Total Nitrogen Removal in a Completely Mixed Membrane Biofilm Reactor for Nitrification and Denitrification

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Abstract

A Hollow-fiber Membrane Biofilm Reactor (MBfR) was used to nitrify and denitrify synthetic wastewater to remove nitrogenous pollutants such as ammonium, nitrate, and nitrite. Aerobic and anoxic MBfRs were combined to oxidize and reduce these nitrogenous pollutants using biofilms as the catalyst for these chemical reactions. The MBfR is capable of converting ammonium (NH₄⁺) to nitrogen gas (N₂), through a series of nitrification and denitrification reactions in a completely mixed environment. For these reactions to occur, oxygen gas must be supplied into the nitrifying reactor (NR) to oxidize the pollutants into nitrate (NO₃⁻). Hydrogen gas must be supplied to the denitrifying reactor (DR) to reduce the pollutants into N₂ gas. Hydrogen is an excellent electron donor because it is inexpensive, nontoxic to humans and leaves no residual in the water. Hydrogen gas is normally a substantial safety hazard, but the hollow fiber membrane’s design eliminates the possibility of an explosion and thereby the danger.

The aerobic/anoxic MBfR was tested to determine the nitrogen removal at several different O₂ pressures while H₂ pressure was held constant. The reactor was also tested at different H₂ pressures while constant O₂ pressure was maintained.

The aerobic/anoxic MBfR was successful for nearly total nitrogen removal (97.1%) from the 50 mg NH₄⁺-N/l medium, which represents the typical nitrogen content of municipal wastewater. Total nitrogen removal was strongly influenced by the O₂ pressure, in that low O₂ pressure (1.25 psi) caused poor nitrification and high O₂ pressure (6.0 psi) caused poor denitrification. Total nitrogen removal was slightly influenced by H₂ pressure, although increasing the H₂ pressures to 5.0 psi provided better denitrification. The optimal gas pressures for O₂ and H₂ gas were determined to be 2.1 psi and 2.0 psi respectively.
Removal of ammonia and nitrate pollution from water bodies is becoming a substantial environmental concern. In general, elevated concentrations of ammonium (NH$_4^+$) and nitrate (NO$_3^-$) threaten aquatic organisms, which are immersed in these pollutants. Ammonia is produced though the formation of fertilizers and chemicals. As a result, large amounts of ammonia are present in agricultural runoff and discharges from industry. Pollution from nitrate is attributed to over-fertilization and intensive livestock farming. These activities contribute to the leaching of nitrate into groundwater supplies and as such have become a health issue. Elevated levels of nitrate in drinking water often cause methemoglobinemia in infants (Maxcy, 1950). As a result, the USEPA set the maximum containment level (MCL) for nitrate to 10 mg NO$_3^-$-N/L.

In most wastewaters, particularly in municipal sewage, the nitrogen pollution is found in the reduced oxidation state as ammonium. In order for reduced nitrogen to be removed, nitrification must take place to oxidize ammonium. If ammonium is released into the environment, this form of reduced nitrogen will consume dissolved oxygen and thus create an oxygen deficiency near the wastewater discharge. Not all wastewaters contain strictly reduced forms of nitrogen. Some industrial wastewaters and agricultural run-off contains a combination of NH$_4^+$ and NO$_3^-$.

In order to remove ammonia and other nitrogenous pollutants from water, two reactions must take place: nitrification and denitrification. Nitrification occurs by the following reaction:

$$\text{NH}_4^+ + 2\text{O}_2 (g) \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$$

The oxidation of ammonium to nitrate creates two acid equivalents (H$^+$) per mole of nitrogen oxidized. Oxygen is required for the oxidation of ammonium and is used as the terminal electron acceptor by the nitrifying bacteria.

In order for denitrification to proceed, nitrate must be reduced. This half reaction is:

$$\text{NO}_3^- + 6\text{H}^+ + 5\text{e}^- \rightarrow \frac{1}{2} \text{N}_2 (g) + 3\text{H}_2\text{O}$$

For the other half reaction an electron donor is required. Electron donors that are often used include acetate, methanol and ethanol. In this study, H$_2$ (g) will be used as the electron donor. The half reaction for H$_2$ oxidation is:

$$\text{H}_2 (g) \rightarrow 2\text{e}^- + 2\text{H}^+$$

By combining these half reactions, the overall denitrification reaction for this experiment is:

$$\text{NO}_3^- + 2.5\text{H}_2 (g) \rightarrow \frac{1}{2} \text{N}_2 (g) + 2\text{H}_2\text{O} + \text{OH}^-$$

The reduction of nitrate to nitrogen gas creates one base equivalent (OH$^-$) per mole of nitrogen reduced.

As illustrated above, nitrification produces two acid equivalents and denitrification produces one base equivalent. When these reactions function individually, large quantities of pH buffers are required to compensate for these acidic and basic products. When these reactions are combined, the acid/base equivalents are partially neutralized, leaving the pH near neutral and reducing the need for a strong buffer.
An innovative process has been developed for the removal of these nitrogenous pollutants using biofilms to carry out nitrification and denitrification reactions. This process uses a Hollow fiber Membrane Biofilm Reactor (MBfR) to treat these pollutants. In order for biofilms (and all microorganisms) to utilize the substrate (nitrogen species), the reactions require a direct gas (H₂ or O₂) delivery into the biofilm. The MBfR is a configuration of hollow fibers that deliver gas to biofilms that grow on the outside of these fibers. The fibers are composed of a 1 µm thick nonporous, hydrophobic polyurethane layer located between a micro-porous polyethylene walls. The dense polyurethane layer forces gas to diffuse through the membrane without forming bubbles. Figure 1 shows a schematic of the hollow fiber membrane.

The high bubble-point pressure allows for a bubble-less transfer of gas to the biofilms located outside of the membrane. The biofilm serves as a place where the substrate in the water and the gas from the membrane meet. The biofilms can directly uptake the gas and the pollutants to produce the desired chemical reaction for pollutant removal. The hydrophobic nature of the membrane prevents fiber clogging from liquid and biofilm infiltration, thereby maintaining the efficiency of the gas distribution.

One incredible benefit of the hollow fiber membrane is that it provides a means of safely utilizing H₂ gas as an electron donor. Normally, concentrations of hydrogen gas create an explosive environment and thus a substantial safety risk (the explosive range for hydrogen is 4 to 74.5% in air). The hollow fiber membrane wall ensures nearly 100% efficiency of H₂ (and O₂) use, thus it eliminates the possibility of hydrogen gas from forming bubbles and sparging from the liquid, and reduces the inherent safety risk of hydrogen gas usage.

Utilizing H₂ gas as an electron donor has many advantages. Of the electron donors available, hydrogen gas is the least expensive donor per e⁻ supplied. Hydrogen gas does not leave a residual in water since it evolves from water when it is at equilibrium with the atmosphere. Hydrogen gas is also nontoxic to humans.

The MBfR is not a new concept. Several papers have been published on its effectiveness with several different types of contaminants. The MBfR has proven to be effective at removing oxidized contaminants such as perchlorate (ClO₄⁻) (Rittmann et al, 2002), nitrate (NO₃⁻) and nitrite (NO₂⁻) (Lee & Rittmann, 2000; Lee & Rittmann, 2002). Up until this point, the MBfR had only been used for oxidized pollutants.
The next evolution for the MBfR was to create a completely mixed MBfR system where the nitrification and denitrification reactions could occur simultaneously. To do this, two MBfRs were combined to complete these reactions in a completely mixed manner. One MBfR was supplied with oxygen gas, to compete nitrification, and the other MBfR was supplied with hydrogen gas, to complete denitrification.

The objectives of this report were to 1) create a completely mixed MBfR system in which nitrification and denitrification would occur simultaneously, 2) determine the effectiveness of the design at different oxygen gas pressures, 3) determine the effectiveness of the design at different hydrogen gas pressures, and 4) evaluate the optimal gas pressures for the design that would provide the highest nitrogen removal from the water.

**Methods and Materials**

**Medium**

The medium used for this experiment was synthetic wastewater. The list of ingredients is found in Table 1. The ammonium concentration was set to be 50 mg NH₄⁺-N/L. The alkalinity of the medium was 525 mg/L as CaCO₃. The buffer capacity of the water was added in the form of 0.252 g/L HCO₃⁻, 0.136 g/L H₂PO₄⁻ and 1.134 g/L HPO₄²⁻. It was assumed that no other buffer was available from the atmosphere (from the carbonate system), since the medium was contained in a N₂ gas sparged bottle.

**Reactors**

The recycling rate in the Nitrifying Reactor (NR) was 50 ml/min and the recycling rate in the Denitrifying Reactor (DR) was 150 ml/min. The wet volume of the individual reactors was 10 ml (with the fibers inside) each.

**Design Configuration**

The series configuration of the reactors is shown below in Figure 2. Each reactor was assumed to be a completely mixed system because of the recycling rates and reactor arrangement. The influent pumping rate was set to 0.11 ml/min. The detention time of the MBfR was approximately 3 hours.
Testing Methods

The parameters measured were NH$_4^+$, NO$_3^-$, NO$_2^-$, and pH. The analytical methods are summarized in Table 2. Each method was calibrated against known standards to ensure measurement accuracy. The Spectronic Spec 20 was the spectrophotometer used to measure the absorbance of the NH$_4^+$, NO$_3^-$ and NO$_2^-$ samples.

Table 2: Summary of Analytical Methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Reference</th>
<th>Range</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$</td>
<td>Chromotropic Acid Method</td>
<td>Hach Method 10020</td>
<td>0-30.0 mg/l NO$_3^-$-N</td>
<td>+/- 1%</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>Diazotization Chromotropic Acid Method</td>
<td>Hach Method 8507</td>
<td>0-0.500 mg/l NO$_2^-$-N</td>
<td>+/- 2.3%</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>Salicylate Method</td>
<td>Hach Method 10031</td>
<td>0-50.0 mg/l NH$_3$-N</td>
<td>+/- 2%</td>
</tr>
<tr>
<td>PH</td>
<td>Electrometric Method</td>
<td>Standard Methods (1998) – 4500-H$^+$</td>
<td>0-14 pH</td>
<td>+/- 0.1 pH</td>
</tr>
</tbody>
</table>

Experimental Procedure

The system was supplied medium for several weeks while the NR and DR biofilms attached to the outside of the hollow fiber membranes. Once a visible layer of biofilms was present, the system was assumed to be in steady state and ready for testing. Experiments were run to determine the nitrogen removal under different gas pressure conditions in the MBfRs. Under constant H$_2$ pressure (2.5 psi), the removal of nitrogen was determined for the following O$_2$ pressures: 1.25 psi, 2.0 psi, and 6.0 psi. Under constant O$_2$ pressure (3.6 psi), the removal of nitrogen was determined for 1.25 psi and 5.0 psi H$_2$ pressure. We allowed the system to reach steady-state performance before adjusting the O$_2$ or H$_2$ pressure. We originally maintained the H$_2$ gas pressure at 2.5 psi while varying the O$_2$ pressure. Next, we varied the H$_2$ pressure while the O$_2$ pressure was maintained at 3.6 psi.

We took samples at least 3 samples for each gas pressure, with a minimum of 9 hours between samples. We filtered each sample (0.45-µm membrane filter) to remove any suspended microbes that detached from the biofilm.

Results and Discussion

Changing O$_2$ Pressure

The first 85 hours of the experiment, which serve as the baseline conditions, are shown in Figure 3 where the H$_2$ pressure was 2.5 psi and the average O$_2$ pressure was 2.5 psi. Figure 3 also illustrates the general trend for nitrate and ammonium as we changed the oxygen pressure between 85 to 280 hours. The ammonium concentration in the effluent
was greatly increased by a low oxygen pressure (1.25 psi). This was due to the limited supply of oxygen in the aerobic reactor (NR), which left ammonium unoxidized. On the other hand, the nitrate concentration was high when the oxygen pressure was increased to 6.0 psi. This was attributed to excess dissolved oxygen concentrations from the NR that was carried over to the DR, where oxygen inhibited denitrification (conversion of $\text{NO}_3^-$ to $\text{N}_2$). Nitrite concentrations were always considerably smaller than ammonium and nitrate and contributed negligibly to the total nitrogen concentration in the effluent. The total nitrogen concentration in the effluent was greatly dependent on the nitrogen species that was most affected by the change in $\text{O}_2$ pressure.

![Graph showing nitrogen species concentrations](image)

**Figure 3:** $\text{O}_2$ pressure effects on nitrogen species concentrations when the $\text{H}_2$ pressure was 2.5 psi. The first 85 hours represent the baseline operation.

Controlling the $\text{O}_2$ pressure to the aerobic reactor was essential for good total nitrogen removal. Proper control of the $\text{O}_2$ pressure allowed excellent removal of all nitrogen species ($<1 \text{ mg N/L}$) and total N ($<3 \text{ mg N/L}$), as shown by the results in the first 85 hours.

*Changing $\text{H}_2$ Pressure*

Figure 4 presents the results when we varied the $\text{H}_2$ pressure while we maintained the $\text{O}_2$ pressure at 3.6 psi. Varying the hydrogen pressure did not alter the concentrations of ammonium and nitrate as dramatically as did the oxygen pressure. While ammonium and nitrite were not affected by the hydrogen gas pressure changes, nitrate concentrations increased substantially when the hydrogen gas pressures were low (1.25 psi). A deficiency in $\text{H}_2$ gas slowed the denitrification in the anoxic reactor, thus leaving $\text{NO}_3^-$ unreduced. On the other hand, increasing the quantity of $\text{H}_2$ gas available in the anoxic reactor did not negatively impact nitrification in the aerobic reactor producing larger nitrogen removal. The total nitrogen concentration paralleled the $\text{NO}_3^-$ concentration.
Gas Pressure Effects on Nitrate and Nitrite

Figure 5 summarizes how the O2 pressure affected the NO3\textsuperscript{−} and NO2\textsuperscript{−} concentrations for all experiments. Clearly, nitrate removal decreases with increasing O2 pressure (Figure 5a). This is due to excess oxygen from the high O2 pressure (6.0 psi) in the NR infiltrating into the bulk solution and carrying over into the DR. Since denitrifying bacteria are facultative anaerobes, they will use the available oxygen as an energy source, instead of hydrogen gas. As a result, they no longer reduce nitrate, allowing it to accumulate in the effluent.

Nitrate removal is also dependent on H2 pressure. As H2 pressure increases, the concentration of nitrate decreases (see Figure 4). At pressures greater than 1.25 psi H2, nitrate removal greatly increases. The hydrogen gas gradient must be great enough to reach the denitrifying bacteria on the water-biofilm interface. If H2 does not reach far enough into the biofilm the rate of removal is substantially less.

Figure 4: H2 pressure effects on nitrogen species concentrations when the O2 pressure was held constant at 3.6 psi.

Figure 5: The effects of O2 pressures on a) nitrate (NO3\textsuperscript{−}) concentration and b) nitrite (NO2\textsuperscript{−}) concentration.
There is essentially no relationship ($R = -0.18$) between $O_2$ pressure and $NO_2^-$ concentration (Figure 5b). Nitrite is an intermediate in the nitrifying reaction and is never a substantial contributor to the total nitrogen in the effluent compared to ammonium and nitrate.

**Gas Pressure Effects on Ammonium**

Figure 6 shows that ammonium concentrations drastically decrease when $O_2$ pressures are greater than 1.25 psi. When the $O_2$ pressure is 1.25 psi or less (Figure 6a), oxygen gas was insufficient to allow full nitrification in the nitrifying reactor. As a result, the removal rates for $NH_4^+$ are low. Once the $O_2$ pressure was greater than 1.25 psi (Figure 6b), ammonium removal did not correlate strongly with $O_2$ pressure, although there is a slight downward sloping trend between the two variables ($R = -0.23$).

There is no apparent relationship between $H_2$ pressure and $NH_4^+$ concentrations (see Figure 4). This is a logical conclusion since the nitrifying bacteria (obligate aerobes) in the nitrifying reactor use oxygen as their electron acceptor and are unaffected by $H_2$ gas.

**Nitrogen Removal Efficiency**

Figures 7 and 8 summarize the total nitrogen removal efficiencies with a fixed influent concentration of 50 mg $NH_4^+$-N/L. At constant $H_2$ pressure (2.5 psi), the total nitrogen removal from the medium is shown in Figure 7. Clearly, an $O_2$ pressure of 2.0 psi produces the largest removal of nitrogen (97.1%). At 6.0 psi, the nitrogen removal is 91.9%. By far, the poorest removal is achieved with at 1.25 psi with a nitrogen removal of 58.1%. This low rate of removal can be attributed to the effect of low dissolved oxygen concentrations in the nitrifying reactor, which was too low for extensive nitrification to occur.
At steady O₂ pressure (3.6 psi), the total nitrogen removal from the medium is shown in Figure 8. At 5.0 psi, the removal is 95.1%. At 1.25 psi, the nitrogen removal is slightly less (92.2%), due to less denitrification. The difference between these pressures is subtle but there is a slight advantage when using a slightly larger H₂ pressure. Controlling H₂ availability in the denitrifying reactor is important for achieving maximum total nitrogen removal, but is not as sensitive as O₂ availability to the nitrifying reactor.

**Optimal Gas Pressures**

To determine the optimal O₂ gas pressure, the average concentrations for nitrite, nitrate, and ammonium were graphed against O₂ pressures. Trend lines from these values helped to visualize the gas pressures that would provide the highest nitrogen removal. Graphically, the optimal O₂ pressure is approximately 2.1 psi. From past experimentation (Lee & Rittmann, 2002), the optimal H₂ pressure ranges were determined to be between 2-4 psi. The optimal gas pressures were found to be 2.1 psi for O₂ and 2.0 psi for H₂.

**Conclusions**

The aerobic/anoxic MBfR was successful for nearly total nitrogen removal from the 50 mg NH₄⁺-N/L medium, which represents the nitrogen content of typical municipal wastewater. Total nitrogen removal was strongly influenced by the O₂ pressure, in that low O₂ pressure (1.25 psi) caused poor nitrification and high O₂ pressure (6.0 psi) caused poor denitrification. Total nitrogen removal was slightly influenced by H₂ pressure, although increasing the H₂ pressure to 5 psi provided better denitrification. The optimal gas pressures for total nitrogen removal were determined to be 2.1 psi for O₂ and 2.0 psi for H₂.

The aerobic/anoxic MBfR has proven to be an effective method of nitrogen removal and deserves further exploration.
References


