Characterization of the Biological Processes Resulting in Decreased Nitrate Concentrations seen in DMWW Off-River Storage Reservoirs

Dennis Hill - 2004

The nitrate reducing phenomenon that occurs in DMWW's off-river storage reservoirs; however, it has yet to be determined just what organisms are truly involved in nitrate reduction. This study was designed to address the question. It also summarizes what we have learned from our studies this year, and what findings may be applied at DMWW to help us produce more quality water and save money.

The experimental pond study performed during 2004 used Crystal Lake water spiked with Raccoon River water. The study suggested that native bacteria were primarily responsible for the reduction in nitrate concentrations. The nitrate concentration decreased steadily as the green algal blooms waxed and waned. Bacteria that were isolated from the water and tested in the laboratory proved to be able to consume nitrate, but no further correlation was drawn in that study. This, however, did not eliminate the role of algae from the complex biology of nitrate reduction in natural waters.

Method:

To help delineate algal and bacterial nitrate reduction, four flasks of Crystal Lake water were prepared. The lake water was spiked with phosphate at 0.3mg/L and nitrate at 5mg/L. Small amounts of these nutrients were already in the lake water.

One flask was filled with natural (unaltered) Crystal Lake water; another was filled with filter-sterilized Crystal Lake water, which was then inoculated with *Pseudomonas aeruginosa* and *E. coli*; another flask was filled with autoclaved Crystal Lake water, which was then inoculated with *Pseudomonas aeruginosa* and *E. coli*; and the last flask was filled with autoclaved Crystal Lake water, which was kept in a sterile state.

The unaltered lake water was included to study its microbial and chemical characteristics.

The filter-sterilized water was used as a lake-water medium without the original microorganisms, yet with an inoculum of defined nitrate-reducing bacteria as an

attempt to determine nitrate reduction by bacteria. Its organic compounds were saved from any heat-degenerating effects.

The autoclaved water was used as a lake-water medium without the original microorganisms, and with an inoculum of defined bacteria as was the preceding water. Its organic compounds may have suffered from some heat degeneration; however, autoclaving was necessary to eliminate non-filterable bacteria or archaea (a newly characterized microbe with unique and largely unstudied properties).

The autoclaved water without bacterial inoculation was used as a control to detect the possible presence of nitrate consumption via an unanticipated pathway.

After sampling the flasks for anion studies, all of them were then placed outdoors in the sun to incubate under moderate summertime sun and temperature conditions.

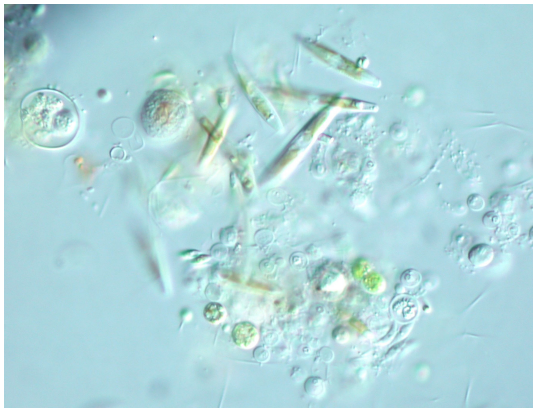
Samples for nitrate determination were taken at three days and at ten days. At ten days, the water was also cultured for bacteria to determine what species were present in the water of the flasks.

Conventional media support the growth of most bacteria of present concern to our industry; however, they do not support some fastidious bacteria and archaea. To determine the possible presence of these organisms, the unaltered lake-water was subcultured using a technique this author developed a few years ago to isolate otherwise unculturable organisms. The water is used as the matrix as well as the inoculum, but agar is also added to cause the medium to solidify. The nutrients of this *natural matrix medium* are essentially what are in the water being tested. The microbes surviving and growing in the water are able to grow without nutrient changes that often selectively inhibit the growth of the more fastidious species.

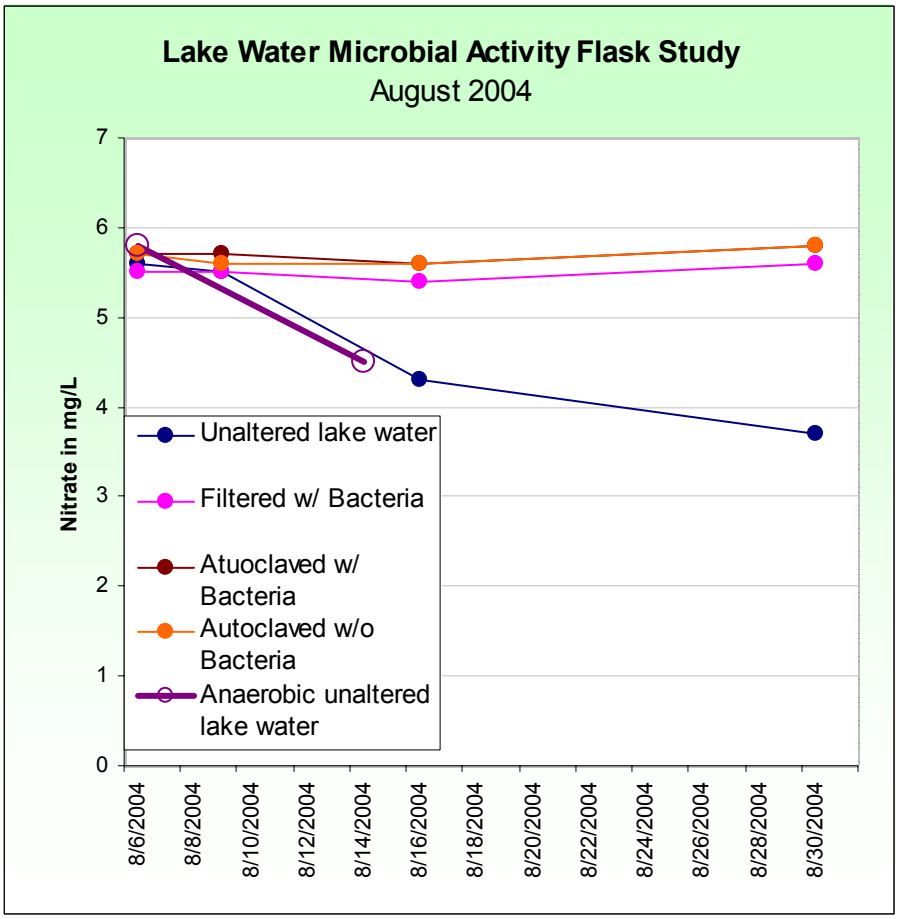
A fifth flask of autoclaved water with added lake bacteria was set up and incubated anaerobically, using a GasPak anaerobe jar system and incubated for 8-days at room temperature.

Results:

After three days incubation, there was no decrease in nitrate values. At ten days, no aerobic flask showed a decrease in nitrate concentration except for the unaltered lake water, which underwent a decrease of 1.2 mg/L, representing a 21% change. The available phosphate for this sample was also used to an undetectable level. The nitrite level did not increase. It was also visually obvious that algal growth had occurred. Microscopically the growth proved to be composed of planktonic algae, protozoa, and bacteria.



Small volumes of water are easily saturated with oxygen, so bacterial denitrification was unlikely in the aerobic flasks. (As shown in an earlier study with the Crystal Lake experimental ponds, once this volume is sufficiently large, such as 10-gallons or more, mid-water anaerobic conditions can easily develop and allow denitrification.) However, nitrate uptake by green algae for amino acid construction does not require a reduced oxygen level and may take place despite the water being shallow or low in volume.



Therefore, since there was algal growth in the lake water with algae (accompanied by bacteria and protozoa), it is apparent that the decrease in nitrate in that flask was due to algal nitrate assimilation.

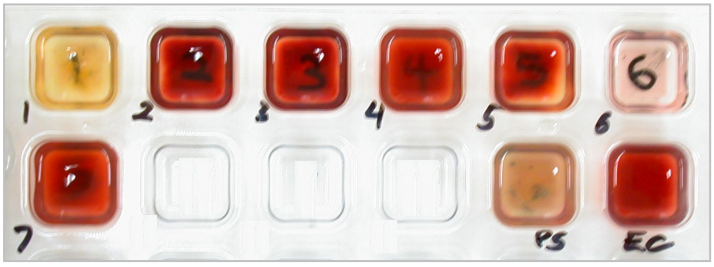
It is important to keep in mind that bacteria are ten times more effective than algae when they do reduce nitrate levels, because the metabolic process is very different. Bacteria use nitrate as a source of oxygen, whereas algae use the nitrogen of nitrate as a nutrient to build amino acids. Bacteria can have a continuous demand for oxygen, while algae have a measured need for nitrogen.

The water from the fifth flask that was incubated anaerobically was tested and showed a reduction in nitrate from 5.8mg/L to 4.5mg/L, a 1.3mg/L decrease. This was accompanied by a nearly equivocal increase in nitrite and no change in the phosphate level. The nitrite indicates that the bacteria present in the flask were unable to complete the conversion of nitrite to nitrogen gas; however, as the nitrate broth studies show, where some bacteria just convert nitrate to nitrite, there are always others present that can complete the process by converting nitrite to nitrogen. Phosphate apparently is not as important for bacterial denitrification as it is for algal nitrate assimilation.

All of the flasks of water were subcultured to conventional media to determine what wild bacteria might be present, and if the *Pseudomonas aeruginosa* and *E. coli* that had been added to two of the flasks had remained viable.

All of the flasks of water grew bacteria, except the autoclaved sample without added bacteria. The *Pseudomonas aeruginosa* and *E. coli* survived but did not thrive; however, other bacteria that were from the lake water apparently proliferated in the membrane filtered sample. They apparently were minute enough to pass through the filter that normally catches coliform bacteria with thoroughness.

Various bacteria were isolated from the subcultures and added to separate wells of nitrate broth. This broth contains nitrate and allows its user to determine a bacterium's ability to reduce nitrate to nitrite and/or nitrogen gas. Reagents are added in a two-step procedure. Red wells indicate bacteria that reduced nitrate to nitrite. Clear wells indicate bacteria that reduced the nitrate all the way to nitrogen gas. This test shows that there are sufficient species in the lake water to accomplish both metabolic processes.



Two new natural matrix medium (NMM) plates were poured using the lake water from the flask. One was incubated at 35 °C in the dark and one was incubated at 25 °C in the light. The plate in the dark grew micro-colonies of bacteria and few algae due to the lack of photosynthesis enabling light. The plate in the light grew micro-colonies of algae as well as of bacteria.

Both NMM's grew many colonies equaling 15,000 colonies/ 100ml and of a mix relative to the fresh water used at the start of this study. The numbers of bacteria and algae were nearly equal.

Summary:

One of the fundamentals of microbiology is that if there are nutrients available, there will be bacteria present to take advantage of those nutrients, despite what or where the nutrients are. We have seen this in our rivers and lakes and I believe that we will be able to make them work to our benefit.

Below are some final conclusions that can be drawn from the various experiments that I have run this year on Crystal Lake water as well as on the rivers and gallery ponds, that may be used as guides for nutrient removal, increased water production, and decreased purification costs. I believe that we can now confidently pursue using Crystal Lake and the gallery ponds as nutrient reducing reservoirs.

2004 Lake and Pond Microbiology Studies Conclusions

1. Experimental pond and flask studies show that both algal nitrate assimilation and bacterial denitrification are readily taking place in the complex biology of lakes and ponds.

2. The anaerobic conditions needed for bacterial denitrification are easily achieved at relatively shallow depths in natural bodies of water, even where there is active surface water aeration and no soil.

3. Sufficient aeration and especially sufficient water movement helps control cyanobacterial growth in favor of beneficial green planktonic and filamentous algae.

4. The algae, cyanobacteria, heterotrophic bacteria and protozoa each may have some limits to their metabolic abilities, but they are able to work together to eventually utilize available nutrients to the maximum potential. An example would be the conversion of the nitrate molecule to nitrite by some microbes, and then the conversion of nitrite to nitrogen gas by others.

Dennis Hill, September 29, 2004