The Use of Anammox Bacteria in Nitrogen Removal from Wastewater

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The amount of reactive nitrogen in the environment has increased by 33 to 55 percent globally since the rise of the twentieth century and especially in recent decades. The onset of the industrial and green revolutions has nearly doubled the rate of creation of reactive, biologically available nitrogen in the environment that would have otherwise been limited by bacterial nitrogen fixation.[1] Because nitrogen is generally a limiting nutrient in the environment, this increase in biologically available nitrogen has led to the acidification of freshwater systems as well as the eutrophication of aquatic ecosystems that results in hypoxia or anoxia.[2] Major sources that introduce inorganic nitrogen into the aquatic environment include agriculture, stormwater, and wastewater effluent.[3] This study focuses on improving nitrogen removal in wastewater treatment facilities, and in particular, the use of anaerobic ammonia oxidizing (anammox) bacteria in municipal wastewater treatment facilities.

The anammox process is the conversion of ammonia to nitrogen gas using nitrite as an electron acceptor in the absence of oxygen; the anammox process is performed by autotrophic anammox bacteria. The anammox process is a relatively new discovery, with the first pilot plant being built in the late 1980s. Since its discovery, anammox has primarily been used to treat high strength industrial wastewaters.[4] Now anammox is being considered for municipal water treatment as a more energy efficient method of nitrogen removal. Conventional municipal nitrogen removal involves a nitrification/denitrification process where nitrification requires a significant amount of aeration and denitrification requires an additional carbon source. Because anammox bacteria are anaerobic primary producers that do not require aeration or an additional carbon source and directly convert ammonia and nitrite into nitrogen gas, using the anammox process is up to 90 percent more efficient than conventional nitrogen removal techniques.[5]

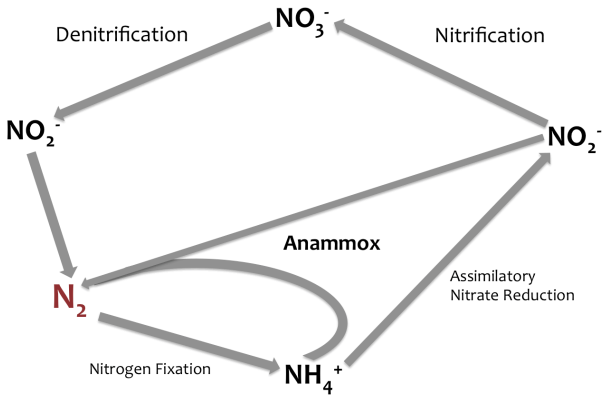

Figure 1: Nitrogen Cycle

For this study, two aspects of the anammox process were examined; the use of anammox bacteria in conjunction with the rock, zeolite, as well as the effects of pharmaceuticals on anammox with and without zeolites. Zeolites are porous aluminosilicate rocks capable of ion exchange and have an especially high affinity for ammonia.[6] The zeolites were used as a mechanism for stabilizing anammox bacterial growth because of their potential to concentrate ammonia in a specific area, making a consistent food source for the anammox bacteria to convert ammonia to nitrogen gas. The anammox bacteria are being tested alongside pharmaceuticals to determine whether the anammox process is inhibited by constituents commonly found in wastewater effluent.

To test these parameters, a series of four conditions were created in triplicate: 1) anammox bacteria alone, 2) anammox bacteria with pharmaceuticals, 3) anammox bacteria with crushed zeolite, 4) anammox bacteria with pharmaceuticals and crushed zeolite. Each of the conditions consist of anammox media that is optimized for anammox bacterial growth and that is developed in an argon headspace.[7] From there, each experimental condition included 5 milliliters of anammox biomass. The conditions with pharmaceuticals consisted of a solution of the 11 most common pharmaceuticals found in wastewater effluent at a concentration of 10 parts per billion.[8] The zeolite conditions each included 4 grams of crushed zeolite. All of the experimental conditions were maintained at a temperature of 37 degrees Celsius in a rotating table incubator to optimize anammox bacterial growth and promote mixing.

Two main aspects were analyzed during this experiment: the nutrient removal rates, as well as the microbial communities in each of the experimental conditions. The nutrients evaluated include ammonia (NH_4^+), nitrite (NO_2^-), and dinitrogen gas (N_2). The ammonia and nitrite were measured through spectrophotometric analysis using HACH reagents, while the dinitrogen gas was analyzed using gas chromatography (GC). The biomass cell communities in each of the bottles were quantified using standard DNA extraction and purification methods that were then processed using quantitative polymerase chain reaction (qPCR) techniques.

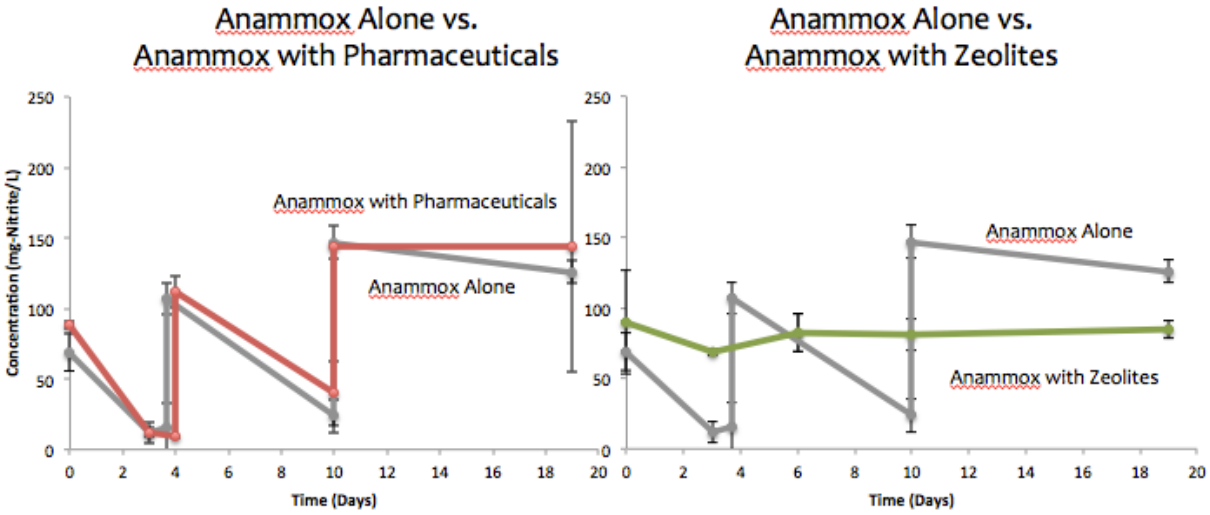

Figure 2: Comparison of nitrite levels between anammox alone, anamoox with pharmaceuticals, and anammox with zeolites

The most interesting results is the comparison of the nitrite levels between anammox alone and anammox with pharmaceuticals, as well as anammox alone and anamoox with zeolites, as seen in Figure 2. Here it is easy to see that the nitrite levels in the anammox with pharmaceuticals are consistent with the nitrite levels in the anammox alone, suggesting that pharmaceuticals have no impact on anammox metabolism. However, the nitrite concentration in the anammox with zeolites varies significantly from the nitrite concentration in the anammox alone, concluding that zeolites influence anammox bacteria's metabolism of nitrite. The cell community quantification results from the qPCR showed that the microbial communities (both anammox and total cells) were mostly stagnant over the course of the experimental period, which may have been due to the use of batch reactors in the experimental design, and suggests that qPCR is not the best method of quantification for this particular style of batch reactors.

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