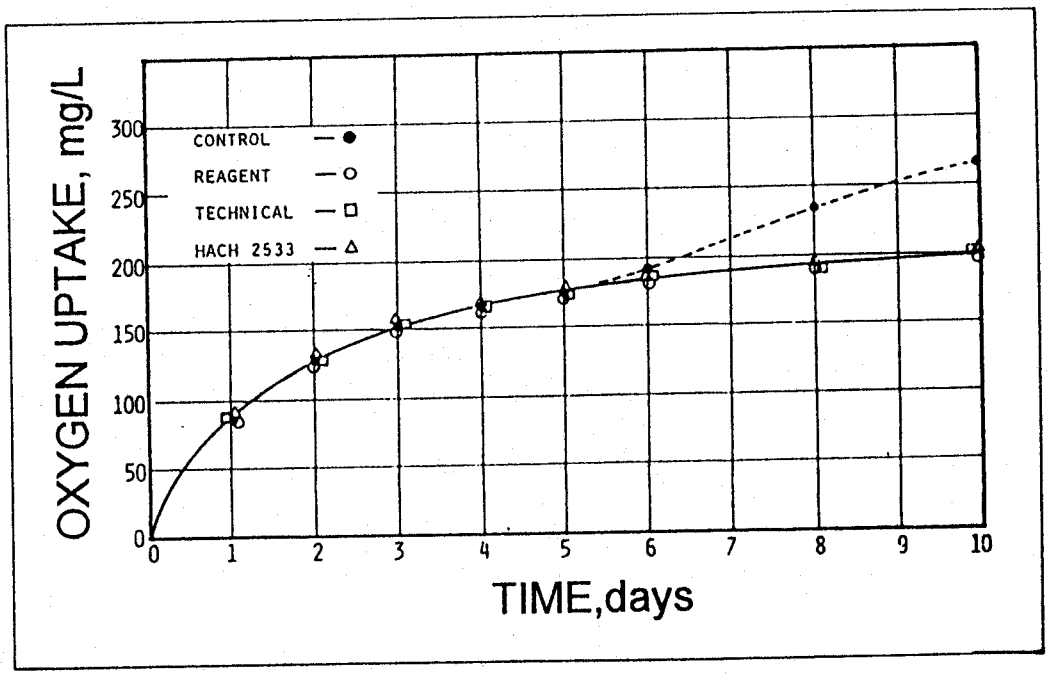

Figure 2. Comparison of oxygen uptake of primary effluent samples when using three forms of TCMP as a nitrification inhibitor (From Young, 1983).

Recent inter-laboratory tests conducted by EPA have shown consistently lower results in GGA controls for BOD tests as summarized in Table 1. The consistency of such occurrence suggests that some bias existed in these tests when using TCMP for nitrification inhibition. While no tests have been conducted to explore the cause of this effect, tests conducted by others do not show this effect (Table 1). In fact, 5-day BOD measurements for the glucose-glutamic acid check more commonly are above 200 mg/L. Kenney (unpublished data, 1986) conducted tests indicating that too little amount of seed produced low

Table 1. Sampling of measurements of five-day BOD of glucose-glutamic acid quality control checks.

SOURCE	GGA Mean Response	Standard Deviation	No. of Measurements
US EPA, 1986: BOD	198	30.5	Inter-laboratory tests: 2 to 112 Laboratories (No nitrification inhibition)
City of Cedar Rapids, Iowa, 1993: CBOD	228	9.82	24 measurements (Using TCMP as a nitrification inhibitor)
Water Quality Institute, Hoersholm, Denmark, 1992: CBOD	210	20	95 Laboratories (Using ATU as a nitrification inhibitor)
USEPA, 1996: BOD CBOD	188.3 162.2	32.7 30.6	46 Laboratories 21 Laboratories (Using TCMP as nitrification inhibitor)

GGA results in both the absence and presence of TCMP (Figure 3). Increasing the amount of seed organisms brought the tests into compliance with essentially no significant difference between inhibited and un-inhibited samples. Tests conducted by Manoharan (1992) showed no adverse effect of TCMP on raw wastewaters or treated effluents through nine days of incubation when using either natural seed or Polyseed (Product of Polybac Corp., Bethlehem, PA) (Figure 4).

Objectives: Because of the importance in resolving conflicts in observations with respect to the effect of TCMP on CBOD reactions, the authors conducted a series of tests designed specifically to investigate whether the use of 2-chloro-6-(trichloromethyl) pyridine for nitrification inhibition creates a problem in analyzing the carbonaceous BOD_5 of industrial wastewater, raw domestic sewage, and primary effluents (Reilly, 1995; Reilly and Young, 1995). Tests included the following variables and conditions:

- oxygen demand measurements using the standard dilution BOD and respirometric methods;
- varying concentrations of TCMP, HACH Formula 2533, and sodium sulfate alone for nitrification suppression;
- the presence of sodium sulfate to determine whether it competes with oxygen as an electron acceptor or inhibits nitrification; and
- varying the concentration of suspended solids to determine their influence on the impact of TCMP on carbonaceous BOD.

METHODOLOGY

The test plan initially concentrated on the use of wastewater from the Louis Rich Company plant in West Liberty, Iowa, because of their concern for the accuracy of CBOD tests at their facility and the impact of nitrification on their sewer surcharge fees. Wastewater from the University Park wastewater treatment plant in State College, Pennsylvania, was included in the later phases of the study. The test plan consisted of a number of runs in which test parameters were varied to further identify problem areas and trends. Both respirometric methods and the standard dilution BOD method were used in most tests to measure oxygen demand (Standard Methods, 1995). In a number of cases, nitrogen species were analyzed to verify the occurrence of nitrification. Seed was obtained from both the plant receiving the industrial wastewater and from the University Park wastewater treatment plant. Generally, the seed consisted of a mixed liquor sample diluted four-fold that was aerated using a magnetic stirrer until used. Tests runs included a control, samples with pure TCMP added (Nitrapyrin, Product of Dow Chemical Co., Midland, Michigan), samples receiving a dose of commercial nitrification inhibitor (Formula 2533: 2% TCMP and 98% sodium sulfate, HACH Co., Loveland, CO), and samples receiving sodium sulfate alone in amounts equal to that in the HACH 2533 formulation.

Respirometer tests were conducted using an AER-200 direct input aerobic respirometer system (Challenge Environmental Systems, Fayetteville, AR). The respirometer system was operated in a temperature controlled room at 25°C. Nutrients and minerals were added to support biological growth and a buffer was used to control pH (Standard Methods, 1995). The respirometer test setup included triplicate samples in which 50 mL of wastewater and five milliliters of seed were added to a 250 mL test reactor. Dilution tests were conducted using standard procedures (Standard Methods, 1995). Dilution tests were conducted by adding 1.0, 1.5, and 2.0 mL of wastewater plus one milliliter of seed to individual BOD bottles followed by filling with BOD dilution water which included appropriate amounts of nutrients and buffers. Seed controls were included with all runs. Nitrification inhibitors and sodium sulfate were weighed individually and added to each bottle. The bottles were then plugged with glass stoppers and capped to achieve an air-tight seal and capped to prevent evaporative losses. Dissolved oxygen was measured using a dissolved oxygen probe and meter (60 second BOD System, Wheaton Scientific, Millville, NJ). Initial DO measurements were taken immediately after the sample was prepared. Subsequent DO readings were taken at intervals throughout 5 to 20-day periods of incubation. Seed corrections were made when necessary. The dilution bottles were kept in a dark, thermostatically controlled incubator at 20°C.

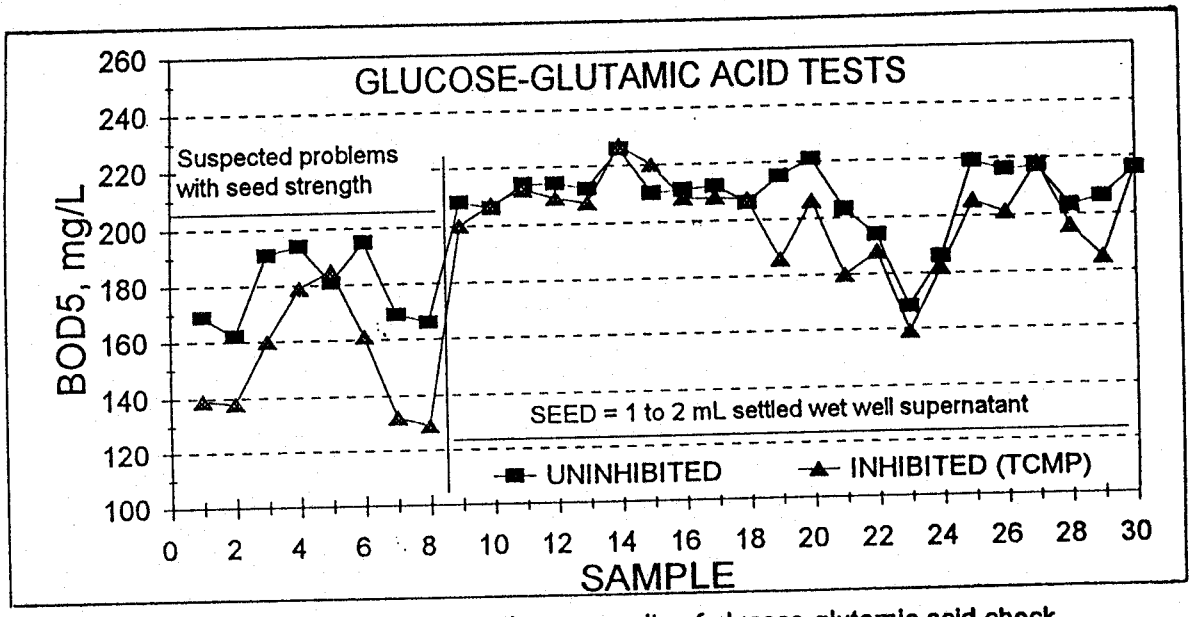

Figure 3. Effect of seed concentration on results of glucose-glutamic acid check
Data from Kenney, 1986.

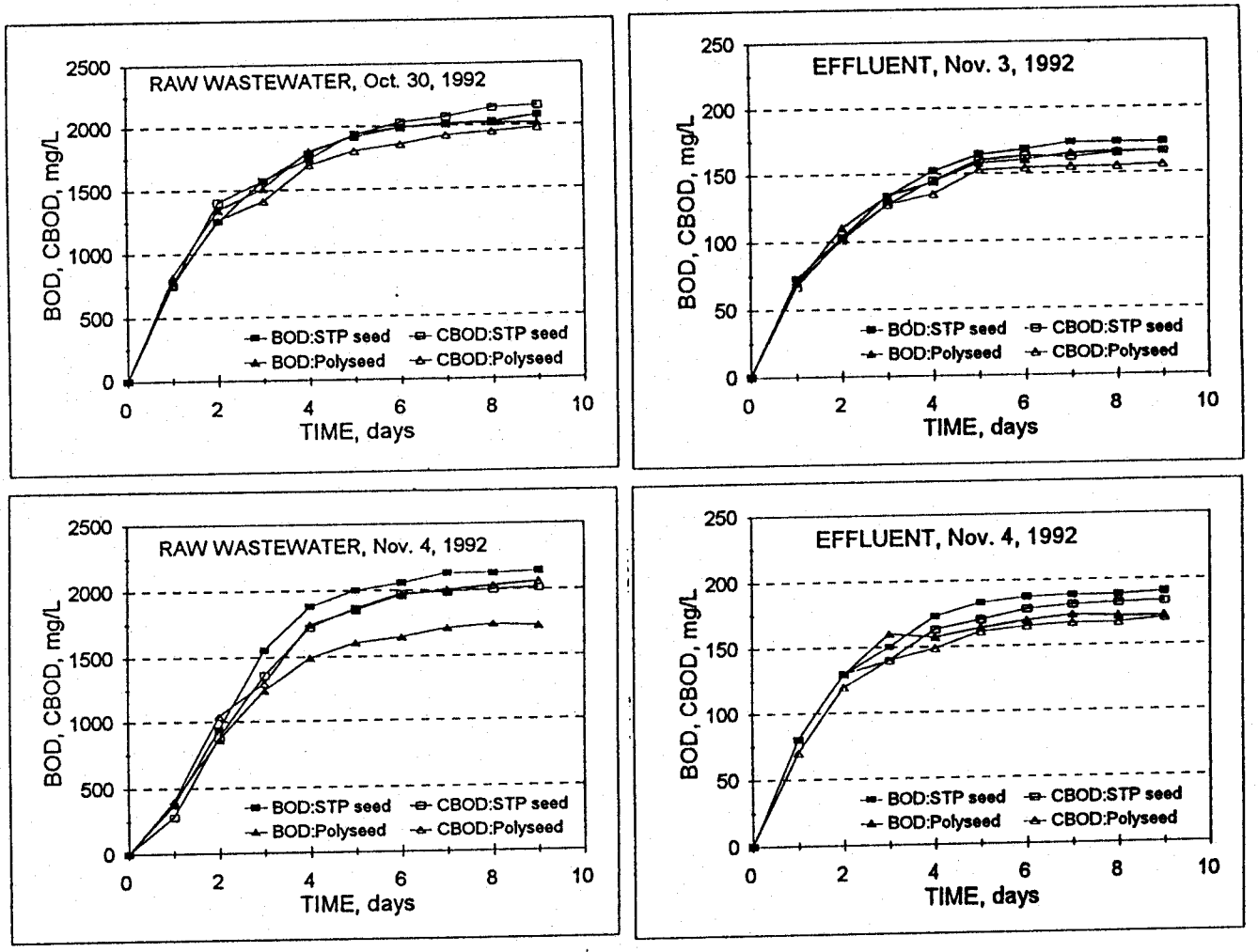

Figure 4. Results of dilution BOD tests using TCMP as a nitrification inhibitor.
Data from Manoharan (1992).

Total and soluble COD measurements were made using the sealed tube digestion method (HACH Company, Method #435, 1989). Total Kjeldahl nitrogen (TKN) initially was measured using HACH Method #8075 (1989) for water, wastewater, and sludge. A 10 mL sample was used for digestion. In later tests, TKN was measured according to the new Test 'N Tube Total Persulfate Nitrogen Method (HACH Company, 1989, 1994). Ammonia nitrogen (NH_3-N) was measured using the Nessler Method (Method #8038, HACH Company, 1989). Nitrite-nitrogen (NO_2^--N) was measured using the Ferrous Sulfate Method (HACH Method #8173, HACH Company, 1989). Nitrate-nitrogen (NO_3^--N) was measured according to the Cadmium Reduction Method (High range Method #8039, HACH Company, 1989). Total and volatile suspended solids were measured using the glass-fiber filter method according to Standard Methods (1989).

Quality control checks and procedures were used throughout the test program to substantiate certain parameters as well as to ensure the accuracy and precision of the procedures being used. For both methods, triplicates were run on controls and test samples to which TCMP, sodium sulfate, and HACH Formula 2533 were added. Measured and calculated NOD values were used to verify that differences between the controls and test samples were caused by nitrification.

RESULTS AND DISCUSSION

Four sets of tests were conducted using the dilution and respirometric methods to determine if there was evidence of inhibition of carbonaceous BOD by TCMP, HACH 2533, or sulfate. Another series of tests was conducted to assess the effect of sodium sulfate. Characteristics of the wastewater samples used for these tests are summarized in Table 2. The first three tests emphasized the industrial wastewater because of the concern expressed by the company and because nitrification was not expected to occur within the five-day standard incubation period.

Table 2. Characteristics of wastewaters used for inhibition tests by the dilution method.

PARAMETER	Series 1 and 2	Series 3	Series 4	Series 6
TCOD, mg/L	1,577	1,450	1,400	382
sCOD, mg/L	665	1,030	560	232
TKN, mg/L	94	110	112	
VSS, mg/L	912	420	640	150

The dilution tests in Series 1 and 2 showed essentially no difference between the control or samples receiving TCMP, HACH 2533 or sodium sulfate (Figure 5). Series 3 and 6 tests did show lower BODs in samples receiving TCMP and HACH 2533, but the effect could have been caused by nitrification rather than inhibition of the carbonaceous reaction. The BOD was essentially the same for both the TCMP and HACH 2533 samples. Additional tests using a mixture of industrial wastewater and raw sewage were conducted to further evaluate the effect of the specific inhibitors on the CBOD reactions. These tests were set up as shown in Table 3.

Table 3. Chemicals added to dilution and respirometer bottles during Series 5 tests.

CHEMICAL	CONCENTRATION, mg/L	
Dilution BOD Tests	TCMP	Sodium Sulfate
Control	0	0
TCMP	10, 20, 30	0
Sodium Sulfate	0	523, 1046, 1569
HACH 2533	10, 20, 30	523, 1046, 1569
Respirometer Tests	TCMP	Sodium Sulfate
Control	0	0
TCMP	10	0
Sodium Sulfate	0	523, 1569
Sodium Sulfate + TCMP	10	523, 1569

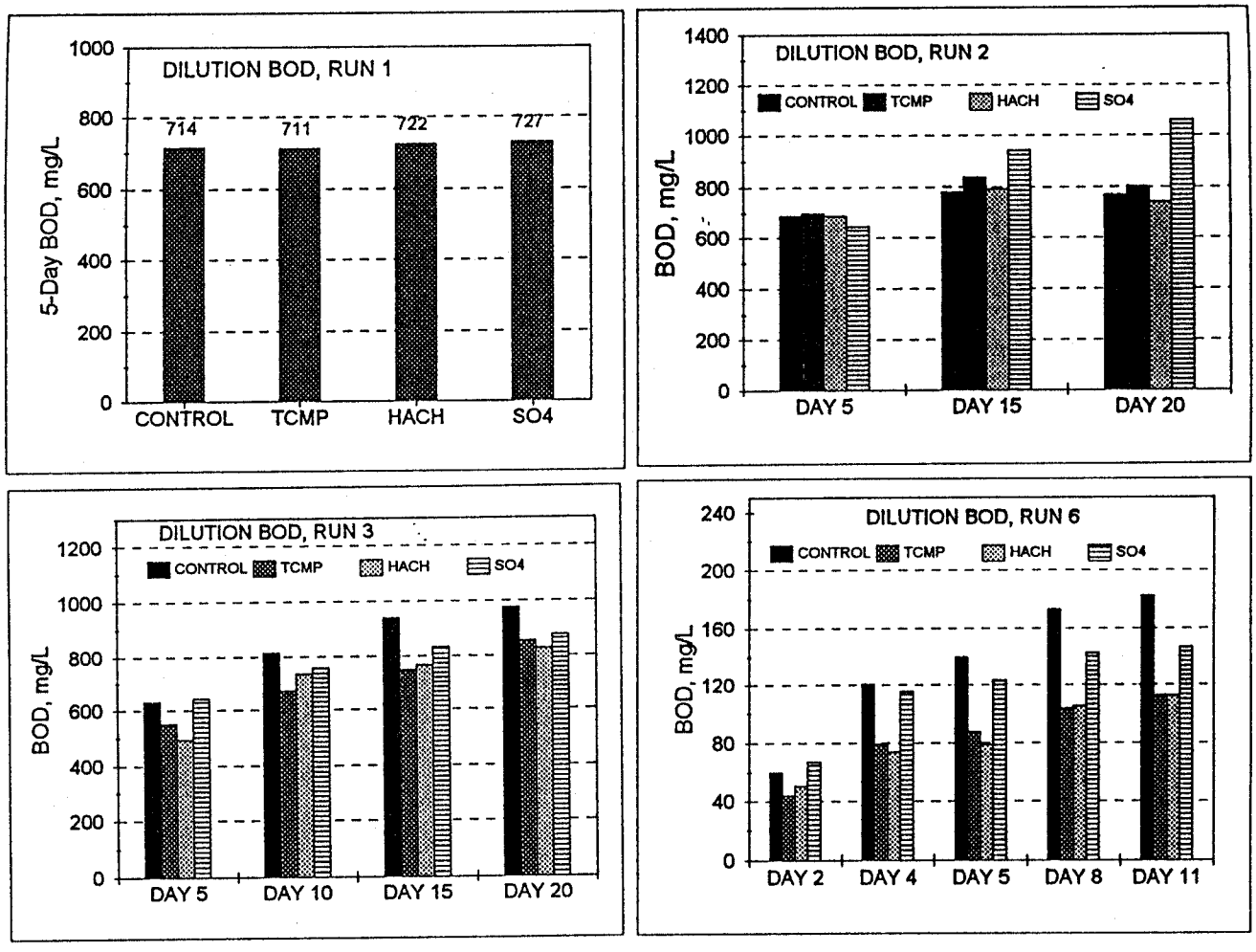

Figure 5. Dilution BOD results for industrial and domestic sewage samples for four test series.

Dilution test data for the samples containing 10 mg/L of pure TCMP, 533 mg of HACH 2533, and 523 mg/L of sodium sulfate showed significantly lower BOD values than the control starting on day-2 (Figure 6A). Nitrification began in the control on the first day of incubation but was delayed until day 3 in the samples containing sulfate. The TCMP and HACH inhibitors produced essentially identical results throughout the 10-day incubation period. The fact that the BOD of the samples containing sulfate were the same as the samples containing TCMP through day 3 indicates strongly that TCMP did not inhibit the carbonaceous BOD. Samples receiving pure TCMP showed a small but noticeable decrease in BOD with increasing chemical dose above 10 mg/L (Figure 6B). Sodium sulfate showed increasing reduction in BOD at concentrations varying from 523 to 1,569 mg/L (Figure 6C). The HACH 2533 inhibitor seemed to have essentially the same effect as the equivalent amount of pure TCMP and the presence of sodium sulfate did not seem to enhance this effect (Figure 6D).

Respirometric tests conducted during series 4 tests yielded oxygen uptake measurements that indicated essentially no occurrence of nitrification through 13 days of incubation (Figure 7A). This lack of nitrification may have been due in part to the seed source and the freshness of the sample. The Control and TCMP-dosed samples showed slightly higher oxygen consumption than the HACH 2533 or sulfate-dosed samples. These results indicate that there were no toxic effects to the CBOD due to TCMP or HACH 2533.

The oxygen uptake results were verified through an analysis of nitrogen species in samples removed from the respirometer bottles. The ammonia concentration remained essentially the same through day-9 in all the tested samples (Figure 7B). (The slight decrease in the ammonia concentration of the TCMP group is not statistically significant). The slight increase in the nitrite plus nitrate-N concentration in the

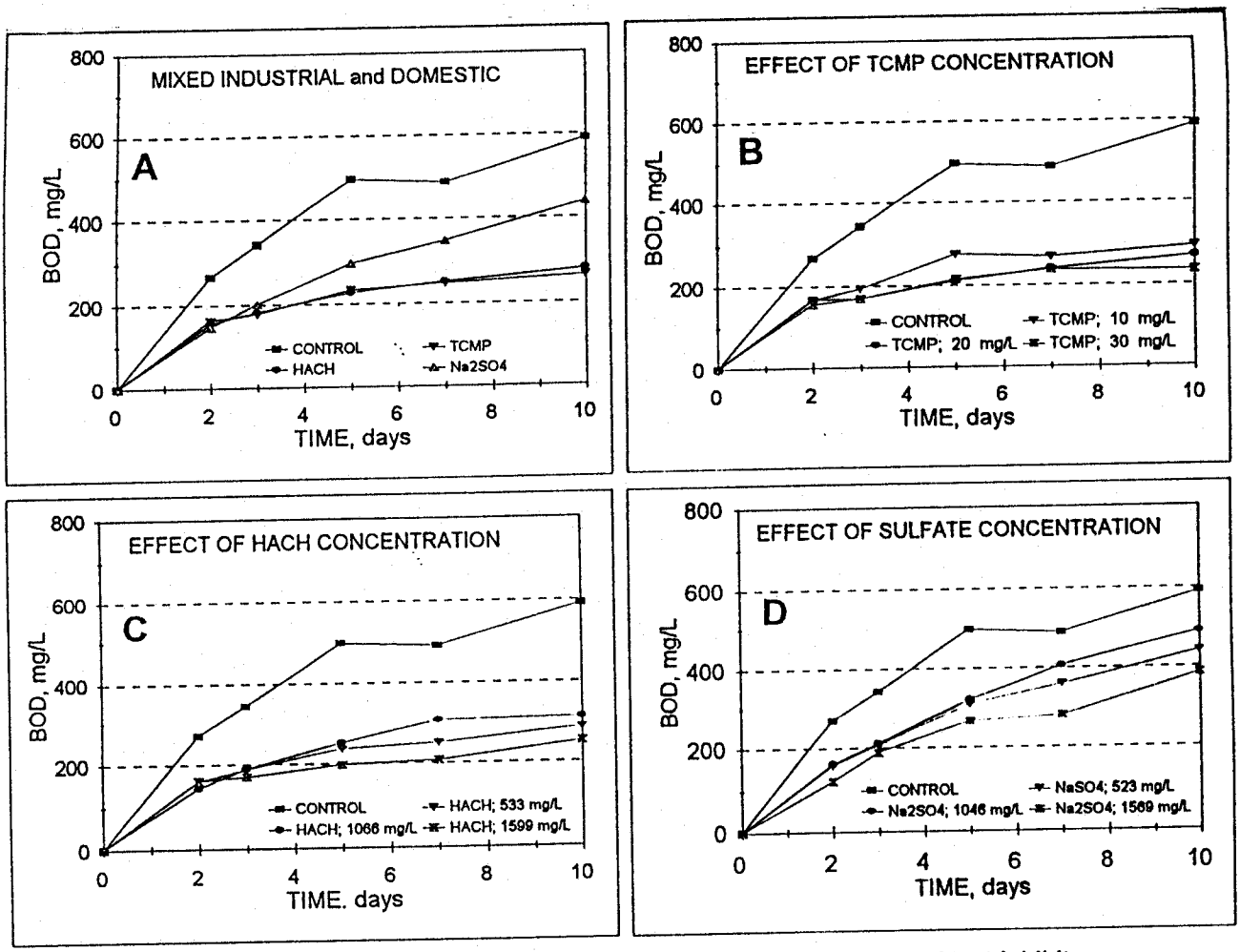

Figure 6. BOD tests results showing effect of varying concentrations of test inhibitors.

control sample by day 9 corresponds with the slight increase in oxygen uptake over the HACH and sulfate-dosed samples (Figure 7C). Also, it should be noted that there were no signs of nitrification in the sulfate-dosed sample indicating that sodium sulfate again may have delayed nitrification. Because of the close agreement between oxygen uptake measurements for the control and samples containing inhibitors, it was concluded that TCMP did not adversely affect the carbonaceous BOD in the respirometric tests conducted during test Series 4.

Respirometric tests conducted during Series 5 showed evidence of nitrification in the control beginning during the first day of incubation (Figure 8A). The 523 mg/L sodium sulfate dose showed a slight suppression of oxygen uptake but 1,569 mg/L caused a 3-day delay in the onset of nitrification. Samples receiving 523 and 1,569 mg/L of sodium sulfate plus 10 mg/L of TCMP showed essentially the same oxygen uptake as the sample receiving 10 mg/L of pure TCMP throughout the 14-day testing period. Increasing amounts of sodium sulfate delayed the onset of nitrification in both dilution and respirometer tests and the oxygen uptake for the 1,569 mg/L of sodium sulfate in respirometric tests was essentially equal to that for samples containing TCMP through day 4, thereby indicating strongly that TCMP did not inhibit carbonaceous BOD in these tests.

Ammonia nitrogen analyses during the Series 5 tests showed that a substantial amount of nitrification occurred in the control sample by day-11 when almost all the ammonia nitrogen had disappeared (Figure 8B). The increase in ammonia nitrogen concentration between days 0 and 11 is considered to be due to the conversion of organic nitrogen to ammonia nitrogen during the test. Increases in nitrite plus nitrate were associated with the decrease in ammonia nitrogen (Figure 8C). The 110 mg/L difference in ammonia nitrogen between the control and the inhibited samples represented 476 mg/L of NOD as compared to a difference in oxygen uptake of 450 mg/L.

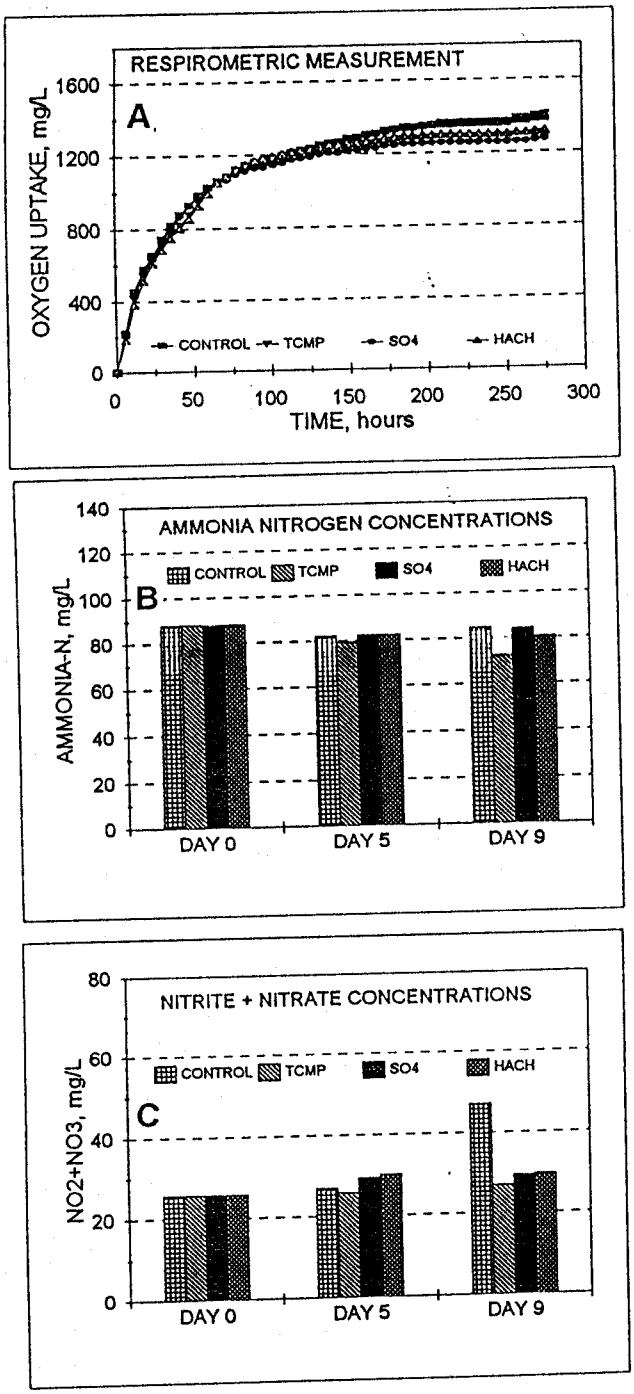

Figure 7. Respirometric oxygen uptake (A), ammonia nitrogen analysis (B) and nitrite plus nitrate concentrations (C) during respirometer Series 4 tests.

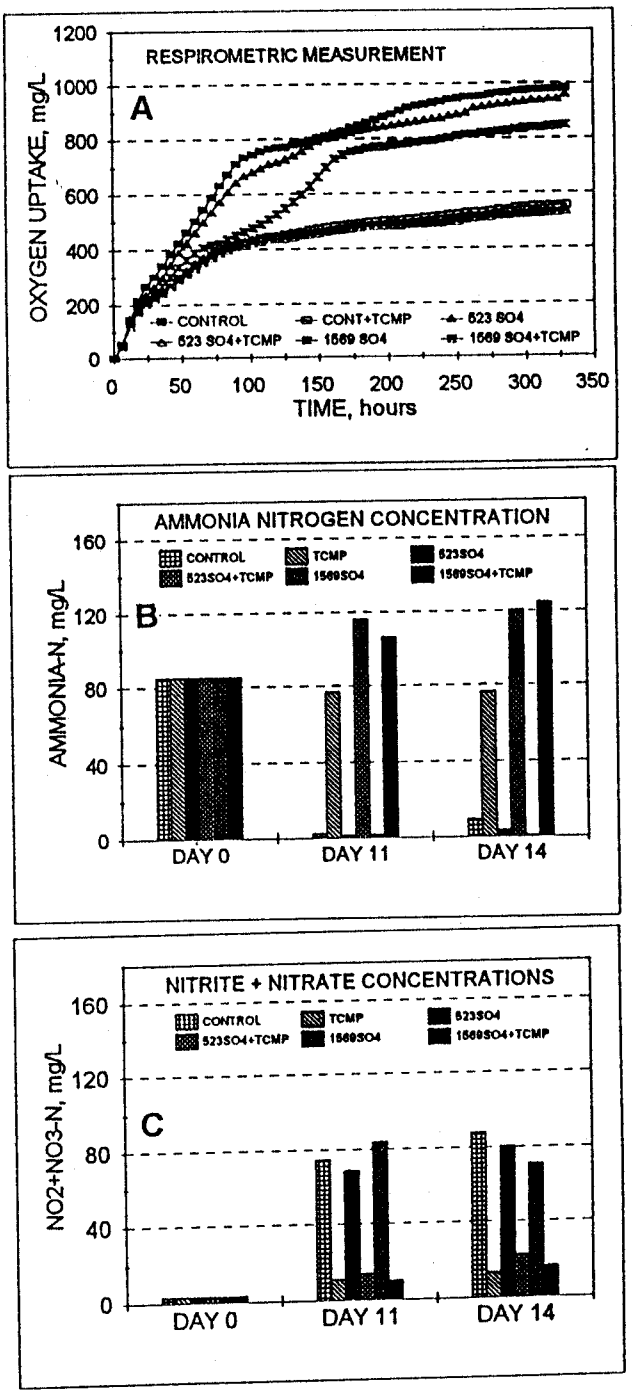

Figure 8. Respirometric oxygen uptake (A), ammonia nitrogen analysis (B) and nitrite plus nitrate concentrations (C) during respirometer Series 5 tests.

Test Series 7 was conducted using primary settled wastewater and seed from the University Park wastewater treatment plant. Only the dilution method was used to measure BOD. The test plan consisted of four groups each containing a Control and a TCMP-supplemented subgroup. The Control group represented the supernatant (decant) from the raw wastewater that had been allowed to settle for two hours. To help improve settling, 30 mg/L of alum was added to flocculate the solids. The 100%

group represented the completely mixed raw wastewater. The 66% group represented a mixture of 34 mL of decant and 66 mL of raw wastewater. The 46% group represented a mixture of 54 mL of decant plus 46 mL of raw wastewater. The wastewater characteristics are listed in Table 4. Samples were run in triplicate using 5, 10 and 15 mL of wastewater per bottle plus 0.5 mL of seed. Only 533 mg/L of HACH Formula 2533 was used as a nitrification inhibitor.

Table 4. Wastewater characteristics for Run 7.

PARAMETER	CONCENTRATION, mg/L			
	100%	66%	44%	Decant
TCOD	360	298	259	180
TSS	170	126	97	40

The only sample for which there was a substantial difference in measured BOD between the Control and TCMP-dosed samples was the decant group (Figure 9A). In this case, the 2-day BOD in the decant Control group without HACH was almost two times that of the decant group with TCMP. By day 5, the difference was reduced to about 25%, but the BOD of the Control still was greater than for the TCMP-dosed sample. Smaller differences were seen in the 46% samples but the 46% Control BOD was still greater than that for the sample containing TCMP. The BODs for the 67% and 100% samples were essentially the same as that for the Control. Overall, the presence of suspended solids seemed to reduce the difference between the BOD of uninhibited and TCMP-inhibited samples.

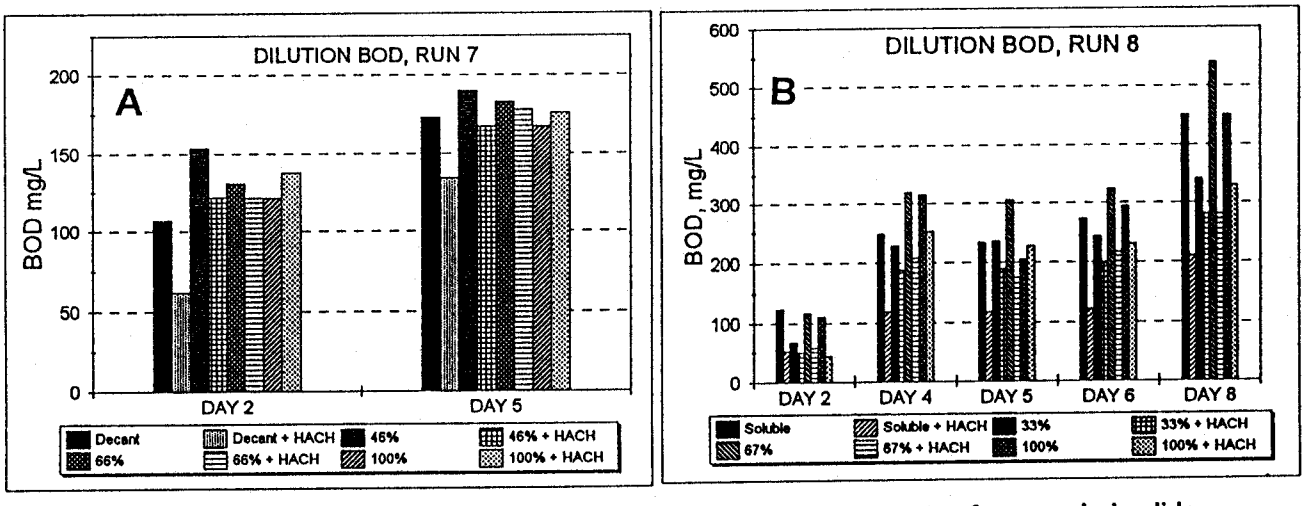

Figure 9. Five day BODs for two test series using different amounts of suspended solids in the test samples.

Series 8 was a repeat of Series 7 to clarify the previous results. Again raw wastewater and seed samples were obtained from the University Park wastewater treatment plant. The wastewater characteristics are shown in Table 5. In this case the low TSS fraction was obtained by filtering the raw wastewater. The raw wastewater was designated as the 100% group and the filtered material was labeled (soluble). The 67% group was a mixture of 33 mL of the filtered material and 67 mL of the raw wastewater. The 33% group was a mixture of 67 mL of filtered material and 33 mL of the raw wastewater. HACH 2533 was used as the source of TCMP for nitrification inhibition.

Table 5. Wastewater characteristics for Run 8.

PARAMETER	CONCENTRATION mg/L			
	100 %	67 %	33 %	Soluble
TCOD	510	447	436	400
sCOD (filtered)	400	400	400	400
TSS	152	102	50	0

Throughout the test period, the 67% group without TCMP exerted the highest BOD, and on most days the 33% group exerted the lowest BOD (Figure 9B). By day-2, there was already a difference between the BODs of the samples with and without TCMP. The BODs of the Controls were consistently higher than for the samples containing TCMP. As in Series 7, the largest percent difference was seen in the soluble group. With the exception of the 33% group, the overall trend was similar to that in Run 7, that is, the differences between the Control samples containing TCMP decreased with increasing solids content. The differences throughout are thought to be due to nitrification since the samples were seeded with the mixed liquor from the University Park wastewater treatment plant. The data from Run 8 did not provide evidence that TCMP inhibited carbonaceous BOD. The differences between the Control and the inhibited samples could have been caused as much by nitrification occurring in the Control as by inhibition of CBOD by the TCMP in the HACH-2533 dose.

CONCLUSIONS

The test program described above indicated that TCMP [2-chloro-6-(trichloromethyl) pyridine] in pure form or in the form of HACH Formula 2533 at recommended dosages did not inhibit the carbonaceous BOD reaction in dilution BOD tests or respirometric oxygen uptake tests using industrial wastewater or raw and primary domestic wastewater. This conclusion is based on several major points:

1. The oxygen uptake of Control samples showing no signs of nitrification, either as increased oxygen uptake or decrease in ammonia nitrogen, agreed closely with that from samples containing 10 mg/L of TCMP either in pure form or as HACH Formula 2533. In these cases, there was no evidence that TCMP inhibited carbonaceous BOD. Respirometric oxygen uptake measurements showed essentially the same relationship between inhibited and uninhibited samples as did dilution test measurements. In essentially all cases, analyses of nitrogen species verified that differences in oxygen uptake between Control samples and those dosed with TCMP or HACH Formula 2533 were associated with nitrification.

2. Sulfate alone often delayed the onset of nitrification. This became clear when the BOD curve of the sulfate-dosed samples initially followed the pattern of the TCMP and HACH-dosed samples but then recovered and began approaching the BOD of the Control samples. When 1,569 mg/L of sodium sulfate – three times the amount used in HACH Formula 2533 – was added to a sample, BOD reactions initially proceeded as if dosed with pure TCMP. Analysis of ammonia and nitrite and nitrate nitrogen verified the inhibition of nitrification by both TCMP and sodium sulfate.

3. There was no evidence that TCMP adversely affected the hydrolysis of suspended solids. In fact, greater differences occurred between the Control and TCMP-dosed samples containing low TSS concentrations than between samples containing high suspended solids concentrations.

4. Data collected by the authors and other investigators indicate that low five-day BOD values for glucose-glutamic acid quality control samples are caused by improper test set up – very likely, using too little seed concentration – rather than by inhibition of carbonaceous BOD by the TCMP. When CBOD tests are set up properly, TCMP is an effective nitrification inhibitor that does not adversely affect the CBOD reaction.

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