Relationship of Various Indices of Water Quality to Denitrification in Surface Waters[1]

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Abstract

Water samples were collected monthly from three farm ponds and from three locations on the Wabash River near Lafayette, Indiana, to determine the actual and potential rates of denitrification in such water systems. Denitrification may serve as an important mechanism for nitrate removal from surface waters. Water parameters which may affect denitrification were estimated at the time of sampling and then related to the denitrification rates observed. Actual and potential denitrification rates were normally small unless an energy source was added, indicating that the low amount of dissolved carbon as well as a high dissolved O_2 content may be the factors limiting denitrification in surface waters. Water temperature, pH level, nitrate level, and numbers of denitrifying bacteria appeared suitable for denitrification during most of the year. Higher levels of denitrifying bacteria, nitrate, and phosphorus existed in the river than in the ponds, while the ponds had slightly higher dissolved carbon levels. The nitrate-N levels did not exceed the United States Public Health Service standard of 10 parts per million, and the river and pond surface water remained aerobic throughout the year. The levels of contaminants studied in the river were little affected by the municipal and industrial effluents that were added between the river locations.

Introduction

The pollution of rivers, lakes, and ponds is a problem of increasing concern. The commercial and recreational use of and the esthetic quality of bodies of surface water are being threatened by the addition of inorganic contaminants, such as nitrate. Nitrate, which has industrial, urban, and agricultural sources, encourages excessive growth of algae when present in surface waters at low concentrations (0.3 ppm nitrate-N) and poses a health problem to infants and ruminant livestock when a contaminant of drinking water in concentrations greater than 10 ppm of N. In view of the possible consequences of increased nitrate loading of surface waters, it is imperative that more be known about nitrate transformations in natural waters and factors which influence these transformations. Denitrifications, *i.e.*, the biological reduction of nitrate to nitrogen gas, is being viewed as a possible mechanism for the removal of nitrate. Denitrification is known to proceed in a variety of environments such as water-logged soil, sewage digestors, and manure pits resulting in the loss of nitrogen from the system. Anaerobic conditions are required for denitrification since bacteria carrying out dissimilatory nitrate reduction are facultative aerobes which use oxygen if available as the acceptor for electrons produced in respiration, but which may use nitrate as the electron acceptor if oxygen is absent. Denitrification may be a significant pathway for removal of excess nitrate from certain aquatic systems if conditions which promote denitrification develop during the year.

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[1] Journal Paper No. 4950. Purdue University, Agricultural Experiment Station. Partially supported by the Indiana Water Resources Research Center (Project No. A-019-IND).

The objectives of the study reported here were: 1) To estimate the actual and potential denitrification rates of surface waters sampled monthly from the Wabash River and three farm ponds; 2) To measure various indices of water quality which may affect denitrification; and 3) To attempt to relate the parameters studied to the rates of denitrification observed.

Literature Review

Although no studies concerned with the distribution of denitrifying organisms in surface waters have been conducted, several studies dealing with total bacterial populations in surface waters have been reported. In a 4-year study in Lake Mendota, Wisconsin, Fred, Wilson, and Davenport (5) found that total bacterial numbers fluctuated widely. There was not always a satisfactory explanation for the extreme fluctuations. Graham and Young (8) reported bacterial numbers ranging up to 8,000/ml in Flathead Lake, Montana, with the counts being lower in the surface water than in the water a few feet below the surface. Similar results were found by Hughes and Reuszer (10) in three southern Indiana farm ponds. The surface water usually contained fewer bacteria than the water 6 inches from the bottom. Although bacterial counts up to 120,000/ml were observed, large seasonal fluctations in the bacterial population was evident. From the investigation of 7 West Virginia farm ponds, Wilson, Miller, and Thomas (17) reported bacterial numbers ranging from 7,000 to 45,000/ml.

Not only have seasonal variations been found, but as reported by Stark and McCoy (15), striking variations in bacterial numbers in different areas of the same lake have been observed. The variations were correlated to the level of weed and algal growth.

Certain chemical characteristics of surface water have been reported in conjunction with some bacterial population studies. Stark and McCoy (15) recorded total organic carbon values ranging from 5.5 to 15.5 ppm for 7 Wisconsin lakes. Hughes and Reuszer (10) reported 7-28 ppm of organic carbon and pH values ranging from 6.8-8.0 on a seasonal basis. A similar pH range, 6.87-8.34, was reported by Wilson *et al.* (17).

Only a few studies have dealt with denitrification rates in aquatic systems. In the anoxic hypolimnion of an island bay in the equatorial Pacific Ocean, Goering and Dugdale (6) found denitrification rates from 12 to 18 μg of N_2/l/day at depths of 125 or more. No denitrification was found at lesser depths. Goering and Dugdale (7) reported that during the winter anoxic period in a subarctic lake studies using $N^{15}O_3^-$ revealed evolution of N_2 at a rate of 90 μg N_2/l/day in the first 3 days from a water over sediment system. About 15 μg of N_2/l/day evolved from water collected 1 m below the surface and incubated at 5° C.

In a study of Lake Mendota, Wisconsin, Brezonik and Lee (4) reported that the denitrification rates ranged from 26 μg of N_2/l/day at 22 and 23 m to 8.4 μg of N_2/l/day at 14 m. The lower depths were influenced by sediments. Although some denitrification occurred at 11 to 13 m, there was not enough to change the denitrification estimate for the entire lake. Anaerobic conditions existed at this latter depth, but not for extended periods. The denitrification estimate reported accounts for only 11% of the total annual input of nitrate.

Materials and Methods

Water samples were collected from three sites along the Wabash River and from three farm ponds in the West Lafayette area. The river sites were located such that one site (R-N), near the State Street bridge, was north of most of the industrial and municipal outlets along the Wabash; the second site (R-SO) was a sewer outlet basin connected with the river just south of the first location; and the third sampling point (R-S) was south of Lafayette near the Fort Ouiatenon area. The first of the three ponds sampled was on the Purdue Horticulture Farm (P-HF) just north of Indiana Highway 26 about 1½ miles west of West Lafayette. This pond has a sod cover on its surrounding banks. The second pond, designated as Pond A (P-A), was Miller's Pond and located in a small wooded area south of Indiana Highway 26 about 1 mile west of West Lafayette. The final pond investigated was Blackbird Pond, labeled Pond B (P-B). Located northwest of the Purdue University campus, Pond B also had sod banks except on the west side where a gravel roadway served as a dam.

The water samples were collected from each site once a month for 1 year. The sampling apparatus was similar to that described by Hughes (9), a modification of the Wilson (16) apparatus, except that sterilized, 1-pint bottles were used. The samples were stored at 5° C until analyses could be performed.

The temperature and the dissolved oxygen content of the water were measured *in situ* with a Yellow Springs Instrument Co. oxygen meter, model 51A. The pH was determined in the lab with a glass electrode. The population of denitrifying bacteria was estimated in each sample on the date of collection using the most probable number (MPN) method as described by Alexander (1). The culture medium used was that suggested by Alexander (2) for denitrifying bacteria.

On the day following collection of the sample, 10-day incubation studies of denitrification rates in unamended, nitrate-amended, and nitrate plus glucose-amended samples were initiated. The unamended denitrification experiments were set up by adding 50 ml of the water sample to a 125 ml Ehrlenmeyer flask, bubbling N_2 through the sample for 10 min, and sealing the flask with a stopper secured with electrical tape. The flasks were then incubated at room temperature (approximately 23° C) and analyzed at selected intervals for nitrogen components. The nitrate-amended denitrification experiments were set up by adding 5 ml of a 200 ppm KNO_3-N solution to 45 ml of the water sample (20 ppm NO_3^--N in the 50 ml solution) and proceeding as described above. The nitrate plus glucose-amended system was composed of 45 ml of the water sample treated with 2 ml of a 500 ppm

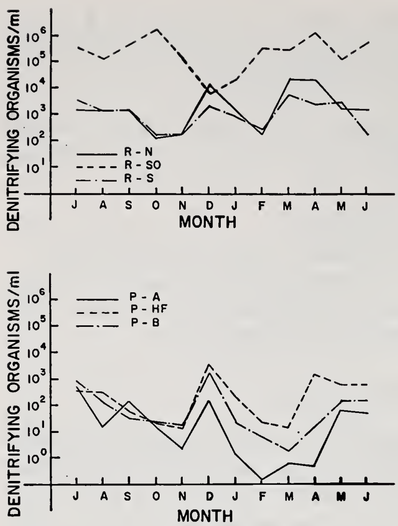
KNO_3-N solution, 2 ml of a 625 ppm glucose-C solution, and 1 ml of deionized water. The resultant solution contained 20 ppm NO_3^--N and 25 ppm glucose-C and was incubated as described above.

Ammonium-N and nitrate-N in samples were determined by steam distillation as described by Bremner and Keeney (3). Filtered (0.45 μ) samples were analyzed for soluble orthophosphate by the Murphy and Riley (13) procedure. The amount of dissolved carbon was determined by refluxing 15 ml of the filtered (0.45 μ) water with 5 ml of 0.025 N $K_2Cr_2O_7$ and 30 ml of concentrated H_2SO_4 for 30 min. Upon cooling, the excess $K_2Cr_2O_7$ was back-titrated with approximately 0.010 N $Fe(NH_4)_2(SO_4)_2$ using ferrion as the indicator.

Results and Discussion

The levels of denitrifying organisms in water samples collected throughout the year are shown in Figure 1. The number of denitrifying bacteria at the two river sites did not greatly differ from each other. Both the northern river site (R-N) and the southern location (R-S) show decreased numbers in the fall but show peaks in denitrifying bacteria in December and again in March. The northern river sampling point, having slightly higher peak values, reached counts of 3.5×10^4 denitrifying bacteria per ml. The low numbers for February can, at least in part, be related to low temperature, as shown by Figure 2. The bacterial populations in the sewer outlet (R-SO) were much greater than those found at any of the other sampling areas. Fluctuations in counts for the sewer outlet did not vary in the same manner as did the bacterial levels at the other locations since peaks in the number of denitrifying bacteria occurred in October and April.

The pond samples had variations in bacterial levels similar to those of the river in which the values decrease in the fall but peaked in December and again in the spring. The pond near the Purdue Horticulture Farm had its second peak nearly a month later than the rivers, while Pond A and Pond B were approximately 2 months later in reaching their second peaks. The bacterial levels in the ponds tended to parallel each other, with the Horticulture Farm Pond generally being the highest and Pond A being the lowest. A few of the points plotted in Figure 1 for Pond A indicating less than 1 organism/ml are merely statistical points, resulting from the MPN method. The number of denitrifying bacteria were so low that a numerical value was essentially meaningless. In general, the ponds had lower numbers of denitrifying bacteria than did the river. Owens (14) found that even when the initial bacterial numbers were as low as 4.4×10^{-2} organisms/ml, enough denitrifying bacteria were produced in 10 days to result in appreciable denitrification if an appropriate level of organic C was present as a source of energy for the organisms. Thus, the denitrifying population of most water samples appears adequate for significant denitrification to occur.



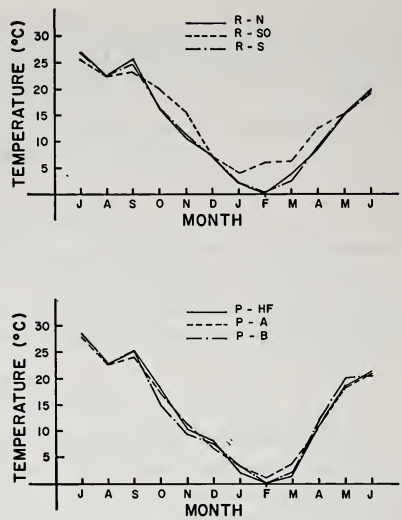
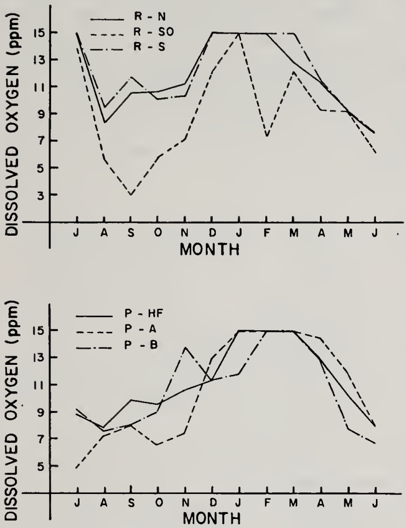


FIGURE 1. *Levels of denitrifying organisms in river and pond surface water samples during a 1-year period.*

FIGURE 2. *Temperature of river and pond surface waters during a 1-year period.*

The water temperatures of both the river sites and the farm ponds were very similar throughout the year, as is shown by Figure 2. The maximum temperatures, which were above 25° C, were reached in July and the minimum temperatures, 1° C and lower, were observed in February. The ponds were ice covered in January, and all the sites except the sewer outlet were ice covered in February. The sewer outlet tended to be slightly warmer than the other sampling points except during the summer months. A study of the effect of the temperature on the rate of denitrification in surface water (14) showed that denitrification occurred at 10° C, but not at 5° C, indicating that from December to March the water temperature was unfavorable for denitrification.

Seasonal variations in the dissolved oxygen content of the surface waters are shown in Figure 3. The highest values were observed during the winter months as expected since the solubility of O_2 in water increases with decreased temperature. The river at the northern location ranged from 7.6 up to 15 ppm (the upper limit measured by the YSI oxygen meter). The R-S site yielded similar values, ranging from 7.7 to 15 ppm dissolved O_2. A high level of dissolved oxygen (15 ppm) was also observed at each river site in July. The oxygen level at the R-SO site, ranging from 3 to 15 ppm, never exceeded the levels in the river and, in fact, was much lower than values for the river during the fall. The ponds were somewhat more sporadic in the variation of oxygen levels. The Horticulture Farm Pond, Pond A, and Pond B ranged from 7.8 to 15, 4.8 to 15, and 6.7 to 15 ppm, respectively. Pond A tended to have a lower dissolved oxygen content in the fall than the other two ponds and slightly higher content in the spring. All of the dissolved oxygen levels measured are above the level of 1 ppm which was found to be the upper limit for denitrification in surface waters (14). Thus the presence of high dissolved oxygen will likely limit denitrification in surface waters although some aquatic systems are known to develop anaerobic zones during stratification or when high BOD effluents are added.



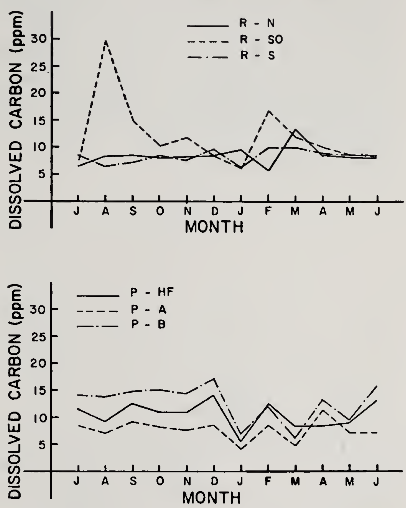


Figure 3. *Dissolved oxygen content of river and pond surface waters during a 1-year period.*

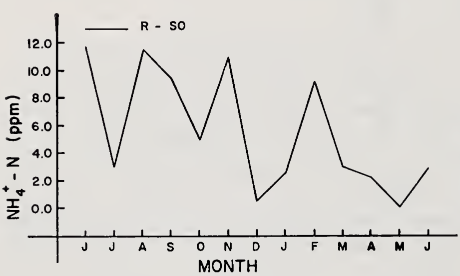
Figure 4. *Dissolved organic carbon content of river and pond surface water samples during a 1-year period.*

There was very little variation in pH throughout the year at any of the six sampling sites. The individual pH values of all samples ranged from 7.4 to 8.4, and the mean annual pH was 7.8 for the sewer outlet and Pond B and 8.0 for the southern river location and Pond A. Since denitrification has been shown to proceed from pH 5.5 to 8.0 (14), the pH values of the surface water from the various sampling sites were in the range which had no inhibitory effects on denitrification.

The characterization of the surface water for dissolved organic carbon is shown in Figure 4. The carbon levels in the river remained fairly constant throughout the year. The southern river location varied from 6.2 to 10.1 ppm throughout the year, while a mild fluctuation in later winter causes the levels of the northern site to range from 5.8 to 13.5 ppm. The sewer outlet had a high level of dissolved carbon (29.7 ppm) in August and dropped off before a smaller peak (17.0 ppm) in February. The carbon levels in the ponds ran rather parallel to each other with Pond B having the highest level and Pond A having the lowest. The level of dissolved carbon in Pond B, the

Horticulture Farm Pond, and Pond A over 1 year ranged from 6.3 to 15.6, 5.5 to 13.2, and 4.4 to 11.3 ppm, respectively. In light of a previous study (14) which showed that at least 30 ppm of dissolved organic C must be present for significant denitrification to occur, it is likely that the dissolved carbon levels observed would be a limiting factor for denitrification in the water systems studied.

The ammonium-N levels in the sewer outlet were highly variable as shown by Figure 5. The individual values ranged from 0 to 11.7 ppm and the mean annual ammonium-N content was 5.3 ppm. The river ranged from 0 to 0.5 ppm NH_4^+-N and averaged 0.2 ppm for both the R-N and R-S sites. For the Horticulture Farm Pond, Pond A, and Pond B, the ammonium-N content ranged from 0 to 0.6 ppm (with the exception of 2.8 ppm value for July), 0 to 0.3 ppm, and 0 to 1.0 ppm, respectively. The mean annual values were 0.2, 0.1, and 0.4 ppm NH_4^+-N, respectively.



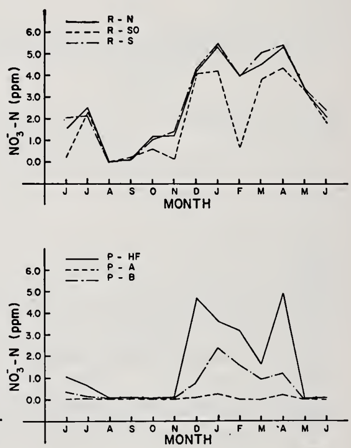
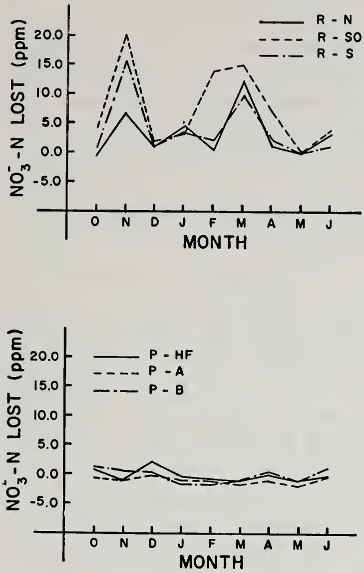


FIGURE 5. *Ammonium-N level of sewer outlet basin surface water samples during a 1-year period.*

FIGURE 6. *Nitrate-N content of river and pond surface water samples during a 1-year period.*

The nitrate-N levels in the river samples were very similar. As shown by Figure 6, the nitrate levels were lower in the summer and fall (minimum of 0 ppm in August) and higher in the winter and spring (maxima near 5.5 ppm in both January and April). The sewer outlet showed a similar trend, but had a much lower nitrate content in February than was observed in the river samples. The mean annual nitrate-N level for the sewer was 2.1 ppm, while for the northern and southern river sites, it was 2.8 and 2.9 ppm, respectively. Like the river, the ponds had lower nitrate levels in the summer and fall with higher levels in the winter and spring. However, the winter and spring nitrate levels differed noticeably from pond to pond. The Horticulture Farm Pond ranged from 0 to 4.9 ppm nitrate-N with a mean of 1.5 ppm, Pond B from 0 to 2.4 with a mean of 0.6 ppm, and Pond A from 0 to 0.25 with a mean of 0.04 ppm. Previous research (14) has shown that there is no apparent minimum nitrate concentration required for denitrification to be initiated.



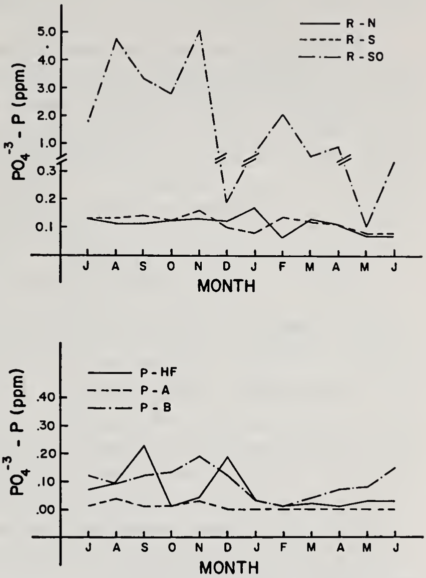


FIGURE 7. *Nitrate-N lost from glucose-amended, anaerobic river and pond surface water samples collected during a 9-month period.*

FIGURE 8. *Orthophosphate-P content of river and pond water surface water samples during a 1-year period.*

In the nitrate-amended denitrification rate experiments, nitrate-N loss did not exceed 1.0 ppm in any of the samples. Likewise, no nitrate loss was observed in the unamended denitrification rate experiments. When this trend was noticed, the unamended denitrification rate experiments were discontinued and the nitrate plus glucose-amended system was initiated. Figure 7 shows the loss of nitrate from the nitrate plus glucose-amended samples collected during the period October to June. The nitrate-N lost from the river samples ranged from 0 to 12.2, 0 to 15.5, and 0 to 19.7 ppm for the R-N, R-S, and R-SO sites, respectively. Months of greatest loss were November and March for all three locations. Explanation of these peaks is difficult since, with the exception of the sewer outlet, the denitrifying bacteria numbers were high only during the March period and dissolved carbon contents were not especially high at times of large nitrate loss. Denitrification was not limited by temperature or dissolved oxygen parameters since all samples were incubated at room temperature (23° C) under complete anaerobic conditions. In samples amended with glucose the numbers of denitrifying bacteria should not be a limiting factor due to their rapid proliferation in enriched media. However, at the level of carbon present, the rate of organism growth is uncertain.

The constancy of the carbon levels in the river water indicates that there is a factor in addition to carbon which influences denitrification since great variability in denitrification was observed in samples having similar organic C contents. Nitrate loss was not appreciable from the pond water samples even in the systems amended with carbon.

Although most surface waters apparently have a low capacity to carry out denitrification, recent studies conducted in our laboratory and elsewhere (11) have indicated that denitrification readily occurs in lake sediments. In fact, denitrification rates reported for sediment are higher than those commonly reported for anaerobic soils due to the presence of large amounts of organic carbon (12) in most sediments. It may be that sediments serve as a sink for nitrate in aquatic systems resulting in an overall reduction in the nitrogen status of lake systems.

Though not of particular significance to nitrogen transformations, phosphorus is an important factor in the eutrophication of surface waters. Figure 8 shows monthly fluctuations in the orthophosphate-P content of water samples from the six sampling points. The two river sites were fairly constant with a mean annual phosphorus content of 0.11 and 0.13 ppm for the northern and southern sites, respectively. The sewer outlet, which may have a laundromat as one of its contributors, had much higher phosphorus levels, ranging from 0.10 to 5.07 ppm with a mean of 1.90 ppm. The phosphorus content in the ponds was not as constant as the river sites, but the ponds had lower annual mean levels of 0.10, 0.06, and 0.01 ppm for Pond B, the Horticulture Farm Pond, and Pond A, respectively.

Conclusions

Denitrification in surface waters was negligible unless samples were amended with organic carbon to serve as an energy source for denitrifying bacteria. When glucose was added to increase the dissolved carbon content by 25 ppm, denitrification was observed in river samples collected during certain periods of the year but not in pond water samples. The number of denitrifying bacteria, pH, and nitrate concentration did not limit denitrification. High dissolved oxygen content of water limited denitrification at all times and water temperature limited denitrification during winter periods. Some unmeasured parameter apparently controlled denitrification in some laboratory experiments.

Characterization of the surface waters in the Wabash River showed that the site near the State Street bridge, north of most of the municipal and industrial effluent outlets, was very similar in composition to the sampling point near Fort Ouiatenon, south of Lafayette indicating that the levels of the contaminants studied were little affected by the effluents that were added between the two locations.

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