Bacterial Examination of Four Borrow Pit Lakes in East Central Indiana

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Introduction

One aspect of aquatic ecosystems often overlooked is the role played by bacteria in nutrient cycling and decompositional processes within the water column. Various bacterial types are involved in transformation of N and S within the water column. Vanderpost (8) has enumerated such physiological types of bacteria as nitrifiers, denitrifiers, ammonifiers, and sulfate reducers within Lake Ontario sediments and waters. Saprophytic bacteria are often considered synonymous with ammonifying bacteria within the water as mentioned by Rodina (6). These are very active in decomposition processes within the lake.

The present study was designed with four goals in mind: to provide data on the water for recreational use from a bacteriological standpoint by MPN values for total coliforms; ascertain bacterial potential for cycling of N and S within the system by determining numbers of sulfur oxidizers, sulfate reducers, dentitrifiers, nitrifiers, and ammonifiers; to determine numbers of saprophytic bacteria within the water most responsible for decompositional processes; and to determine if bacterial stratification exists during the summer months in Dumpert's Lake, the deepest of the four lakes examined. The results of these studies will be related to various environmental parameters as they might affect bacterial potential and numbers.

Description of Study Area

Four borrow pit lakes were selected along I-69 in Indiana for variation in surface area, depth, and general conditions. The lakes are named according to the individual owning the land on which they are located. Clark Lake consists of 1.5 acres with a mean depth of 2.75 m and maximum depth of 3.7 m. Cardinal Lake is 17.2 acres with mean and maximum depths of 1.9 m and 2.5 m respectively. Dumpert Lake is 8 acres having a mean and maximum depth of 2.4 m and 5.5 m, respectively. The fourth lake is referred to as Walter's and has mean and maximum depths of 1.4 m and 1.8 m respectively and a surface area of 4.5 acres. The first three are located in north west Delaware County, Indiana while Walter's is located in southern Grant County, Indiana.

Methods

Water samples were taken at least monthly over the 1976 calendar year. A 2-1 Kemerrer was used for collection of water samples at the water-sediment interface where maximal bacterial activity is known to occur. All samples were taken close to the deepest spot in the four borrow pit lakes examined. Samples were placed in sterile medicine bottles for transport to the laboratory. Samples for the stratification study were taken with a J-Z bacteriological sampler

(Rigosha Co., Rigosha, Japan). This apparatus allows for sample collection at a given depth without contamination from water from other depths within the water column.

Most probable number (MPN) technique was used to estimate numbers of ammonifiers, denitrifiers, sulfur oxidizers, sulfate oxidizers, sulfate reducers, nitrifiers, and total coliforms. Triplicate tubes were inoculated with the appropriate dilutions for MPN determinations. The MPN procedure followed was outlined in *Standard Methods for the Examination of Water and Wastewater* (3).

Appropriate dilutions of the following media were inoculated and incubated at 28-30° C for 3 weeks before results were recorded: Postgate's medium (5) for sulfur oxidizers; Starkey's medium (7) for sulfate reducers; Alexander and Clark's medium (2) for nitrifying bacteria using ammonium as an energy source; Alexander's medium (1) for denitrifying bacteria; and Vanderpost's medium (8) for ammonifying bacteria. Total coliforms were obtained by standard procedures using lactose broth as a presumptive media with incubation temperatures of 37° C.

Results

Of the more than 48 samples taken of the 1976 calendar year only twice did total coliform values exceed one organism per ml. The June sample in Clark's Lake and the October sample in Cardinal Lake, both reached 150 coliform/100 ml sample. Samples taken at drainage ditches and field tiles at their entrance into the lake never surpassed 1 coliform/ml. These sites were only sampled a few months of the year when water was observed flowing in them.

A positive test for sulfate reducing bacteria was the presence of a black precipitate in the screw cap tube filled with Starkey's medium (7). Values obtained over the year (Figure 1) show relatively high values in the shallower

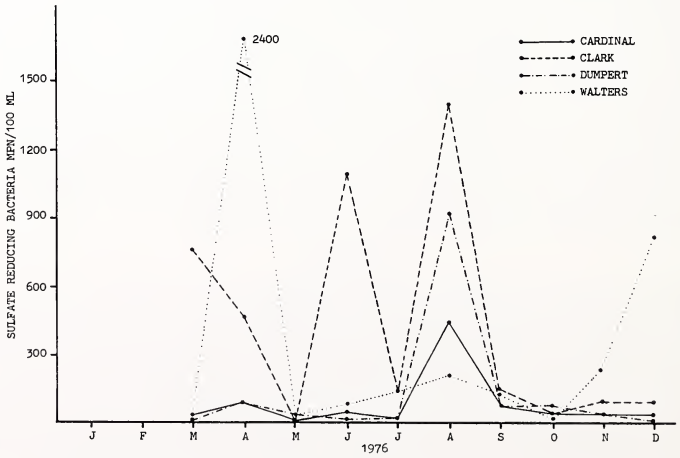

FIGURE 1. *Sulfate reducing bacteria at the sediment-water interface in borrow pit lakes during the 1976 calendar year.*

lakes (Walter's and Clark's). A very black mud sediment was visible in Walter's Lake with the generation of sulfide evident by smell and color. This was not the case in Clark or the other lake sediments.

Sulfur oxidizers were determined by a drop in pH and the presence of a red precipitate in the media described by Postgate (5) for enumeration of these organisms. Initial pH values of 7 and 5 were tried but no positive results were obtained. Members of the genus *Thiobacillus* could be determined by this medium.

The presence of gas and an alkaline reaction were considered positive tests for denitrifying bacteria in Alexander's medium (1). In all lakes greatest numbers were obtained in September (Figure 2). These bacteria utilize NO_3 as a terminal electron acceptor in the limitation or absence of oxygen. In general, numbers decreased substantially during the colder months following the September peak.

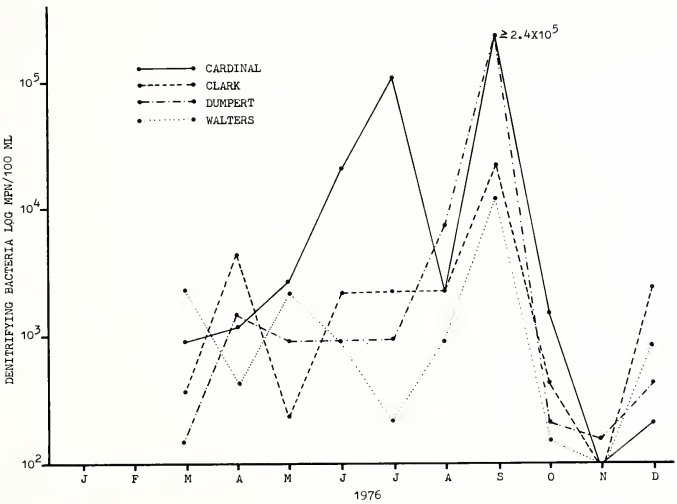

FIGURE 2. *Denitrifying bacteria at the sediment-water interface in borrow pit lakes during the 1976 calendar year.*

A blue color upon addition of Griess-Ilosvay reagent to Alexander and Clark's medium (2) was a positive test for ammonium oxidizing bacteria. Although the organisms were not detected in all samples, highest values (240-1100/100 ml) were observed in April samples. These autotrophic, highly aerobic organisms may have lacked proper oxygen during the experiment although samples were vortexed every third day.

Ammonification, the release of ammonia from nitrogenous compounds, can be performed by a diverse group of bacteria. A positive test for ammonifying bacteria in the MPN tubes was the appearance of an orange precipitate on the addition of Nessler's reagent to Vanderpost's medium (8). The highest values for ammonifying bacteria occurred primarily in the summer months. December

samples also showed increased values. Values equal to or greater than 2.4×10^6 bacteria/100 ml occurred in the summer months with values of 9.3×10^4/100 ml in Cardinal to 1.1×10^6/100 ml in Clark and Walters as obtained in December. Dumpert had an MPN of 2.4×10^5/100 ml in December. Samples in January and February were equal to or greater than 2.4×10^4 to 2.4×10^5 respectively in the four lakes.

Stratification studies conducted on Dumpert's lake showed no real stratification of the bacterial types studied (Table 1). The 4.5 m sample represents the sediment-water interface. Care was taken to collect the water samples for stratification from the surface to the deepest sample, in that order to avoid mixing of the column by the sampling procedure.

TABLE 1. *Enumeration of Bacterial Types (MPN/100ml) in Dumperts Borrow Pit Lake at varying depths during June and July 1976.*

June sample Depth (M)	Coliforms	Nitrifiers	Denitrifiers	Ammonifiers	Sulfate reducers
0	4	<30	90	1,100,000	3
1.5	9	<30	230	210,000	21
3	4	<30	110	21,000	240
4.5	< 3			2,400,000	53
July sample					
0	15	<30	—	2,400,000	9
1.5	9	<30	70	150,000	93
3	4	<30	90	150,000	93
4.5	< 3	<30	< 30	12,000	150

Discussion

Data obtained on coliforms indicate the borrow pit lakes to be relatively free from sewage pollution. The recreational value of these lakes are not jeopardized by contamination from domestic or human sewage. However, further tests should be conducted to determine the safety of the water for drinking and swimming purposes.

The bacterial cycling of sulfur in the lake is exemplified by the sulfate reducing bacteria. These bacteria metabolize most efficiently in the shallow, relatively small borrow pit lakes examined (Clark and Walters). The values obtained for these organisms will probably increase with age of the pits as silt and other debris slowly fill in the pit. The high numbers point to the accelerated eutrophy most likely occurring in these bodies of water. Their presence also points to possible taste and odor problems, as well as corrosive qualities, often associated with members of this group.

Sulfur oxidizers such as *Thiobacillus novellus* are probably active in the bodies of water, although no results were obtained to indicate this. Enrichment studies have shown the existence of photosynthetic bacteria possibly active in this S transformation.

The increased numbers of denitrifying bacteria obtained over the warmer months supported studies conducted by Owens and Nelson (4) on selected farm ponds in Indiana. They reported the effects of various parameters on denitrification processes in surface waters. Using water from the sediment-water interface shows higher numbers (4, 7) and also a greater potential for microenvironments of an anaerobic nature required for identification. Nitrate runoff from adjacent fields may also have stimulated the increased numbers obtained during the warmer summer months.

The peak in nitrifying bacteria in April may be associated with application of ammonium fertilizer to nearby fields with subsequent runoff into the borrow pit lakes.

The number of ammonifying bacteria were high in nearly all samples taken. This is indicative of the high counts of saprophytic bacteria existing in lakes (7). This group of bacteria display potential degradative qualities that may be active in the borrow pit lakes.

Typical bacterial stratification in lakes should show highest bacterial numbers at the sediment-water interface with second highest values at the water-air interface. This is not evident with the physiological types examined. However, oxygen sensitive organisms, such as sulfate reducers, do show an increase with increasing depths.

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