Nitrification in the Wabash River

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Nitrification, the oxidation of ammonium to nitrate by specific autotrophic bacteria (*Nitrosomonas* spp. and *Nitrobacter* spp.) occurs in soil, water, and sewage when aerobic conditions are present. The conversion of one mole of ammonium to one mole of nitrate involves consumption of two moles of oxygen. In recent years considerable attention has been devoted to the effects of nitrification on the dissolved oxygen status of moderately polluted streams and rivers. Of most interest are nitrification rates in rivers downstream from municipal and industrial discharges containing substantial concentrations of ammonium, particularly during periods of low river flow. Nitrogeneous oxygen demand (NOD) in a stream would be most significant under conditions of high water temperatures ($> 20°$), low flow, long residence time, and high substrate (ammonium) concentrations. As Tuffy *et al.* (1974) have stated, "nitrification occurring at a level significant enough so that it must be included in a dissolved oxygen or water quality model, does not occur along the entire length of a polluted river, but does occur in identifiable zones.

Many of the models used to predict the dissolved oxygen status of a stream incorporate a NOD term. When dissolved oxygen models are appiled to many Indiana rivers, the oxygen uptake associated with ammonium oxidation appears to be a very significant factor in the overall oxygen balance in a river. Furthermore, high predicted NOD values in turn restrict the allowable discharge of carbonaceous oxygen demanding substances. Although models predict that NOD is an important component in the dissolved oxygen status of Wabash River, little is known about actual nitrification in water or sediments. Therefore, the objective of the work reported here was to determine the populations of *Nitrosomonas* spp. and *Nitrobacter* spp. associated with water, periphyton and bottom sediments in two segments of the middle Wabash River and to interpret the population data in terms of nitrification potential.

Materials and Methods

Three sampling trips were conducted (during June, July and September, 1977) on each segment of the River studied. Table 1 presents locations where samples were taken. Water samples were collected 15 cm below the surface using sterile 250 ml glass bottles. Samples of bottom sediments were collected by sucking the upper 1 cm of sediment into sterile 500 ml filtering flasks. Periphyton samples ($\sim$ 1 g dry weight) were taken by scrapping the slimy layer (present at or just below the water level) of rocks, logs, and plants located near shore into 100 ml of sterile water contained in glass jars. Samples were taken from the center and both sides of the river at each sampling station.

TABLE 1. *Locations of sampling sites for water, periphyton, and bottom sediments on the Wabash River.*

Site No.	Approximate mile point	Descriptive location
1	313	Mascouten Park, W. Lafayette
2	309½	200 yards above Lilly outfall, Lafayette
3	309	100 yards below Lilly outfall, Lafayette
4	308	900 yards below Lilly outfall, Lafayette
5	307	Fort Quiatenon
6	303	Granville Bridge
7	300	4 H Center
8	298	Black Rock
9	240	Montezuma
10	238	Cottages
11	237	Big Bend
12	236½	100 yards below Lilly outfall, Clinton
13	236	900 yards below Lilly outfall, Clinton
14	235	1 mile below Lilly outfall
15	230	Clinton RR Bridge

All samples were placed on ice and stored at 4°C until analyses could be carried out (normally within 24 hours).

Sediment and periphyton slurries were homogenized by a high speed blender using a sterile blade. Duplicate samples were taken from homogenized slurries. One set of samples was dried at 105°C for 24 hours, weighed, and the solids content calculated. Water samples and the other set of homogenized slurries were serially diluted in sterile dilution blanks (0 to 10^{-4} and 10^{-1} to 10^{-5} dilutions for water samples and slurries, respectively). The populations of nitrifying bacteria were then determined by the MPN methods as described by Matulewich (1974), except that 5 replicate tubes per dilution were used. All values are averages of duplicate determinations. Abundance of nitrifying bacteria associated with sediment and periphyton is expressed as viable cells per mg of oven-dry solids, whereas bacterial populations in the water column are expressed as cells per ml.

Results

Table 2 presents data on the seasonal average nitrifier population in Wabash River taken near Lafayette. The high standard deviation results from averaging data collected throughout the summer and fall. In most cases, the numbers of nitrifying bacteria were low and the population of *Nitrosomonas* exceeded the *Nitrobacter* concentration by five to ten fold. There were indications of increased *Nitrosomonas* populations downstream from the Lafayette sewage treatment plant and Eli Lilly Company outfalls; however, the data are not conclusive. There was a strong tendency for the *Nitrosomonas* population to increase as the summer period progressed. However, this observation may be an artifact of the sampling scheme because samples collected late in the season may have been influenced by more recent runoff from agricultural land and would have been enriched with nitrifiers relative to samples collected in June.

TABLE 2. *Nitrifying bacteria in Wabash River-water near Lafayette, Indiana.*

Sampling site	No./Location	Nitrosomonas spp.*	Nitrobacter spp.*
	river mile	Organisms/ml	
1	313	3 ± 1	3 ± 2
2	309½	19 ± 18	2 ± 1
3	309	51 ± 90	4 ± 3
4	308	46 ± 51	4 ± 2
5	307	69 ± 138	2 ± 1
6	303	31 ± 49	5 ± 4
7	300	23 ± 21	3 ± 1
8	298	57 + 92	2 ± 2

* Average and standard deviation for all samples analyzed.

Nitrifying bacterial populations in water samples collected from the Clinton segment of the Wabash River are given in Table 3. The data obtained from the Clinton area were similar to that of the Lafayette segment confirming that the water phase contains low *Nitrosomonas* and *Nitrobacter* populations. This finding suggests that nitrifying bacteria are present in low concentration throughout the middle Wabash River. There was no apparent trend toward increased population of nitrifying bacteria downstream from the Lilly Laboratory at Clinton. Populations of *Nitrobacter* spp. were uniformly low at all sampling periods and samples collected in September did not differ appreciably from those taken in June and July.

TABLE 3. *Nitrifying bacteria in Wabash River water near Clinton, Indiana.*

Sampling site	No./Location	Nitrosomonas spp.*	Nitrobacter spp.*
	river mile	Organisms/ml	
9	240	16 ± 11	3 ± 2
10	238	28 ± 26	2 ± 1
11	237	18 ± 12	2 ± 1
12	236½	51 ± 98	3 ± 2
13	236	27 ± 19	5 ± 4
14	235	20 ± 16	2 ± 1
15	230	17 ± 13	1 ± 1

* Average and standard deviation for all samples analyzed.

Lafayette segment sediment samples collected from the water-bottom interface had substantial concentrations of nitrifying bacteria (Table 4). The population size was equal to or exceed the numbers found in agricultural soils, which suggests that sediments may be active sites of nitrification. Samples collected downstream from known ammonium discharges tended to have higher populations of *Nitrosomonas* than those upstream of the discharges. Samples collected in September had very high densities of *Nitrosomonas* spp. ($\sim 10^3$/mg). Sediment samples collected from the Clinton segment contained lower populations of nitrifying bacteria than samples collected near Lafayette (Table 5). There was a slight tendency for increased *Nitrosomonas* populations in

sediment samples collected downstream from the Lilly discharge (June and July samples) as compared to upstream samples. On the average, samples collected in September contained higher populations of nitrifying bacteria than samples collected earlier in the summer.

TABLE 4. *Nitrifying bacteria in bottom sediments near Lafayette, Indiana.*

Sampling site	No./Location	Nitrosomonas spp.*	Nitrobacter spp.*
	river mile	Organisms/mg sediment	
1	313	334 ± 481	47 ± 51
2	309½	2753 ± 5747	117 ± 94
3	309	2047 ± 1740	59 ± 49
4	308	3498 ± 6711	120 ± 51
5	307	2502 ± 3581	137 ± 70
6	303	705 ± 898	86 ± 94
7	300	893 ± 1184	29 ± 21
8	298	1798 ± 3036	20 ± 9

* Average and standard deviation for all samples analyzed.

On the average there were 1435 ± 2170 and 380 ± 436 *Nitrosomonas* spp. cells per mg of periphyton for samples in the Lafayette and Clinton segments, respectively. In addition, there were 139 ± and 163 and 93 ± 42 *Nitrobacter* spp. cells per mg of periphyton in samples collected at Lafayette and Clinton, respectively. Although significant nitrifying bacterial populations are associated with periphyton, the low amount of periphyton present (it was difficult to even obtain enough periphyton for samples at many stations) in the middle Wabash precludes this river component from a significant role in nitrification.

TABLE 5. *Nitrifying bacteria in bottom sediments near Clinton, Indiana.*

Sampling site	No./Location	Nitrosomonas spp.*	Nitrobacter spp.*
	river mile	Organisms/mg sediment	
9	240	581 ± 1010	16 ± 10
10	238	155 ± 114	21 ± 6
11	237	137 ± 145	45 ± 36
12	236½	282 ± 261	13 ± 6
13	236	342 ± 287	66 ± 75
14	235	302 ± 280	4 ± 1
15	230	419 ± 369	14 ± 6

* Average and standard deviation for all samples analyzed.

Discussion

The nitrification potential of the Wabash River is very low because of the low populations of nitrifying bacteria. The overall average population density of nitrifiers in the Wabash River was 38 *Nitrosomonas* spp. per ml and 2.8 *Nitrobacter* spp. per ml (NS/NB ratio was 13.6). Matulewich (1974) reported that the Passaic River in New Jersey contained an average of 476 and 32 *Nitrosomonas* and *Nitrobacter* per ml (NS/NB ratio was 15). Tuffy *et al.* (1974) found that 1300 *Nitro-*

somonas per ml of Mine Brook water, a tributary of the Raritan River in New Jersey. Further study on the Passaic River (Finstein *et al.* 1977) suggested that *Nitrosomonas* and *Nitrobacter* cell densities were 775 and 84 per ml, respectively. The low *Nitrosomonas* populations in the Wabash River suggests that nitrification in the water phase is insignificant. This fact is pointed out by the finding of Tuffy *et al.* (1974) indicating that at least 10^4 *Nitrosomonas* cells per ml are required before nitrification becomes rapid enough to exert a measureable oxygen demand.

Kinetic data calculations may be used to estimate ammonium oxidation potential of *Nitrosomonas* in Wabash River water. McLaren (1971) has shown that the nitrification rate can be described mathematically by the relationship in Equation 1:

$$\frac{d(NH_4^+)}{dt} \sim = Cm \qquad [1]$$

where C is the rate constant for ammonium oxidation (under ideal conditions it is about 7×10^{-6} μg N per cell per day—Knowles *et al.*, 1965) and m is the population of *Nitrosomonas;* for example 10^2 cells/ml.

$$\frac{d(NH_4^+)}{dt} = (7 \times 10^{-6}\ \mu g\ N/cell/day)\ (10^2\ cells/ml)$$

$$\frac{d(NH_4^+)}{dt} = 7 \times 10^{-4}\ \mu g\ N/ml/day$$

Since it requires about 3.22 μg of O_2 per μg of NH_4^+–N oxidized, the oxygen consumption rate would be about 2.25×10^{-3} μg O_2/ml/day. If the Wabash River contained 10^4 *Nitrosomonas* cells per ml the oxygen consumption rate would theoretically be about 0.225 μg O_2/ml/day (0.225 mg O_2/l/day), a measurable oxygen demand in BOD tests. However, the average *Nitrosomonas* population in the Wabash River is only 32 calls/ml. This finding suggests that the low nitrifying bacteria population in Wabash River water eliminates nitrification as a significant sink for added NH_4^+.

Averaging the numbers of nitrifying bacteria in all sediment samples collected gave 1009 *Nitrosomonas* and 32 *Nitrobacter* cells per mg of sediment (NS/NB ratio was 31). Matulewich (1974) reported that the *Nitrosomonas* and *Nitrobacter* populations in the Passaic River averaged 462 and 17 cells per mg of mud-water interface sediment, respectively. An average of 3370 *Nitrosomonas* cells per mg of sediment was observed in Mine Brook, whereas the Passaic River sediment contained 264 *Nitrosomonas* cells/mg (Tuffy *et al.*, 1974). In further studies of the Passaic River, Finstein (1977) reported that surface sediments contained an average of 3400 *Nitrosomonas* and 460 *Nitrobacter* cells per mg (NS/NB ratio = 7.4).

The upper layer of bottom sediments in the Wabash River supports an active nitrifying population. The problem to be rationalized is how

much nitrification actually occurs in the sediment phase or at the sediment-water interface. Crude estimates of benethic nitrification under ideal conditions can be arrived at if many assumptions are made.

Assumptions

1. About 10% of the river bottom area supports an active nitrifying bacteria (most of the surface is sand and gravel having limited nitrifying bacteria).
2. The average river width is 200 m and the average depth is 1 m (a segment of the river 1 m long would contain 200 m^3 of water).
3. The average concentration of NH_4^+–N in the water column is 0.2 μg/ml (a 1 m segment of the river would contain 40,000 mg of NH_4^+–N).
4. Only the NH_4^+–N in the lower 10 cm of the water column can interact with the sediment.
5. The bulk density of sediment is 1.25 g/cm^3.
6. The average *Nitrosomonas* population of bottom sediment is 10^6/g.

Case I. The bottom 10 cm of the water column and the upper 4 cm of sediment behave as a slurry.

The rate of reaction is not dependent upon diffusion of NH_4^+–N to the nitrifiers and diffusion of O_2 from the overlying water. Chen *et al.* (1972) found that under ideal conditions the maximum rate of NH_4^+–N oxidation in aerated, stirred sediment slurries was 25μg NH_4^+–N/1/day. Therefore, using the assumptions above the maximum nitrification rate in a 1 m segment of the river bottom surface can be calculated as:

200 m x 1 m x 0.1 = 20 m^2 of bottom surface with nitrifiers

20 m^2 x 0.14 m depth = 2.8 m^3 of sediment slurry

= 2,800 1 of sediment slurry

2,800 1 of slurry x 25 μg NH_4^+–N/1/day = 70 mg NH_4^+–N/day

(1.8% of NH_4^+–N in the slurry could be nitrified).

Calculated oxygen demand would be: 70 mg NH_4^+–N x 3.22 mg O_2/mg NH_4^+–N

= 225.4 mg O_2 consumed in 1 m segment

= 225.4 mg O_2/2800 of slurry = 0.008 mg O_2/1 of slurry/day.

Case II. Nitrification occurs at the water-sediment interface and the nitrifying bacteria involved are present in the upper 1 cm of sediment.

Nitrification in a 1 m segment of the river may be calculated under ideal conditions as:

200 m x 1 m x 0.1 = 20 m^2 of bottom sediment with nitrifiers.
(20 m^2) ($10^4 cm^2/m^2$) (1 cm deep) = 20 x 10^4 cm^3 of sediment with nitrifiers.
(20 x $10^4 cm^3$) (1.25 g sediment/cm^3 sediment) = 25 x 10^4g sediment with nitrifiers.
(25 x 10^4g) (10^6 nitrifying bacteria/g = 25 x 10^{10} nitrifying bacteria.

(25 x 10^{10} bacteria) (7.15 x 10^{-9} mg NH_4^+–N/cell/day)
= 1,790 mg NH_4^+–N/day nitrified.

About 4,000 mg NH_4^+–N are present in the lower 10 cm of the water column (40,000 mg NH_4^+–N are present in the 1 m deep water column). Therefore, about 45% of the NH_4^+–N in bottom 10 cm of water could be nitrified under ideal conditions. If this were the case, the O_2 demand in the bottom 10 cm of water would be: (1790 mg NH_4^+–N) x (3.22 mg O_2/mg NH_4^+–N)
= 5764 mg O_2 consumed per day/2 x 10^4 l of water.
= 0.29 mg O_2 consumed per liter of water per day.

If all NH_4^+–N in water had a chance to interact with sediment, under ideal conditions only 1790 mg NH_4^+–N could be nitrified. Therefore, only about 4.5% of NH_4^+–N in the water column would be nitrified under ideal conditions. In this case, the O_2 demand in the water would be:

(1790 mg NH_4^+–N nitrified per day) (3.22 mg O_2/mg NH_4^+–N)
= 5764 mg O_2 consumed per day / 2 x 10^5 l of water.
= 0.029 mg O_2 consumed per liter of water per day.

Analysis using Case I suggests that benthic nitrification exerts little O_2 demand on overlying water, whereas Case II analysis indicates that a small but measureable O_2 demand may occur near the bottom as a result of nitrification at the sediment: water interface under ideal conditions. The actual nitrification rate and oxygen demand are likely something between the two extremes illustrated by Cases I and II. Therefore, it seems unlikely that under normal conditions benthic nitrification exerts a significant O_2 demand on the Wabash River although in some localized areas, the O_2 demand may be measureable. Support for this conclusion is given by a series of studies (data not reported) conducted in which water was passed over the surface of Wabash River bottom sediment cores and NH_4^+ uptake and NO_3^- release were measured. Although NH_4^+–N was assimilated by benthic hetrotrophic bacteria, no NO_2^-–N or NO_3^-–N was liberated, suggesting that nitrification was not a significant sink for NH_4^+–N in the Wabash River bottom sediments.

The findings of this study suggest that nitrification rates in the Wabash River are low and that NOD is not a significant factor in the oxygen status of the River. These findings are somewhat difficult to rationalize with other recent studies (Tuffey *et al.*, 1974 and Feinstein and Matulewich, 1977), which suggest significant nitrification potential in shallow streams having rocky bottoms covered with bacterial slimes. However, the Wabash River differs greatly from the conditions observed in the above listed studies and it seems likely that the Wabash River is a relatively poor habitat for nitrifying bacteria. Ammonium disappearance in the Wabash River may likely be explained as a combination of several biological and chemical processes: (i) nitrification, (ii) uptake by aquatic biomass, (iii) ammonia stripping, (iv) absorption by cation exchange sites on sediment and suspended particles, and (v) assimilation by benthic heterotrophic bacteria.

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