Adapting Algae to Ammonia Rich Environments for Wastewater Treatment

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Abstract

Cyanothece algae were adapted to have a tolerance of high ammonia environments (about 1200 mg/L) for wastewater treatment. The effects of keeping algae of the same generation in containers with various levels of ammonia content was studied. As that generation was given ample time to adapt to each ammonia-rich environment, the seed from the algae able to survive in the highest concentration was then used as the next generation and was exposed to higher ammonia concentrations (this process is explained later in the paper). The algae and its housing was tested daily to ensure the conditions of each vial remained constant throughout the experiment. Salinity in each jar was kept to 30 g/L, pH was kept at 6.8-7.8. Optical density (ODE) and dry weights were taken to measure algae growth within each container. It was determined in this experiment that the algae could be adapted to an ammonia level of about 1200 mg/L. This experiment was important to the ultimate goal of using the algae for wastewater treatment in that it could be used as a sustainable source in areas where they would normally not be able to survive.

Background

The objective of this research project was to adapt algae to high concentrations of ammonia (1200 mg/L) which ordinarily in nature they would not survive in. The purpose of acclimating the algae is for wastewater treatment. The algae eat nutrients (nitrogen and phosphates) from the waste water which will prevent algal bloom.

Figure 1: example of algal bloom [7]

An algal bloom is a rapid manifestation of algae in a marine ecosystem that can be recognized by the discoloration of the water pigments [7]. These algal blooms can lead to fish dying off, cities having to cut off water to civilians, and the closing of fisheries [7]. These types of blooms which are detrimental to animal and marine life as well as the ecology are called "harmful algal blooms" (HAB) [7]. Without prior treatment of the wastewater, an accumulation of phosphates can occur in nature. As the algae feeds off these nutrients they multiply until they essentially block all sunlight from reaching through the water. This in turn kills plant and animal life of which lives in the ecosystem.

Ammonia (NH_3) is both man-made and found naturally. About 80% of ammonia is used in agriculture as fertilizer but is also used as a refrigerant and in the manufacturing of plastics, explosives, textiles, pesticides, dyes, and more [2]. It is a colorless gas with a sharp odor and easily dissolves in water, where it generally becomes ammonium (NH_4^+). It is easy for ammonia to switch between is gaseous and aqueous forms [1]. We are regularly exposed to ammonia at levels between 1 and 5 ppb (parts per billion) in air [1]. The level of ammonia is rivers and bays are generally under 6 ppm (parts per million) and in soil about 1-5 ppm [1]. OSHA recommendations for the Permissible Exposure Level (PEL) to be 50 ppm as an 8-hr time-weighted average (TWA) [3]. High levels of ammonia pose a health risk if inhaled, ingested, or absorbed through the skin with potential for death. Ammonia GHS classification is shown below [3].

A large portion of ammonia comes from fertilized soils and livestock. This can contribute to nutrient pollution in farmland. Fertilizers and manure are both rich in nitrogen and phosphorus, which can cause an impact on water quality after rains when the chemicals run off into near waters or sink into ground water [4]. This agriculture air pollution generally comes in the form of ammonia from the livestock waste and fertilized fields [5]. After combining with the nitrogen oxides and sulfates from power plants, vehicles, and industrial processes a small aerosol about 2.5 micrometers in diameter can be inhaled and cause pulmonary disease where there are at least 3.3 million deaths per year [5]. Vast amounts of excess fertilizer washes of the fields which creates an ammonia rich environment that makes it difficult for the algae to survive.

Ammonia as mentioned plays a huge role in agriculture. Nitrogen (N) is essential to plant growth and although is abundant in nature, the amount available to use for plants in a minute fraction. What lacks in the soil is made up for by adding fertilizer to promote these nutrients. The most available source of nitrogen for plants is in the form of nitrate (NO_3^-) and ammonium (NH_4^+) which are derived from the nitrogen comprising about 78% of the earth's atmosphere [6]. This derivation is through atmospheric electrical discharges, biological fixation of soil microorganisms, and manufacturing fertilizers [6]. It is this nitrogen that has allowed our population to grow to such an extent that has resulted in doubling global cereal grain in the last 40 years [6].

Current Methods

Biological Nitrification Denitrification

Denitrification is the removal of nitrogen by a two-step chemical process [8]. The 1st step is nitrification in which ammonia is converted to nitrite then to nitrate [8]. This happens naturally with any aerobic-biological process at small organic loadings with suitable environmental conditions [8]. In this step it is essential to control the net rate of accumulation of nitrifying bacteria. In the second step, denitrification the nitrate is converted to nitrogen and whatever other gas as a product [8]. For this to happen there must be three conditions: 1) Nitrogen is present as a nitrate; 2) there must be an organic carbon source; and 3) an anaerobic environment. Some disadvantages of this process in that there is a significant amount of storage space needed for this type of waste system. As well, ambient temperature plays a role in the performance of the system as well as the lifespan of its biological components [8].

Ozonation

Another method ammonia is removed from water is through oxidation. Ozone can form hydroxide (OH^-) which has a strong oxidative ability [9]. The ozone can be introduced to the wastewater as microbubbles which will dissolve and eventually oxidize the ammonia. This mechanism is show right in figure 2. Research has shown the higher the initial pH level, the higher the removal of ammonia [9]. A disadvantage of this is how basic the solution would need to be to get a high efficiency.

$$NH_4^+ + OH^- \leftrightharpoons NH_3 + H_2O$$

$$3O_3 + NH_4^+ \rightarrow NO_2^- + 2H^+ + H_2O + 3O_2$$

$$NO_2^- + O_3 \rightarrow NO_3^- + O_2$$

$$4O_3 + NH_4^+ \rightarrow NO_3^- + 2H^+ + H_2O + 4O_2$$

$$3O_3 + NH_3 \rightarrow NO_2^- + H^+ + H_2O + 3O_2$$

$$4O_3 + 3NH_3 \rightarrow NO_3^- + H^+ + H_2O + 4O_2$$

$$6 \cdot OH + NH_3 \rightarrow NO_2^- + H^+ + 4H_2O$$

$$NO_2^- + 2 \cdot OH \rightarrow NO_3^- + H_2O$$

$$NH_3 + 8 \cdot OH \rightarrow NO_3^- + H^+ + 5H_2O$$

Figure 2: Ozonation of ammonia [9].

Precipitation Method

Magnesium ammonium phosphate (MAP) is used to control the release of fertilizer to recycle ammonia [10]. In this method for ammonia wastewater treatment, the precipitant Mg^{2+} and $PO43^-$ reacts with the ammonium (NH_4^+) to create a precipitate in the wastewater as the salt $NH_4MgPO_4 \cdot 6H_2O$ (struvite) [10]. In this method after precipitation occurs, this can be removed physically. One downfall to this method is that there is limited research done as to the efficiency of this method.

Algae

The used of algae in wastewater treatment has the capability of providing a tertiary biotreatment as well as producing a valuable biomass [11]. Due to their capability to remove heavy metals and toxic organic compounds, algae don't have any secondary pollution which is a plus [11]. In wastewater treatment, removing biochemical oxygen demand (BOD), nutrients (nitrogen and phosphates), suspended solids, coliform bacteria, and toxicity are the main components to treat and purify wastewater [11]. This BOD can deplete the dissolved oxygen in the water and lead to killing fish and anaerobosis.

Algae proves to have a great advantage over other wastewater treatments because they are easily available, which is a plus in developing countries. Because algae undergo photosynthesis, this type of wastewater treatment is ideal with high sunlight exposure and temperatures. It removes organic matter, nutrients (including phosphorus and nitrogen which is the main cause of eutrophication in water), and contaminants and pathogens that could prove harmful to the ecosystem and human/animal health [12]. After the algae is used up, the biomass could prove a great opportunity in a variety of applications including use as a fertilizer.

Process/Results

The end goal of this research was to adapt algae to be able to thrive in environments where naturally they wouldn't be able to survive. In this case, the algae were to be adapted to about 1200 mg/L of ammonia within an aqueous environment. The first generation was introduced to ammonia environments of 50, 100, 150, 200, 250 and 300 mg/L simultaneously and monitored to see if they could survive. After letting it settle and it was assessed that the algae were thriving in its current environment, from the highest concentration of ammonia that the algae could survive the next generation. This next generation has a higher concentration. This process in shown in the flow chart below in figure 4. Each generation was tested for about 10 weeks to make an assessment where it was then moved to the next level of ammonia concentrations.

Figure 3: Housing unit for the algae kept at various ammonia concentrations.

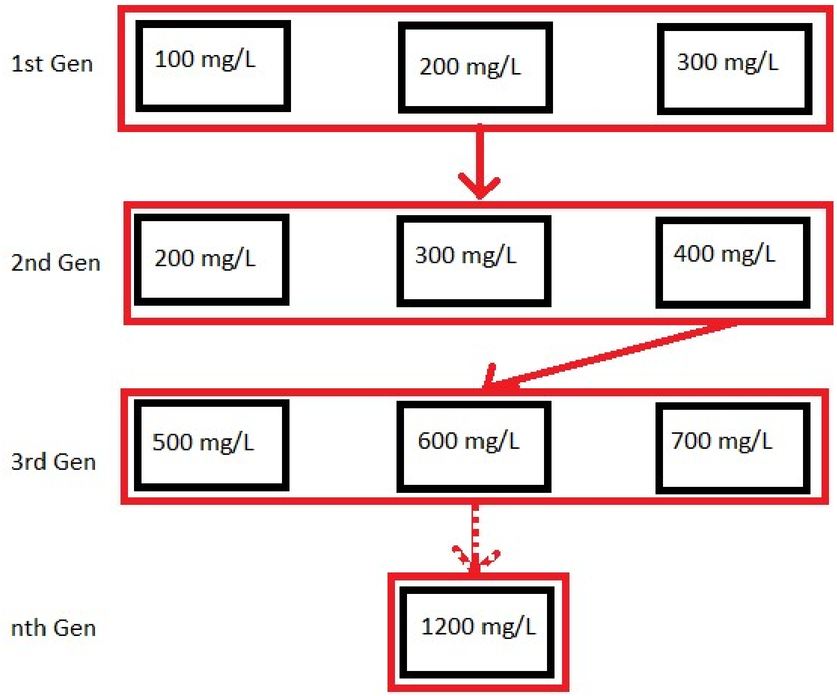

Figure 4: Flow chart of climatization of algae. Each mg/L refers to ammonia concentration.

Various tests and experiments were done to each algae specimen to determine how they were performing in each various concentration levels. To set a base, the ammonia concentration was the only factor that varied between each vial. Salinity levels were kept to around 30 g/L, pH was kept at 6.8-7.8 and each test specimen was sparged with air to ensure the algae was kept moving within each vial and as well to allow the algae to breath.

Figure 5: Spectrometry used to measure ODE.

Each day, a 1 mL sample was taken and put in a small vial which was then put into a spectrophotometer for optical density testing (ODE). This device measured the concentration of the algae according to Beer's Law ($A = \epsilon bc$). The wavelength (ϵ) was set to 540 nm on the spectrophotometer which matched with the general color of the algae (green). The length of the tube was a constant and the absorbance was measure using the machine. From this the concentration was directly related to the absorbance. Through comparison of the data from day to day and week to week, the condition of the algae could be assessed to see if they were surviving in the environment. One issue that arose with this test is the maximum concentration the machine could measure was 1000 mg/L. Due to this, the original sample was diluted to 5 times and 10 times with DI water where they were stirred together uniformly. Through this dilution the concentration could be known due to the machine being calibrated with the DI water.

After ODE testing was complete, salinity levels needed to be measured to ensure it was a good environment for the algae. As mentioned, salinity needed to remain at 30 g/L. A salinity refractometer was used for this purpose. It was important to keep a record of these levels due to the evaporation of water from the containers. As water evaporated, the salt would remain in the container in about the same amount so salinity levels would rise. From this we could make a dilution by adding DI water.

Figure 6: Salinity sight glass.

pH was then taken of each specimen. 2 mL of algae water was put into a vial and pH was determined using a probe. The algae preferred an environment pH of around 6.8-7.8. This number could drop too low due to the algae breathing. As CO_2 was released by the algae and it was absorbed by the water, the fluid becomes more and more acidic. If this number gets too low (around 4) the algae would die.

Dry weight of each specimen was taken once a week. This proved to be the most accurate way to telling the true concentration of the algae. To do this a 10-mL sample of algae solution from a well-mixed jar was put into a vial that way then centrifuged. The algae would precipitate at the bottom of the vial. The supernatant was removed and water was added back to the algae to dissolve it again where it was then put back into the centrifuge. This was done about three times to remove inorganic salts. After this process a small amount of water was added back to the algae to dissolve it where it was then put in an oven at 550°C for 2 hours to move all water. From here the dry weight could be taken and compared to the last sample.

Conclusion

It was found that climatization of the algae to tolerate ammonia an in aqueous environment of 1200 mg/L could be achieved. After this part of the research was complete it was viable to move forward with more research on the actual wastewater treatment using the algae. The implementation of this algae could greatly reduce the threat of harmful algal blooms which if devastating to itself and surrounding ecosystems. As well the spent algae could prove to be a valuable biomass that can be used in numerous applications.

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