

Nitrogen Mass Balance of the ABSR System, and Comparison of Nitrate Removal of its Two Modes

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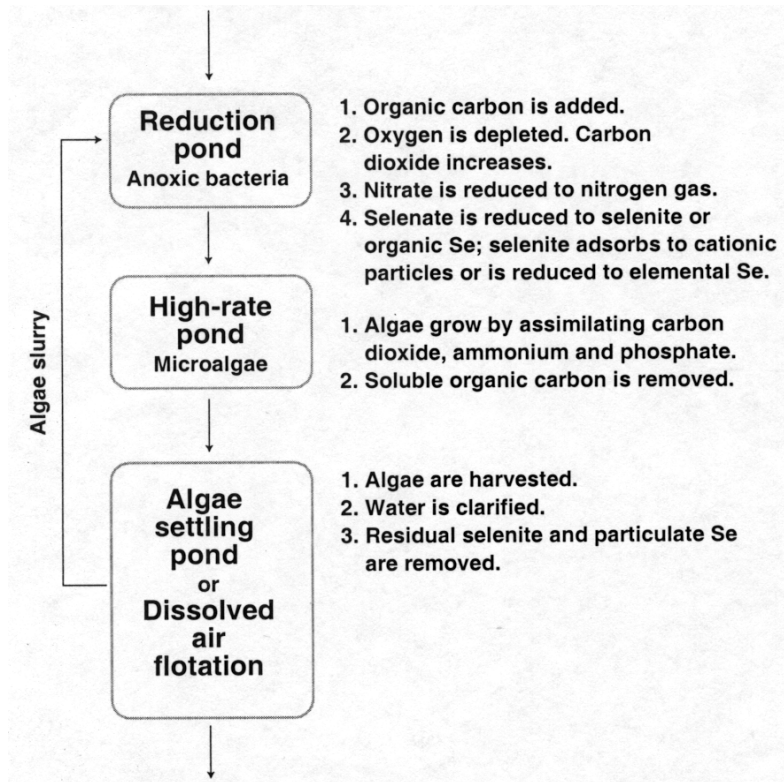
Abstract A technology known as Algal-Bacterial Selenium Removal (ABSR) may solve the problem of removing selenium, a toxic metalloid, from agricultural drainage water. However, to remove selenium, nitrogen compounds must also be removed. This study focused on nitrogen removal and involved two experiments. In the first, I developed a mass balance equation of nitrogen for a high-flow-rate system of the ABSR facility to determine the path of influent nitrate, which comes from fertilizers. Water samples were tested for concentrations of nitrate, nitrite, and ammonia, and the composition of samples of gas bubbling out of the system was measured. The mass balance equation revealed that 30.5% of influent nitrate was unaccounted for in the effluent. The unaccounted nitrate should be nitrogen gas, but nitrogen gas production was calculated to be only 14.4% of the amount expected, indicating a problem with gas collection locations. In the second experiment, nitrate concentrations for a second low-flow system were determined to provide comparisons of the low-flow and high-flow systems under different weather and inflow rates. The low-flow system's effluent nitrate concentration was lower, at 21 mg/L versus 34 mg/L, but it removed a smaller mass of nitrate per day because its inflow rate is 4.5 times lower. Since selenium levels in agricultural drainage water are regulated in terms of mass, the high-flow system is better in terms of selenium removal, because it removes a higher mass of nitrogen, and therefore a higher mass of selenium. The low-flow system is still useful when a low nitrogen concentration is sought to minimize toxicity to wildlife.

Introduction

Selenium (Se) is a metalloid, a chemical element with properties in between those of metals and nonmetals (Wilber 1983). It is required in trace amounts for animal and human nutrition, but it is toxic in large concentrations (Bainbridge et al. 1988). The toxic threat of selenium in agricultural drainage water, in the form of the soluble ion selenate (SeO_4^{2-}), has been recognized since the 1980s, when deformed waterfowl embryos were discovered in the Kesterson Reservoir in the western San Joaquin Valley of California (Ohlendorf et al. 1986). In response, the California State Water Resources Control Board has instated a selenium concentration limit of 5 $\mu\text{g/L}$ for the San Joaquin River, but selenium in agricultural drainage water continues to be well over this level on a regular basis, and enforcement of such regulations is anticipated to become more stringent. This has led to research to find a technological method that removes selenium from the water, but no economically feasible method has yet been found (Presser et al. 1994, LBNL 2000).

One technology that is hoped to be economically feasible is Algal-Bacterial Selenium Removal (ABSR), which was proposed in 1985 by William J. Oswald, professor at the University of California (UC), Berkeley, and is now being tested on a pilot basis (Quinn et al. 2000). The ABSR facility consists of a series of ponds designed to grow algae and bacteria for the main goal of converting selenate to the more easily removable selenite (SeO_3^{2-}) and elemental selenium (Lundquist et al. 1994). The high-rate pond (HRP) is a ring-shaped pond with paddles to circulate the water, in order to maximize algal productivity and bacterial oxidation of dissolved organic matter (Oswald 1988). The reduction pond (RP) is a dark or covered area that allows oxygen depletion, necessary for selenium removal, as well as reduction of nitrate to nitrogen gas by bacterial respiration. This allows for bacteria to convert selenate to selenium precipitates which settle in the RP, or take selenate up in their cells. Before the water leaves the facility, it goes through an algae settling pond, or dissolved air flotation, which harvests the settled algae and pumps it back into the RP for bacterial consumption, and also filters out residual selenite and particulate selenium (Quinn et al. 2000, LBNL 2000). The ABSR facility consists of two systems, South and North, or modes one and two respectively, that represent two different means of selenium removal (Fig. 1).

North system



South system

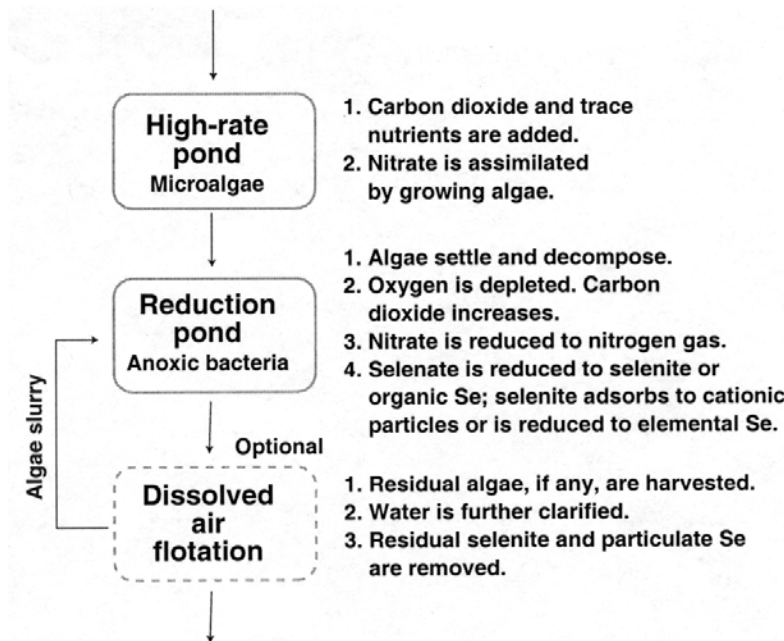


Figure 1. Flowchart of the ABSR system, and the functions of its ponds (Quinn et al. 2000).

First is the South system, which puts the HRP before the RP. The North system, on the other hand, puts the RP before the HRP. The two systems differ in that the North system receives a supplement of molasses for bacterial consumption, whereas the South system does not. Additionally, in the North system, bacterial metabolites from the RP, such as ammonium, phosphate, and carbon dioxide, flow into the HRP and help algae growth. In contrast, the HRP algae of the South system do not receive these metabolites because the RP is placed after the HRP. Carbon dioxide, phosphate, and trace nutrients must be manually added to the HRP to have maximum algal growth (Table 1). The addition of molasses adds to the cost of operation of the North system, but allows it to handle an inflow rate that is 4.5 times higher than that of the South system (Lundquist 2002 pers. com.).

	North	South
Inflow rate (L/day)	54130	11920
Bacterial food sources	Carbon from molasses and algae	Carbon from algae
Algal food sources	Bacterial metabolites directly from RP	Manual addition of CO ₂ , phosphate, and trace nutrients
Molasses added?	Yes	No
HRP conversions	Unconverted nitrate, nitrite, ammonia, biomass N	Unconverted nitrate, nitrite, ammonia, biomass N
RP conversions	Same as HRP along with nitrogen gas	Same as HRP along with nitrogen gas

Table 1. Summary of the similarities and differences of the North and South ABSR systems (Quinn et al. 2000, Lundquist 2002 pers. com.).

Besides removal of oxygen, another condition that must be met to remove any selenium from agricultural drainage water using ABSR is that nitrogen must also be removed (Gerhardt et al. 1991). This is because bacteria gain more energy from reducing oxygen and nitrate than from reducing selenate, so they preferentially reduce oxygen and nitrate, which hinders removal of selenium (Lundquist 2002 pers. com.). Nitrate is abundant in agricultural drainage water due to the use of fertilizers (Blevins et al. 1996). The ABSR facility is designed to remove both selenium and nitrogen from the water. The nitrate entering the facility can leave in several ways. In both the North and South systems, denitrification occurs in the RP and the resulting nitrogen gas escapes into the atmosphere. In both ponds of both systems, some of the nitrate is

assimilated by the algae and bacteria, which are filtered out of the effluent. These two pathways are not problematic. However, in both ponds of both systems, some of the nitrate is converted to nitrite and ammonia, and some of the nitrate remains unconverted (Lundquist 2002 pers. com.). This is problematic because nitrate and nitrite inhibit selenium removal, and nitrite and ammonia are toxic to aquatic species. Nitrate also causes harm to several species and is the main contributor to eutrophication (Johansson et al. 2001, Bruning-Fann and Kaneene 1993, McIssac et al. 2001).

The Applied Algal Research Group of UC Berkeley (AARG) has continuously recorded the level of nitrate and selenium removal of both the North and South systems. During 1997-98, nitrate levels were reduced by more than 95%, and soluble selenium levels were reduced by 92%, but effluent selenium concentration of 32 $\mu\text{g/L}$ was still over the 5 $\mu\text{g/L}$ limit (Quinn et al. 2000). One way to investigate the shortcomings of the ABSR system is to create a comprehensive mass balance equation of the system. Although many measurements on nitrate and nitrite levels have been made by the AARG, no measurements on nitrogen gas have yet been taken. Secondly, these past measurements were taken under different inflow rates and weather conditions than those of my study, necessitating new measurements to construct the mass balance equation, and new nitrate removal comparisons. This is because the new flow rates affect hydraulic residence times of the facility, and thus change the amount of time bacteria have to react with the selenium, nitrate, and molasses. Also, the weather affects temperature and solar insulation, which are important variables affecting growth rates of the algae and bacteria.

My study is part of ongoing work by the AARG, and the goals of my study are to construct a comprehensive mass balance equation of the North system of the ABSR facility, and to compare nitrate removal of both the North and South systems. I hypothesize that a majority of the mass of nitrate as nitrogen (nitrate-N) will be converted to nitrogen gas. I also hypothesize that the North system will remove a higher mass and concentration of nitrate than the South system, because molasses is an easier substrate for bacteria to use than algae. Another reason is that the North system receives 200 mg/L carbon in the form of molasses, whereas the South system receives only 50 mg/L carbon in the form of algae (Lundquist 2002 pers. com.). It is hoped that this study will give clues toward the development of an economically successful selenium removal method, which is important because it will help eliminate the toxic threat of nitrogen

and selenium to wildlife and humans, and also help ease the difficulties of compliance with environmental laws related to selenium in agricultural drainage water.

Methods

The AARG collected one set of samples each week, from October 2001 to March 2002, from the pilot ABSR facility in the Panoche drainage district near Firebaugh, CA. A set consists of 1-4 syringes of gas samples, and 3-8 water samples that are collected from the influent and effluent of the various ponds of the facility. This is done to compare the relative performance of the HRP and RP. Due to a pump failure, samples from the South system could not be regularly collected until February, so only data from February and afterward was used for the nitrate removal comparison. In contrast, for the mass balance equation, all of the data collected from the North system that was collected was used. The samples that are collected were brought to the Lawrence Berkeley National Laboratory (LBNL) for analysis.

The water samples were first filtered through a 0.22-micron filter to eliminate color and other interferences to the colormetric tests that are used to determine nitrate and nitrite concentrations. The filtered water was stored in 50 mL plastic vials at 4°C until analyzed. Three drops of hydrochloric acid were then added to lower the pH to 2, and kill any algae or bacteria that could still be manipulating the selenium or nitrate.

For the colormetric tests, a commercially made reagent (Szechrome NAS, Technical Data Bulletin 239, Polysciences, Inc.) was added to the water samples, which were diluted with deionized water. For analyzing nitrate-N plus nitrite-N (hereafter, “nitrate” refers to nitrate plus nitrite unless otherwise noted), 250 μ L of the mixture was then added to the wells of a microplate. The microplate was then put into a microplate reader (Dynatech, Inc.) which determines the concentration of nitrate-N according to the color density of the reagent. To confirm the presence of nitrite itself, nitrite-N concentrations were also determined by a colormetric analysis with optical density read by the microplate reader (Method 4500-NO₂⁻-N; APHA 1995).

To measure gas composition, a gas chromatograph, connected to a chart recorder, was used. The gas chromatograph, through a column, separates the gases that were injected into an input tube attached to the chromatograph using the gas sample syringes. For calibration, syringes of Scotty Standard 234 (containing a 5% nitrogen gas content), and Scotty Standard 237 (containing

a 66.5% nitrogen gas content) were used. The chart recorder marks peaks on a graph over a period of ten minutes, with peak height proportional to the concentration of the individual gas (carbon dioxide, oxygen, nitrogen, or methane). The peak heights of the standards and the samples were used to calculate the gas compositions of the samples.

The ammonia selective electrode method (Method 4500-NH₃; APHA 1995) was used to measure concentration of ammonia as nitrogen (ammonia-N). In this method, a 100 mL sample is transferred into a 150 mL flask. The flask is stirred with a magnetic stirrer, and 1.5 mL sodium hydroxide is added to raise the pH above 11 and convert ammonium to ammonia. The potential measured by the ammonia selective electrode was converted to an ammonia-N concentration. One of the measurements was then randomly selected and taken again for quality control.

After the concentrations of nitrate-N for each sample set were determined, they were averaged, and multiplied by the appropriate water inflow rate to provide a mass. The effluent masses and concentrations were subtracted from those of the influent, and the resulting figures were compared between the two systems using a Wilcoxon matched pairs test.

For the mass balance equation, the effluent mass of ammonia-N was calculated in the same way as that of the nitrate-N, and subtracted from the influent mass figure. The concentration of nitrogen taken up by algae and bacteria (biomass N) was estimated by Lundquist at 5 mg/L. The corresponding mass was also subtracted from the nitrate-N mass figure. The remaining mass that was unaccounted for was expected to be the nitrogen gas that has bubbled out of the system.

Results

	North	South
Inflow rate (L/day)	54130	11920
Influent concentration (mg/L)	68.3	68.3
Effluent concentration	34.1	21.4
Difference	34.2	46.9
Influent mass (g/day)	3699	815
Effluent mass	1843	255
Difference	1856	560

Table 2. Nitrate-N removal comparison of the North and South ABSR systems. Figures are averages. Data from February 2002 and afterward is taken into account.

For the nitrate-N removal comparison between the North and South ABSR systems, it can be seen that because of its higher inflow rate, the North system is superior in terms of mass of nitrate-N removed, but the South system is better in terms of concentration of nitrate-N (Table 2). The Wilcoxon matched pairs tests reaffirm this, with the p-values for both concentration and mass being .0431.

	Mass per day (g)
Influent nitrate-N	3475
Effluent pathways :	
Unconverted nitrate-N	1802
Ammonia-N (estimated)	162
Biomass N (estimated)	271
Nitrogen gas	179
Unaccounted for	1061
Total effluent	3475

Table 3. Nitrogen mass balance of the North ABSR system. Figures are averages. All data (from October 2001 to March 2002) is taken into account.

The mass balance equation for the North system was not complete (Table 3). Subtracting 64.2 mg/L (October-March influent nitrate-N concentration) from 22.9 mg/L (October-March average effluent nitrate-N concentration) yields a difference of 41.3 mg/L, which can be multiplied by the flow rate to give a figure of 2235 g/day. Biomass N was estimated by Lundquist to be 5 mg/L. Technical error has left me with only three ammonia tests with doubtful success because the experimental quality control standards of the ammonia tests were not met. Therefore, Lundquist has estimated the effluent ammonia-N concentration to be approximately 3 mg/L. If we assume that corresponding amounts of the nitrate were converted into ammonia and taken up by the biomass, we can convert the biomass N to 271 g/day, and the ammonia-N to 162 g/day. We can subtract these figures from 2235 g/day, leaving 1802 g/day as the mass of unconverted nitrate. Comparing this with 3475 g/day nitrate-N in the influent, it should be that the remainder (1240 g/day) has been converted into nitrogen gas. However, only 50 mL of gas was collected per week, and approximately 80% of the gas was composed of nitrogen. This can be converted to 40.5 g/day N, or 179 g/day nitrate-N, which can be subtracted, leaving 1061 g/day nitrate-N, or 30.5% of influent nitrate-N, unaccounted for in the effluent. The mass balance results also refute my original hypothesis, because even if all 1240 g/day of the

unaccounted nitrate-N was converted to nitrogen gas, it would mean that only 35.6% of the mass of influent nitrate-N was converted.

Discussion

Sampling There were several shortcomings in the results of the experiment. First is the number of sample sets that were collected. Considering that the sample sets were being collected once a week from October 2001 to March 2002, I anticipated over twenty sample sets to be collected for both the North and South systems, which would provide enough data for a statistical comparison. Unfortunately, the pump failure in the South system has prevented the AARG to collect any samples from it until February, leaving only five matched North-South pairs of data to perform a comparison. Although the Wilcoxon matched pairs test showed significance, it would be best to continue the study until enough matched pairs are available to have normally distributed data, required for a t-test.

Nitrite and Ammonia A missed step in following the procedure for making the colorimetric nitrite test standards ruined the validity of all but one of the nitrite tests that I have performed. The failed nitrite tests can not be repeated because they must be performed as soon as possible after sample collection, because nitrite in the sample will convert into other compounds over time (Method 4500-NH₃; APHA 1995). The one successful nitrite test is still useful, because it confirms the presence of nitrite in the effluent, meaning that the effluent is toxic to fish. This indicates a major shortcoming in the application of ABSR, which must be minimized.

Because of failures in the operation of the ammonia probe and in the making of ammonia test standards, only three ammonia tests were conducted. Unfortunately, their success is doubted because the repeat measurements that were taken for quality control of the experiment did not fall within 10% of each other. This may indicate a faulty ammonia probe or a faulty experimental procedure because only 20 mL of sample was used to conserve the water sample for future studies, instead of 100 mL as in previous studies. The 20 mL may be too little for the ammonia probe to make a correct measurement. Because of this, we have used previous studies to estimate the effluent ammonia concentration at 3 mg/L. With more time, successful ammonia tests can be completed and replicated, allowing for a more accurate mass balance equation.

Selenium and Nitrate Since the California State Water Resources Control Board has set up selenium level regulations in terms of mass (5 µg/L), the North system is better in terms of

selenium removal because it removes a larger mass of nitrogen, thus allowing it to remove a larger mass of selenium. The South system is still useful though, because it yields a lower concentration of nitrate in the effluent, making it useful when a certain concentration of nitrate must be met in order to prevent the threat of eutrophication, and toxicity to wildlife and humans. Either way, selenium removal is still hindered enough by the presence of nitrate such that the selenium level regulations are still not being complied with as of yet.

An interesting observation is that nitrate concentration in the time period of my study was reduced by only 69.4% in the North system, compared to the 95% reductions that were reported in 1997 and 1998 (Quinn et al. 2000). A possible explanation for this is the weather. The 1997 and 1998 reports included summer months in their records of the performance of the ABSR facility, whereas my study is recording performance during non-summer months (October to March). The higher summer temperatures are believed to contribute to higher removal rates in the ABSR facility.

Molasses and Economics It was mentioned that the molasses added to the North system adds to the cost of its operation, which can bring forth a discussion of economic considerations related to ABSR. Unfortunately, the economic considerations involved in choosing either system are not easily resolved. Molasses is available in the San Joaquin Valley at a wholesale price of \$60-90 per ton (USDA 1999). This may seem like a small price, but it must be remembered that the current ABSR facility is only a pilot facility, and construction of several large-scale facilities for agricultural use is envisioned. The molasses contribute to the higher operating costs of the North system, but these higher costs are offset by the sludge disposal costs of the South system, which have not been mentioned until now. Sludge refers to the settled algae and bacteria that collect in the floor of the ponds, and must be removed periodically from the system. It is produced at a faster rate in the South system. The AARG believes that there is a tradeoff between the lower operating costs of the South system and the higher inflow rate and lower sludge removal costs of the North system (Quinn et al. 2000).

Gas Collection The gas collection, which is being performed by AARG for the first time, has presented a few questions of interest. The first problem to be resolved is the fact that the amount of nitrate converted to nitrogen gas is only 179 g/day, instead of the 1240 g/day that was expected. One explanation for this is that denitrification occurs mostly along certain “sweet spots” along the bottom of the reduction pond. Although the gas collector equipment has been

moved along various points along the pond, it seems that the “sweet spots” have not been found yet. Understanding the makeup of the pond, though, gives clues to the location of the “sweet spots.” The reduction ponds are 35 feet wide, and only the middle seven feet of the width of the pond has a flat bottom. The remainder is sloped, meaning that much of the algae and bacteria have slid down to the flat bottom of the middle seven feet, creating the layer of sludge mentioned earlier. Therefore, most of the denitrification is probably occurring over the flat-bottomed area of the pond. Considering that the gas collector apparatus currently being used is not long enough to reach the middle seven meters, it is probably not located at the “sweet spot” of denitrification. For further studies, a gas collector should be constructed that can reach this “sweet spot.”

The next question of interest regarding gas collection relates to the determination of the composition of collected gas. Nitrogen composition was high, at 70% and above. Carbon dioxide composition was very low, from 0-5%. Oxygen composition was also at levels similar to those of carbon dioxide, except for the September 5 sample set, which had a 10-16% oxygen composition, possibly indicating contamination of the samples with outside air. It was anticipated that the only measurable gases would be carbon dioxide, oxygen, and nitrogen. However, compositions of the three gases added up to an average of approximately 80%. One possibility is human error during the experiment. Water drops were present in all of the sample syringes, possibly from storage of the syringes in water during transport to prevent contamination from outside air. The water is difficult to remove completely from the syringe, so some water is injected into the gas chromatograph along with the gas, possibly affecting the results. Another possibility is human error during calibration of the gas chromatograph. Contamination of syringes may have occurred during collection of the Scotty standards, leading to a calibration line with a negative y-intercept. This may explain the negative oxygen concentrations that appeared on the February 28 sample set, which in turn contributed to a total percentage of approximately 80%. The most intriguing explanation is that some, if not all, of the remaining 20% of the gas is composed of something else that the gas chromatograph cannot measure. In fact, in some of the chart recordings, small peaks were discovered. These peaks are believed to correspond to nitrous oxide or nitrogen dioxide. A further study is recommended to determine the identity of the unknown gas formation.

To summarize, the North system has more potential to be useful in terms of complying with the environmental regulations for selenium concentration, because it removes a higher mass of

nitrogen, allowing it to remove a higher mass of selenium in turn. The South system, in contrast, yields a lower concentration of selenium and is useful when a low nitrate concentration is sought to minimize eutrophication and toxic effects on wildlife. Finally, although the mass balance equation of the North system was not complete, the findings are still useful because they provide clues as to what research questions should be pursued in the future.

Acknowledgments

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