Nitrogen Removal from Wastewater in Microalgal-Bacterial Treatment Ponds

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Abstract Much of agricultural and domestic wastewater contains high concentrations of nitrogen, which can affect public health and have harmful ecological impacts. Microalgal-Bacterial treatment ponds have generally been viewed as an effective method of removing nitrogen from wastewater. However, it is not well known under what physical and biological conditions nitrogen can most effectively be removed in the system. This study investigated how seasonal variables, namely water temperature and solar radiation influence the nitrogen removal in microalgal-bacterial treatment ponds. Additionally, the study aimed to elucidate what biological nitrogen removal mechanisms (ammonification, nitrification, denitrification, ammonia volatilization and algae assimilation) were mostly operating in a pond whose wastewater was municipal sewage and in a pond whose wastewater was agricultural drainage. Data of water temperature (°C) and solar radiation (w/m^2) were collected from two Northern California wastewater sources, Panoche and Delhi. Concentrations of nitrogen (mg/L) in four different forms (organic nitrogen, ammonia, nitrite, and nitrate) in the water samples from both sites were calculated. Regression tests were used to predict the relationship between nitrogen removal and seasonal variables. The test showed that the total nitrogen removed from both sites was not significantly correlated with both water temperature and solar radiation. Ammonia volatilization and algae assimilation could be the two major removal mechanisms in ammonia-dominated Delhi, and denitrification was the most important in nitrate-dominated Panoche. This study gives a better understanding of microbial and biological processes in the microalgal-bacterial treatments ponds of Delhi and Panoche.

Introduction

Nitrogen is becoming increasingly important in wastewater management because nitrogen can have many effects on the environment (Halling-Sørensen and Jørgensen 1993). Nitrogen can exist in different forms because of various oxidation states, and it can readily change from one to another depending on the oxidation state present at the time. In the environment, living organisms can accomplish changes from one oxidation state to another. The principal forms of nitrogen are organic nitrogen, ammonia (NH_4^+ or NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) (Reed 1984).

The presence of nitrogen in wastewater discharge can be undesirable because it has ecological impacts and can affect public health. Ammonia is extremely toxic to fish and many other aquatic organisms and it is also an oxygen-consuming compound, which can deplete the dissolved oxygen in water. The Depletion of dissolved oxygen in water is a problem in aquatic ecosystem since maintenance of a high oxygen concentration is crucial for survival of the higher life forms in aquatic ecosystem. Another ecological impact is eutrophication. All forms of nitrogen are taken up as a nutrient by photosynthetic blue-green bacterial and algae. The excessive growth of bacteria and algae due to the increase of the amount of nitrogen discharged into water, contributes to the reduction of the oxygen level in water.

Although nitrate itself is not toxic, its conversion to nitrite is a concern to public health. Nitrite is a potential public health hazard in water consumed by infants (Sedlak 1991). In the body, nitrite can oxidize the iron (II) and form methemoglobin, which binds oxygen less effectively than normal hemoglobin. The resulting decrease in oxygen levels in young children leads to shortness of breath, diarrhea, vomiting, and in extreme cases even death (Kelter et al. 1997). These occurrences are generally associated with disposal of municipal sewage and fertilizer application to agricultural crops (Pinette 1993). The dangers that all these incidents have posed are a clear indication that municipal and agricultural wastewater must be treated before discharge.

The treatment of wastewater in microalgal-bacterial treatment ponds exploits the physical and biochemical interactions that occur naturally in aquatic system to remove nitrogen (Hurse and Connor 1999). The system of microalgal-bacterial treatment ponds has been viewed as effective and low cost method of removing nitrogen from wastewater and are it is widely used all over the world. In north America alone, there are around 7000 microalgal-bacterial treatment ponds in use (Maynard et al. 1999). In theory, the nitrogen in wastewater will be converted to harmless nitrogen gas and will be lost to the atmosphere by going through three major biological transformations during removal of nitrogen in micoralgal-bacterial treatment ponds. The three biological transformations are ammonification, nitrification, and denitrificaion.

In ammonification, microorganisms decompose the organic nitrogen and produce ammonia. In nitrification, microorganisms oxidize the ammonia compounds to nitrite and then to nitrate. The nitrate will be taken up by algae and either be converted to organic nitrogen in their cell tissues (algae assimilation) or will be reduced to elemental nitrogen (N_2) and lost as a gas by denitrification (Oswald 1996). In addition, many studies have been conducted and reported wide variations in the performance of microalgal-bacterial treatment ponds systems. However, there is some disagreement over the mechanisms responsible for the removal of nitrogen in treatment ponds. Hurse and Conner (1991) concluded that nitrification and denitrification were important nitrogen mechanisms in a wastewater treatment pond in Australia. On the other hand, several studies concluded that ammonia volatilization is the main nitrogen mechanisms from wastewater treatment ponds (Silva et al. 1995 and Soares et al. 1996). The differences from these two could be explained by Sørensen's study that explains the advantages and disadvantages of various mechanisms presented for the removal of nitrogen from wastewater. Each mechanism has an effect on a specific form of nitrogen, which can be either organic nitrogen, ammonia or nitrate. Moreover, Gianelli (1971) stated that temperature and light energy were very important factors in microalgal-bacterial treatment ponds because temperature affects the growth rate of algae and algae require sufficient solar energy in order to release oxygen from waster by photosynthesis.

Despite the knowledge from previous studies about the performance of nitrogen removal in microalgal-bacterial treatment ponds, two wastewater treatment lagoons, such as the Advanced Integrated Wastewater Pond System (AIWPSs) of Delhi and the Algal-Bacterial Selenium Removal Facility (ABSR) of Panoche in California, have little date to identify the major nitrogen removal mechanism operating in both ponds and have less examination of the influences of water temperature and solar radiation on the nitrogen removal in the ponds. Therefore, this study is designed to determine which is the major nitrogen removal mechanism for AIWPSs whose wastewater comes from municipal sewage and for ABSR whose wastewater comes from agricultural wastewater. In addition, I examined how the nitrogen removals in AIWPSs and in ABSR correspond to water temperature and to solar radiation.

My hypothesis for each question is as follows: (1) Denitrification will be the major nitrogen removal mechanism for the AIWPSs of Delhi. It is because the wastewater source in AIWPSs is agricultural drainage and it is dominated by nitrate (Tryg 2000, pers. comm.). Ammonia volatilization will be major mechanism for ABSR at Panoche because the wastewater in ABSR is municipal sewage and according to Puckett (1995), municipal sewage predominantly contains ammonia. (2) The nitrogen removed from AIWPSs and from ABSR will be greater at warmer temperature and higher solar radiation, which will stimulate miroalgal and bacteria to grow in the ponds and remove more nitrogen.

Study site The study took place at two sites, the Advanced Integrated Wastewater Pond Systems (AIWPSs) of Delhi and the Algal-Bacterial Selenium Removal Facility (ABSR) of Panoche in California. In AIWPSs, the source of wastewater is municipal sewage. AIWPSs consist of four ponds in series: Advanced Facultative Ponds (AFP), Algal High Rate Ponds (HRP), Algal Setting ponds (ASP), and Maturation Ponds (MP). The AFP combines both aerobic (algae) and anaerobic (bacterial) process in breakdown of sewage. The anaerobic zone, dominated bacteria, serves the general purpose of reducing biochemical oxygen demand (BOD). Nutrients are released to the aerobic zone to be used by algae. The HRP is designed to optimize algae growth and further recycle nutrients. The removal of pathogens is also accomplished at this state. In ASP, algae are removed and in MP it is a stage for irrigation.

The wastewater of ABSR in Panoche comes from agricultural drainage water. Like the AIWPSs, the ABSR is also divided into four ponds: Reduction Pond (RP), High Right Pond (HRP), Dissolved Air Flotation (DAF), and Slow Sand Filter (SSF). In RP, heterotrophic bacteria are able to metabolize carbon and nitrogen and other elements by extracting oxygen from nitrate to yield nitrogen gas. The HRP is designed to grow microalgae, which release oxygen from water to oxygenate the effluent from the RP. In DAF and the SSF algae are removed.

Methods

500 ml of water samples were collected on a weekly basis by the Algae Research Groups of the University of California’s Engineering Field Station in Richmond with Nalgene sample jars washed by deionized water at the primary inflow point where the wastewater comes into each pond system and at each effluent pint from their corresponding ponds. The water samples were

taken from September 2000 through March 2001. I took water temperature (℃) from each site and use solar radiation (w/m^2) data (California Department of Weather Resource 2001, elect. comm.). In order to see the effects of water temperature and solar radiation on nitrogen removal, I kept substrate dose (g, molasses/L) constant because substrate dose could influence alga growth and it might affect nitrogen removal in the ponds systems as well. It was kept at 0.2g, molasses/L during my experimental period.

I determined the concentration of nitrite, nitrate, ammonia, and organic nitrogen, at each pond from the water samples. Tracing the change of concentration of each nitrogen compound from one pond to another pond acts as a guide to conjecture which nitrogen removal mechanism, such as ammonification, nitrification, denitrification, ammonia volatilization, and algae assimilation, occurs in each pond and which nitrogen removal mechanism works better for each site, AIWPSs of Delhi and ABSR of Panoche. Total nitrogen removed from wastewater is defined by the change of concentration of nitrogen in wastewater between the primary inflow point to the final pond. Therefore, the total nitrogen removed from the two treatment ponds systems can be calculated by subtracting the concentration of nitrogen at the primary inflow point from the concentration of nitrogen at the final pond. The total nitrogen removed was analyzed along with water temperature and solar radiation using regression tests to see how total nitrogen removed and the two variables were correlated.

The principle of nitrite analysis is that sulfanilamide is diazotised by nitrite in an acidic solution (pH2-2.5) (Standard Methods 1995). The intensity of the dye color is proportional to the concentration of nitrite in the solution. In the procedure, sample were first centrifuged for 15 minutes to remove the sediment from the sample water, and then filtered through a 0.2-micron pore size syringe filter. Secondly, the samples were diluted with Mille-Q H_2O to let their concentration reach below 1000µg/L after dilution. Thirdly, 10µL of color reagent were injected into each well of the microplate and added 300µl of each sample. The color reagent was made of 800mL of deionized water, 100ml of phosphoric acid, 10g of sulfanilamide and 1 g of N- (1-napthyle)-ethylene diamine dihydrochloride. After a pause of 10 minutes for the color to develop, the microplate was placed on the Dynatech machine, a microplate reader, to calculate the concentration of nitrite.

The principle of nitrate analysis is that the reagent, a mixture of sulfuric and phosphoric acid, produces an intensely purple color when oxidized by nitrate (Standard Methods, 1995). The density of this purple color was measured for the concentration of nitrate in the samples. The samples needed to be centrifuged for 15 minutes and filtered through a 0.2-microne pore size syringe filter. Then the filtered water needed to be diluted with Mille-Q H_2O to make its concentration reach below 15 mg/L after dilution. Next, took 250 µL of diluted samples and 2.5 ml of reagent into the test tubes. Waited for more than 10 minute to allow the color to develop, then transferred 250 µL from each test tube into microplate and calculated the nitrate using the Dynatech reader.

Organic nitrogen is defined as Total Kjeldahl Nitrogen (TKN) minus NH_3. To calculate TKN, using an 800 ml Kjldahl flask the sample was placed inside and neutralized to pH 7and dechlorinated (Hsieh 2000). Selenized Hengar Granules were then added and 300 ml was boiled off. For digestion, 50 ml Kjeldahl digestion reagent was added to a cooled digestion flask. The flask was then heated until white fumes became visible. Once fumes were cleared by the fume hood, the flask was cooled. Once cooled, the contents of the flask was diluted to 300 ml with water and mixed. The flask was tilted away and 50 ml sodium hydroxide-thiosulfate reagent was added. The flask was then connected to seamed-out distillation equipment and swirled. The contents were distilled and 250 ml distillate was collected. The distillate was added with 50 ml boric acid or 0.04 N H_2SO_4 depending on ammonia measurement method. The titrimetric method then proceeded with the addition of 0.02 N HCl to the distillate flask until the distillate became purple. The amount of HCl needed to change the distillate purple was recorded and used in calculating Total Kjeldahl Nitrogen for the sample.

To measure ammonia levels, the ammonia selective electrode method was used (Hsieh 2000). This required the use of an electrometer, an ammonia-selective electrode, a magnetic stirrer, ammonia-free water, sodium hydroxide, 10 N NaOH/EDTA solution, and standard ammonia solution. A 100 ml sample was placed into a 150 ml flask. The stirrer was then inserted and the electrode inserted once stirring commenced. An amount of 1.5 ml NaOH was added to raise the pH above 11. The electrometer was set measure mV and measurement was recorded when the mV reading stabilized.

Results

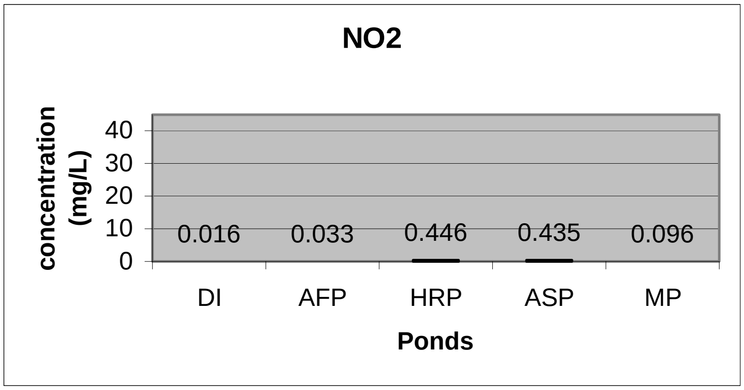
The number of water samples collected during the experimental period in Delhi was 20 and the number of water samples collected in Panoche was 14. Regression tests for each site showed that total nitrogen removed was positively correlated to water temperature and to solar radiation in both AIWPSs and ABSR (coefficients >0) (Table 1). However, they were all insignificance (P-values> 0.05).

	DF	F-value	P-value	R^2	Coefficient
AIWPSs of Delhi Nitrogen vs. Water temperature	Regression=1 Residual=18	1.175	0.2926	0.061	.7
AIWPSs of Delhi Nitrogen vs. Solar Radiation	Regression=1 Residual=18	0.677	0.4214	0.036	0.039
ABSR of Panoche Nitrogen vs. Water temperature	Regression=1 Residual=10	1.317	0.2709	0.116	1.431
ABSR of Panoche Nitrogen vs. Solar radiation	Regression=1 Residual=12	1.606	0.2292	0.118	0.42

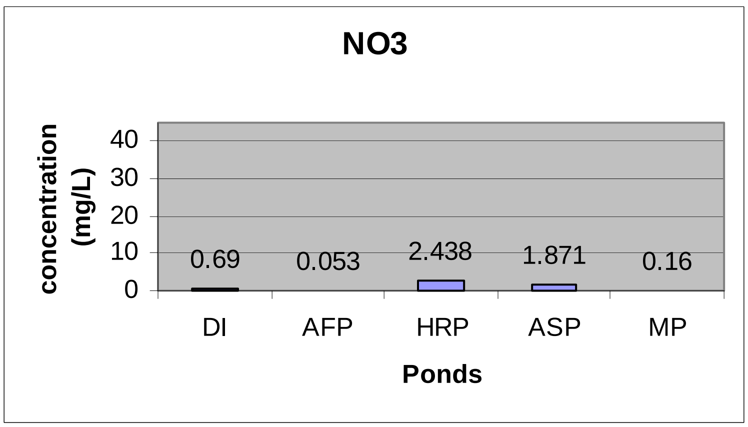
Table 1. The results of regression tests between total nitrogen removed and water temperature, and between total nitrogen removed and solar radiation in Delhi and Panoche.

Figure 1 and 2 summarize the change of average nitrogen concentrations of each nitrogen compound in AIWPSs of Delhi and ABSR of Panoche respectively. In Delhi, the concentration of ammonia at DI (the primary inflow point in Delhi) was the highest of four nitrogen compounds, and it accounts for 76.2% of total nitrogen concentration (Figure 1. (C)). After DI, the concentration of ammonia started decreasing and it was reduced by 65.6 % in the treatment of AIWPSs. The concentration of organic nitrogen, which was the second highest, started increasing after DI and decreased at MP again (Figure 1. (D)). The concentration of organic nitrogen was reduced by 24.3 % in AIWPSs. The concentrations of nitrite and nitrate were very low and were mostly close to zero at all ponds (Figure 1. (A) and (B)).

In Panoche, the concentration of nitrate was higher than other nitrogen compounds at all ponds and it accounts for 83 % of total nitrogen concentration in ABSR (Figure 2. (F)). It was reduced by 58.8 % in ABSR. Similar to ammonia in Delhi, the concentration of nitrate started to decrees after PI (the primary inflow point in Panoche); however, the concentrations of other nitrogen compounds, nitrite, organic nitrogen and ammonia remained at low concentrations throughout the other ponds (Figure 2. (E), (G), and (H)).



(A)



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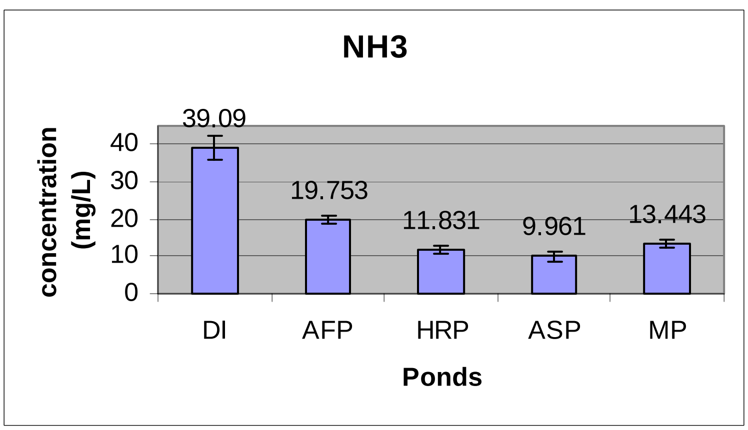
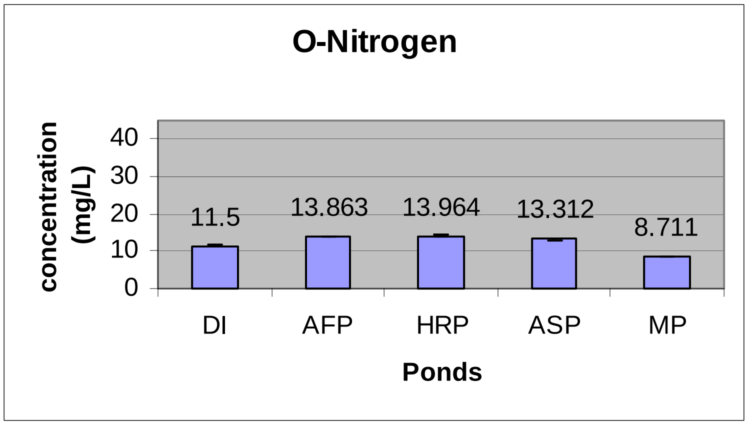
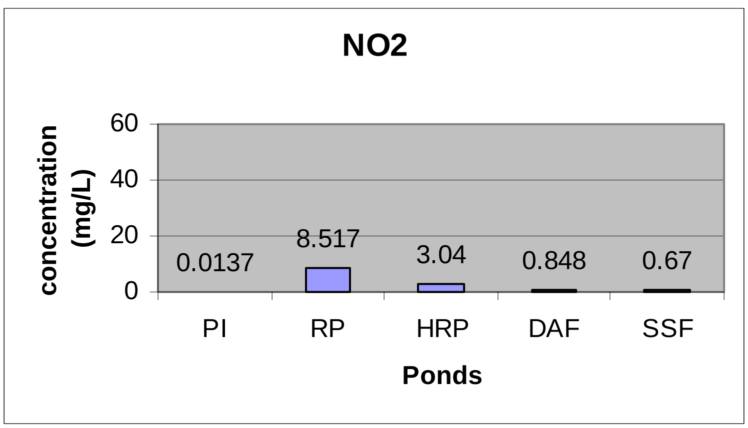


(C)

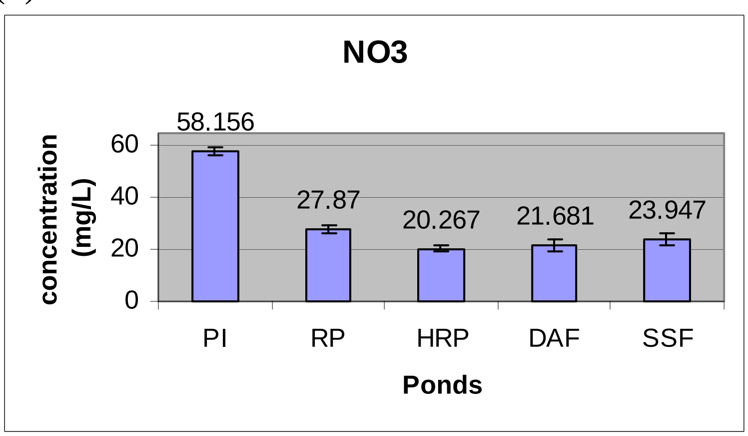


(D)

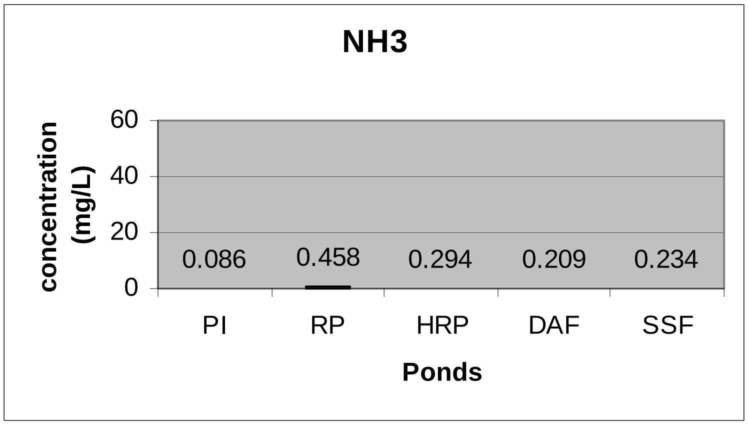
Table. 1 The change of average nitrogen concentrations of each nitrogen compound through five ponds in Delhi. (A) nitrite (B) nitrate (C) ammonia (D) organic nitrogen.



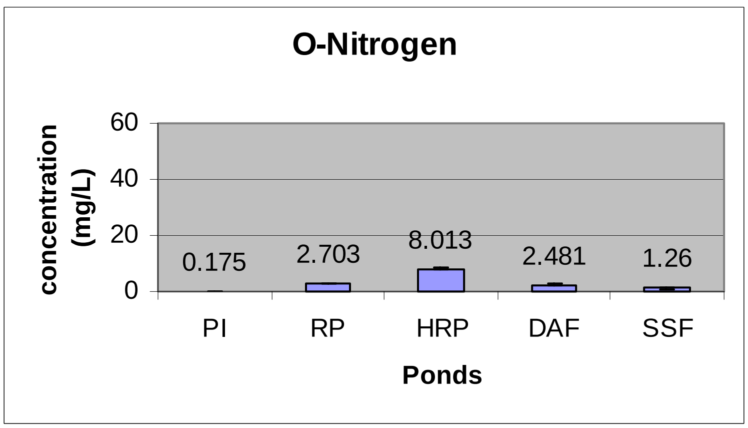
(E)



(F)



(G)



(H)

Figure. 2 The change of average nitrogen concentrations of each nitrogen compound through five ponds in Panoche. (E) nitrite (F) nitrate (G) ammonia (H) organic nitrogen

Discussion

The results of regression tests indicate that the total nitrogen removed in each sites do not increase linearly with either water temperature or solar radiation with significance. Therefore this result rejected my hypothesis, which was that the total nitrogen removed, would be greater at higher water temperature and solar radiation. However there are several considerations that might be the causes of the insignificance result in regression test. It could be because the size of sample was small. Although the water samples were taken every week from September 2000 through March 2001, there were incidents when water samples were not taken from all of the four ponds in the system in both sites. Therefore, the actual number of test samples was small. Additionally, concentrations of nitrogen were not the best way to express how much nitrogen was actually removed from each pond system since it ignored the consideration of flow rate (mass/hydraulic residence time). The flow rates in AIWPSs and ABSR varied during the experimental period. If flow rate is greater, there will be less time for wastewater to be treated in each pond within the system and growth of microorganism rate will be decreased by washing out the microorganisms (Hurse and Connor 1999). It was important to keep the flow rate on the wastewater treatment ponds constant in order to see the effects of water temperature and solar radiation on nitrogen removal since flow rate influences nitrogen removal.

Two major nitrogen mechanisms might be occurring in AIWPSs of Delhi. There was a large reduction of ammonia concentration at HRP while at the same time organic nitrogen concentration increased. Since rapid algae growth was occurring at HRP, it appears that algae are taking up ammonia as their nutrient source. The algae oxidized the ammonia compound to nitrite and then nitrate by nitrification. The reason for the increase of organic nitrogen could be that the nitrate was taken up by the algae and converted to organic nitrogen in cell tissue by algae assimilation. Another possible ammonia reduction is volatilization of ammonia gas. For volatilization to take place, pH values greater then 10 are needed (Maynard et al. 1999). However, pH was not included in the measurement of my study. The pH value from AIWPSs gathered from August 1999 to February 2000 by the algae Research Groups were less than 10 (Tryg 2001, pers. comm.). On the other hand, another study found that in a well-mixed pond, ammonia volatilization would be the major nitrogen removal process even at pH values of 7-8 (Maynard et al. 1999). From these above, it is difficult to conclude where ammonia volatilization is operating or not from the results in my study.

In Panoche, the major nitrogen removal mechanism seems to be denitrificaion. Nitrate would be reduced to elemental nitrogen (N_2) and lost as gas since concentrations of other nitrogen compounds remained at low concentration. However, in HRP organic nitrogen concentration was exceptionally higher then other ponds. It can occur due to the algae growth in HRP since 12 to13 % of alga weight is organic nitrogen (Sedlak 1991). Similarly, in RP nitrite concentration increased as well. It could be because nitrate could be transferred to nitrite since nitrite is an intermediate formation and unstable and can be changed to another form readily (Sedlak 1991). From my study, I can’t prove which pond is the most efficient by simply looking at the pond in which has the largest reduction of nitrogen concentration since I ignored the evaporation and precipitation. These two factors could affect nitrogen removal rate in wastewater treatment ponds. Moreover, there were more variables that might influence nitrogen removal such as dissolved oxygen concentration and alga concentration which were not considered in my study (Halling-Sørensen and Jøgensen 1993).

Overall my study, total nitrogen removal was not significantly correlated to either water temperature or solar radiation. The major nitrogen removal mechanisms in Delhi could be algae assimilation or ammonia volatilization, or both. In Panoche, denitrification was the major nitrogen mechanism. However, more research is required to conclude that whether water temperature and solar radiation influence the nitrogen removal in microalgal-bacterial treatment ponds and what nitrogen removal mechanisms are operating in AIWPSs of Delhi and ABSR of Panoche.

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