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How Can Dangerous Levels of Nitrogen in Water be Remediated using Nanofibers

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ABSTRACT

Freshwater is vital for plant, animal, and human life. Due to increased demand for agricultural and animal products, excess reactive nitrogen species are seen in the environment as synthetic fertilizers. Consequently, increased instances of eutrophication occur in surface waters coupled with increased levels of nitrates in groundwaters. Both of these are common drinking water sources. Current nitrate treatment technologies require expensive infrastructure to build and maintain, have long treatment periods due to biological processes, and can generate copious amounts of waste and byproducts (ex: granular activated carbon and anion exchange resins). Safe, affordable, and sustainable reactive nitrogen removal water treatment technologies are needed. Nanotechnologies are an emerging, novel treatment for the removal of reactive nitrogen species in the form of nitrate in both drinking water and wastewater. In particular, chitosan nanofiber mats with algal substrate show promise in removing nitrate from water sources and have the added benefit of providing a sustainable source of biofuel. This article includes a review of nitrate contamination in the environment and chitosan nanofiber mats with an algal substrate possible solution.

Introduction

Nitrate in water sources can adversely affect the health of both humans and ecosystems. While nitrate is a source of essential nutrients for plant growth, anthropogenic sources of nitrogen, such as nitrate, have resulted in drastic disruptions to the natural nitrogen cycle. In nature, nitrogen can be in the form of nitrate, nitrite, or as ammonium ions (de Farias et al., 2019). Nitrate is highly soluble in water and is naturally occurring. However, nitrate levels are increasing due to anthropogenic influences from agriculture, industrial wastewater, and municipal wastewaters. The most common cause of nitrate contamination in rural freshwater wells are from runoff of agricultural fertilizers (Agency for Toxic Substances and Disease Registry, 2021). Another water source that is high in nitrate is wastewater, both industrial and residential. Most wastewater treatment processes do not completely remove nitrate through their biological processes, only to meet government imposed treatment requirements, resulting in an effluent that still contains high levels of nitrate that is not dilute. Additionally, industrial wastewaters contain concentrations of nitrate that are orders of magnitude higher than municipal wastewaters. These facilities require specific, and expensive, nitrate removal processes at larger scales. The last step is optional in many treatment centers and varies in the level of acceptable ammonia and nitrate levels post treatment (Office of Water, 2000). A smaller source of anthropogenic nitrate is from fossil fuel usage and animal waste runoff (Ward et al., 2018).

Excess nitrate is a precursor to algal blooms, which die off quickly, resulting in dead zones from critically low oxygen levels (Water Resources Mission Area, 2019). This process is called eutrophication, and occurs because decomposers utilize the oxygen in the water to break down the dead algae. Runoff from fertilizer usage, animal waste, and wastewater treatment plants are expelled into the environment. The effects are twofold: increase nitrate in freshwater wells and groundwater, and increase nitrate in water systems which lead to eutrophication. Both are harmful to living creatures and plants. It is imperative to decrease nitrate contamination of the environment for the health of all living organisms.

Nitrate in drinking water is associated with methemoglobinemia of infants. A medical emergency, its hallmark sign is the “Blue Baby Syndrome”. Nitrate oxidizes iron in hemoglobin from ferrous iron (Fe_2^+) to ferric iron ($Fe\ _3^+$), respectively. The resulting lowering of iron’s affinity to O_2 (Oxygen) gives the baby’s blood a blue hue due to hypoxemia. Additionally, nitrate can be reduced to nitrite in the body. Nitrite is associated with other human health issues such as birth defects, thyroid disease, and certain types of cancer (colorectal, breast, and bladder) (*Nitrate/Nitrite Toxicity*, 2021). Nitrite is a precursor to NOC (n-nitroso compounds), which are known carcinogens (cancer causing) and teratogens (birth defects causing). As such, in the U.S, the Environmental Protection Agency (EPA) has set the level of nitrate deemed acceptable to human health in drinking water at 10 mg/L of nitrate as nitrogen (NO3-N), i.e. the Maximum Contaminant Level (MCL). One study by Ward et al estimates that 2% of public wells and 6 % of private wells in the U.S. have nitrate levels above the 10 mg/L (NO3-N) MCL. This same study estimated 21% of agricultural private wells had nitrate concentrations above the MCL (Ward et al., 2018).

Globally, the World Health Organization (WHO) has set the MCL for nitrate at 11.3 mg/L NO3-N. For comparison, Europe sets the MCL for nitrate at 4 mg/L NO3-N, whereas Canada sets the MCL at 10 mg/L NO3-N. In one study, Abascal E. et al found that the nitrate level of groundwater sources in underdeveloped countries are higher than in developed countries (Abascal et al., 2022). For example, Senegal has nitrate levels of 42.9 mg/L NO3-N. In India, 39% of 79 groundwater samples around Krishna Delta had nitrate levels above 11.3 mg/L NO3-N, the highest reading being 30.5 mg/L NO3-N (Prakasa Rao et al., 2017). Nitrate contamination of water is not contained to the United States, nor is it only an issue for underdeveloped or developed countries. It is a global issue and affects all life on earth.

The cost of removing nitrate from drinking water is out of reach for many. For a small town in Kansas, with a population of 3,300, installation of a new water treatment plant was estimated at 3.5 million. This is a major cost to the people, but a necessity as the nitrate levels were above the MCL in their drinking water (Environmental Working Group, 2020). In one study, the Central California San Joaquin Valley had contaminated drinking water wells due to agricultural runoff and animal waste. The area is populated by lower income working class inhabitants. Installing water filters would increase the total household water cost by 1.5% per month, an expense that is difficult for the families residing in the area.

Projects such as a new well, consolidation, or installation of a treatment facility was estimated to cost $150 million. Additionally, the burden of this cost would be carried by the inhabitants. Clean, safe drinking water is a right for all human beings, but often the limiting factor is cost, especially for rural and/or low income areas. Globally, the United Nations has set 17 Goals for Sustainability, with access to clean water and combating inequities each a separate goal, and seem to be two sides of the same coin (*Water and Sanitation - United Nations Sustainable Development*, n.d.). We must rectify the inequity of clean water availability by investing in water treatment in areas with high environmental pollution and low resources.

Nanotechnology and Water Treatment

The need for low cost, sustainable water treatment systems is important as safe, clean drinking water is a right for all humans (United Nations Sustainability Goals). In addition, maintaining a sustainable ecosystem is a requirement for the population of the inhabitants on earth. In the U.S., there are an estimated 40 million inhabitants without access to reliably tested drinking water, as private wells are still the main source of drinking water for many people (de Farias et al., 2019). Nanotechnology is an emerging and exciting science to treat wastewater and to specifically eliminate harmful levels of nitrate in contaminated water. A brief summary of nanotechnology, nanomaterials, and definitions will follow.

There are many types of nanomaterials, categorized by shape and substance. Nanoparticles have a size between 1-100 nm and can be metallic, semiconductors, or polymers. They have a high surface area to volume ratio and are excellent catalysts. Nanoshells are a different shape consisting of a dielectric core with a thin metallic film covering. Nanotubes are hollow tubular structures. Carbon based nanotubes (CNT) can function as a catalyst, co-catalyst, or absorbant. They are versatile and have high absorption rates. Of note, CNTs need surface modification due to it being hydrophobic for purposes of water treatment (Tyagi et al., 2018). Nanoclusters are just what the name implies: many atoms clumped together. The clumps' overall shape are spherical. Unfortunately, for the purpose of nitrate removal from water via nitrate reduction, nanoclusters produced the most ammonia instead of the desired nitrogen gas (Tyagi et al., 2018). Nanofibers are structures with diameters of less than 100 nm. Their properties include high surface area to volume ratio, safe, an extremely long length, easily incorporated into any media/lattice/matrix, large porosity, and adjustable functionality. Nanofibers are more versatile than nanoparticles since they can be reused and more easily separated from any solution medium than nanoparticles (de Farias et al., 2019). Typical nanofibers are composed of chitosan or carbon, and show promise in applications for water treatment.

There have been many advances in the research using nanotechnology for water treatment. Similar to why a single cell is very small and has a high surface area to volume ratio, it provides more area for reactions to occur. In one study, engineered nanomaterials, or ENM, are used to disinfect wastewater. As seen in *Figure 1*, using different mechanisms such as electrostatic interaction, cell-wall disruption, or photochemical reactions, pathogens can be destroyed or inactivated.

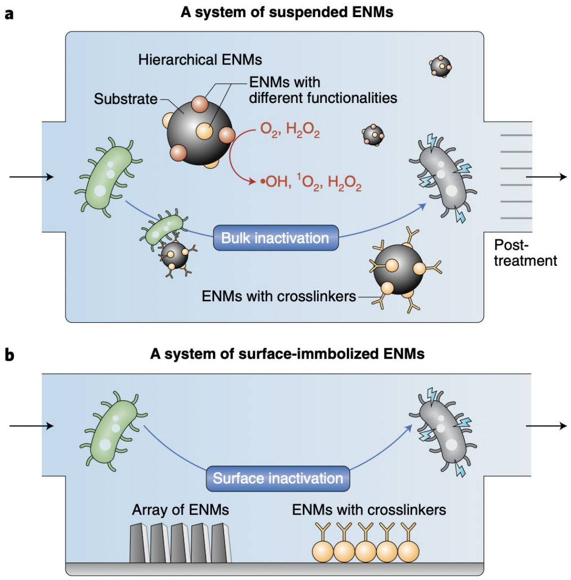

Figure 1. An example of engineering nanomaterials by Mauter, M. S. illustrating their use for water disinfection where (1a) shows the bulk inactivations and (1b) demonstrates surface inactivation of pathogens (Mauter et al., 2018).

There has also been research into using ENMs for delivery of disinfecting agents, with very high specificity to the contaminant. ENMs can even be a more broad spectrum, if that is the need, by using nonselective reactive oxygen species, which disrupts the cell wall functions of pathogens either chemically or physically (Mauter et al., 2018a). The advantages of using ENMs include not having toxic metabolites or by-products such as halogenated by products seen with chlorination, and decreased growth of biofilm due to the nature of ENMs. The disadvantages of

ENMs are the cost of the infrastructure as most of the research is still in laboratories, and limiting the possible environmental impact as some disinfecting agents are not selective to one pathogen and may harm biological systems (Mauter et al., 2018a).

One possible real world application with potential is adding a coating of nanosized magnetite to sand, as sand is used as a water filter material, in water treatment. However, sand has a limitation of not filtering out heavy metals. With the added composite of a nanocoating of magnetite, the sand filter process could potentially remove arsenic in water sources (Alvarez et al., 2018). Another example of nanotechnology is its use in water desalination. Desalination uses reverse osmosis and desalination membranes allowing only water to pass through while solutes cannot. These membranes are called thin-film composite (TFC), and consist of three layers. The first layer is a fabric backing, then a porous polymer, and finally a highly crosslinked polyamide selective layer of 200 nm thickness (Mauter et al., 2018a). These membranes are prone to biofouling, due to its hydrophobic nature which tends to absorb organic substances. One way of decreasing the biofouling would be to use embedded ENMs that provide a hydrophilic surface, thereby preventing organic foulants from absorbing on the surface of the TFC (Alvarez et al., 2018). These methods are still being researched to see if they are affordable and sustainable for large scale production.

Nanotechnology and Nitrate Removal

As previously mentioned, elevated nitrate levels are a specific concern to the health of humans and ecosystems. The use of nanotechnology to decrease nitrate levels in a sustainable process could be a viable and profitable solution. The two mainstays of nitrate removal by nanomaterials involve either reduction or absorption as illustrated in *Figure 2*.

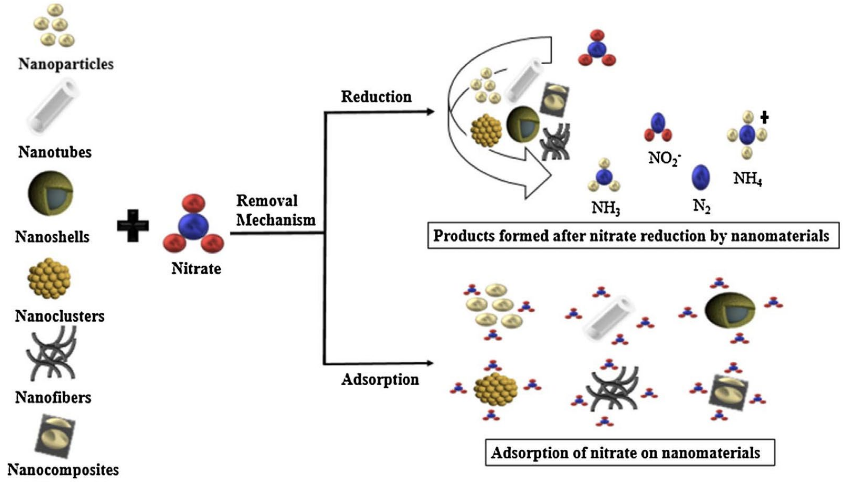

Figure 2. An example of the different types of nanomaterials and the two mechanisms of nitrate removal: reduction and adsorption by Tyagi et al (Tyagi et al., 2018).

During reduction as seen in *Figure 3*, the nitrate is reduced by the reducing agent to produce NH3, N2, NO2-, or NH4+. The reducing agent can be many things, such as active metals, borohydride, formate, hydrazine, or iron to name a few (Tyagi et al., 2018). Depending on the type of nanomaterial, the reducing capacity varies. During absorption, the nitrate molecule is bonded to the surface via physisorption or chemisorption. Physisorption is a weak and reversible process driven by Van der Waals forces , and around 40 kJ/mole is needed to break a physisorption hold. Chemisorption is a stronger and irreversible chemical bond, and needs around 80 kJ/mole to break that bond (Kabita

Sharma, 2022). The type of nanomaterial determines the absorption capability and efficacy, and follows isotherm and kinetic models (Tyagi et al., 2018).

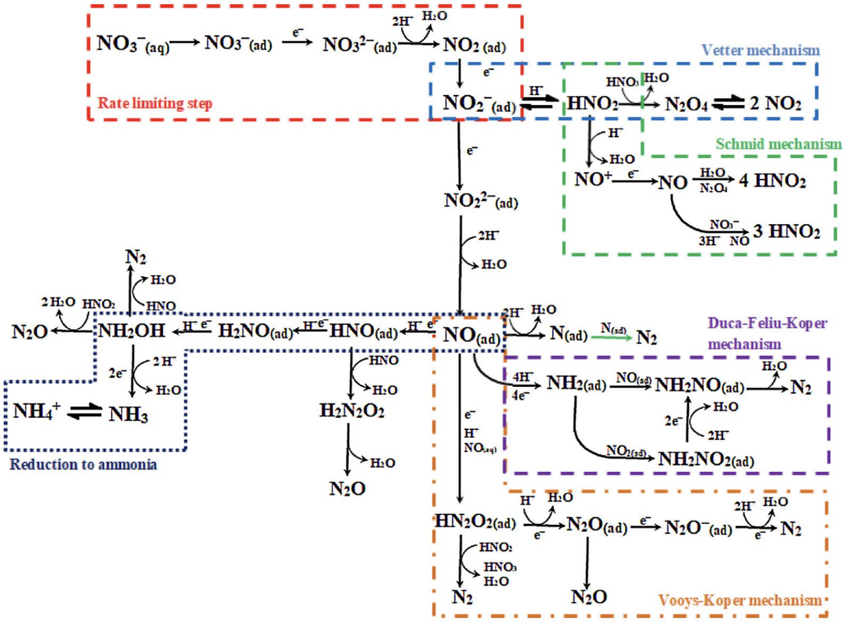

Figure 3. A flow chart of the nitrate reduction chemical process. By Garcia-Segura, Applied Catalysis B, 2018 Nitrate reduction pathways (Garcia Segura et al., 2018).

Ryu, A. et al showed how a Nanoscale Zero Valent Iron (NZVI) reduced nitrate to various substrates. A minor problem was aggregation of the nanoparticles, which was decreased by immobilization of the NZVI on supports. Interestingly, Tyagi et al used the same NZVI, but used it to absorb nitrate (Tyagi et al., 2018). Cai, X. et al used bimetallic nanoparticles such as Fe/Ni to remove nitrates, the results showed an increased nitrate removal efficiency from 26% to 40% versus when using single metallic nanoparticles (Tyagi et al., 2018). Motamedi, E. et al compared coating of NZVI with a capping agent or a carbon based oxide layer in nitrate removal through reduction. Making a composite of a metal nanoparticle (Fe, Ni, or Co) coated with graphene oxide results in an increased rate of nitrate reduction due to less aggregation and increases stability of the composite. After 8 hours there was an 83% reduction in nitrate. Of note, graphene oxide is stable, with a large surface area, and has a high oxidizing potential, making it an excellent choice for water decontamination. Another benefit is since graphene is magnetic, the composite can be removed from the water with a magnet (Tyagi et al., 2018). The speed of metal and metal alloy nanoparticles were faster for nitrate removal if the diameter was smaller (Tyagi et al., 2018). However, nitrate reduction results in ammonia as a byproduct, which can be toxic. The challenge for nitrate reduction is improving selective non-toxic nitrogen gas as the product instead of the intermediate reactive nitrogen species of nitrite, ammonia, and ammonium as depicted in *Figure 3*. More research needs to be done to change the main product to non-toxic nitrogen gas (Tyagi et al., 2018). Nanotechnology is increasingly being considered as a potential long term solution to removal of nitrates from contaminated water sources. Having a large surface area to volume ratio, it is optimal for nitrate removal. One particular branch of nanotechnologies that is showing promise as a sustainable, renewable way of nitrate remediation are chitosan nanofiber mats with algal substrate.

Chitosan/Algal Nanofiber Composite for Nitrate Removal

Chitin is the precursor of Chitosan, and it is the second most common naturally occurring polymer on Earth, after Cellulose. Chitin is found in the exoskeleton of crustaceans such as shrimp, crab, and lobsters. Chitosan is a polysaccharide derived from chitin, consisting of *B*-(1-4) linked D-glucosamine and *N*-acetyl-D-glucosamine in a random pattern polymer backbone. It is biodegradable, non-toxic, naturally antimicrobial, and biocompatible. Excess chitosan can even be used as animal food (Cui et al., 2021).

Chitosan has many amino and hydroxyl functional groups which are excellent for absorption of biological contaminants. In particular, nitrates are absorbed on the quaternary ammonium groups on the chitosan polymers (Aneesh P & Jishna M, 2017). Interestingly, chitosan is a compound that can be converted into chitosan nanofibers with relative ease, as the process has been repeated by many laboratories.

Chitosan nanofibers are being explored as a possible solution to nitrate removal in contaminated wastewaters. They are safe, and when coupled with an algal medium, can provide a renewable source of biofuel. Public acceptance and willingness to convert to algae as a biofuel would need to be investigated. There are many options for consumers, such as combustion engines, hybrids, and electric vehicles. Adding biofuel motorized vehicles would need a large infusion of funds to set up manufacturing/converting gas powered vehicles to biofuel, production of biofuel, and accessibility across the nation. However, it is a natural plant and most people are familiar with its nontoxic and sustainable nature.

Chitosan nanofibers are fabricated by a process shown in *Figure 5* called electrospinning. Electrospinning requires high voltage, a capillary tube and needle, and a collecting area (Islam et al., 2019). When the electrostatic field is strong enough, it disrupts the surface tension of a liquid. A Taylor Cone is formed at the spinneret when the electrical field is strong enough, and the liquid medium is expelled in a whipping jet stream (de Farias et al., 2019). Before chitosan is electrospun, it needs to be properly prepared as only higher viscosity liquids are optimal for electrospinning. Once the chitosan nanofibers are spun into a mat, it forms the matrix or scaffold for algal cells.

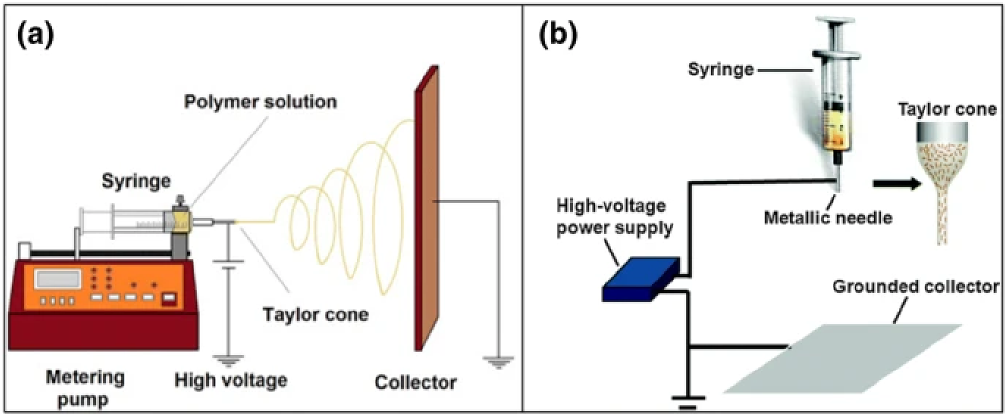

Figure 5. The electrospinning process flow diagram (5.a) illustrating the input polymer solution, the electrical source, and (5.b) the Tayler Cone output and a more specific diagram of the formation of the Taylor Cone using a metallic needle (Islam et al., 2019).

Erolgu et al showed chitosan nanofiber mats supporting green microalgae having promise in nitrate removal (Eroglu et al., 2012). *Chlorella vulgaris* microalgae was cultured while chitosan was prepped for electrospinning. Per the study, powdered chitosan (6 wt%) was dissolved with trifluoroacetic acid (TFA) and dichloromethane (DVM) at a 70:30 vv ratio. Glutaraldehyde solution was added at 5.4% vv (25% in H2O), then the liquid mixture was immediately electrospun (Eroglu et al., 2012). After the mats were fabricated, the *Chlorella vulgaris* cells were introduced. Electrostatic attraction from the positive charge of the chitosan amine group toward the negatively charged surface of *Chlorella* was seen. Of note, *Chlorella* has a negative surface charge due to the dissociation of uronic acid groups on its surface and the existence of sulfate groups on its surface (Eroglu et al., 2012). The composite was placed in an artificial growth medium with nitrates, phosphates, carbonate buffer, and both micronutrients and vitamins (*Figure 6*).

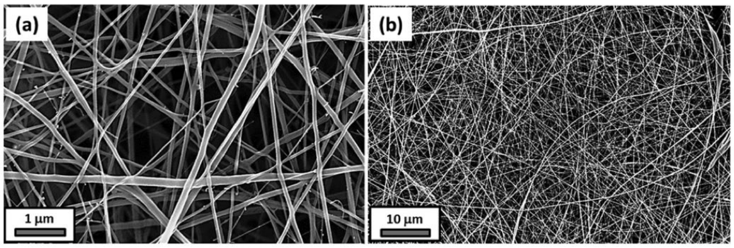

Figure 6: Scanning electron microscope image of chitosan nanofibers (a) lower magnification of 1 *u*m and (b) higher magnification of 10 *u*m, from Eroglu et al (Eroglu et al., 2012).

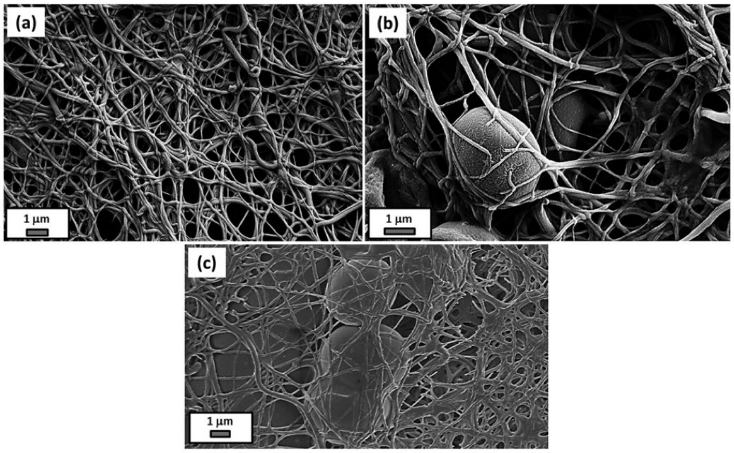

Figure 7: Scanning electron microscope images of chitosan nanofiber mats after (a) 48 hours in a nitrate medium resulting in swelling of the chitosan nanofibers, (b) single algal cell within the chitosan nanofiber matrix, and (c)multiple algal cells in the chitosan nanofiber matrix. From Eroglu et al (Eroglu et al., 2012).

The initial nitrate level was 30 mg/L (NO3-N) which is similar to the concentration of nitrate in wastewater. Results from the composite chitosan mats impregnated with *Chlorella vulgaris* algal cells were obtained. Nitrate concentrations decreased by 30% in 48 hours, due to absorption of nitrate onto the nanofibers. Thereafter, from day 2-10, nitrate concentration decreased even more significantly to 5 mg/L (NO3-N), and that was attributed to the usage of nitrate by the algal medium for cell growth. The control, chitosan nanofiber mats without algal cells, stayed relatively the same after an initial decrease to 21 mg/L (NO3-N). This correlated to the initial decrease of nitrate concentration in the first 48 hours seen in the chitosan/algal composite as the process was driven by physicochemical absorption. Thereafter, the control group remained steady with a concentration of around 20 -22 mg/L (NO3-N), as there were no other processes to drive the reduction of nitrate concentration (*Figure 7*). During the experiment, the nutrient medium became more basic as algae growth alkalinized the medium. This was countered by adding dilute HCl to keep the medium around 6.5-7 ph (Eroglu et al., 2012). The trend seen was while the initial decrease in nitrate was due to the chitosan mat, the continued decrease seen with the composite chitosan/algal mat was due to the algae utilizing the nitrate for cellular growth. Calculated numbers showed a decrease in nitrate was 32 +- 3% and 87 +- 4% for the chitosan vs the chitosan/algae composite, respectively (Agency for Toxic Substances and Disease Registry, 2021). This application of nanofiber technology shows promise in also providing a source of biofuel with the algae substrate.

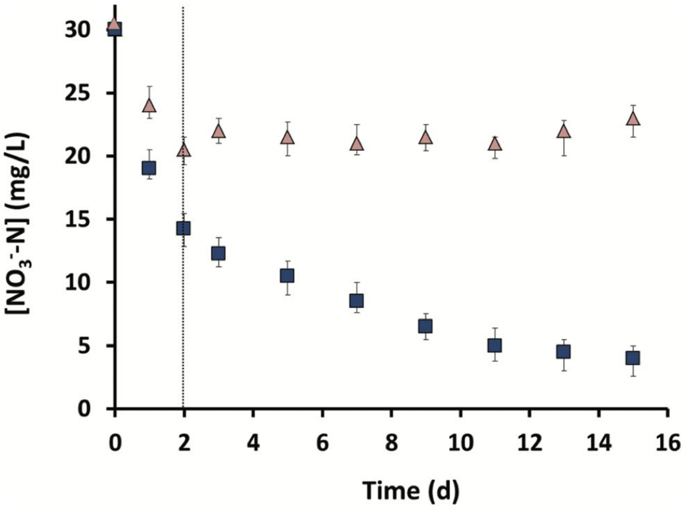

Figure 8. Graph depicting NO3-N concentration over time comparing chitosan nanofibers with and without algal cells. Results showed without algal cells, the max adsorption onto chitosan nanofiber mats was after 48 hours, however with algal cells, nitrate levels continued to decrease over time as nitrate was consumed for algal growth. From Eroglu et al (Eroglu et al., 2012).

Economic and Environmental Implications

Economic Implications

While research gives novel ideas and innovation, the true test is using those ideas on a more practical and industrial scale. Riofrio et al examined the economic profitability of making chitosan from shrimp exoskeletons in Ecuador, and a theoretical business model was constructed. The process of producing chitosan from chitin was examined, and the model started at the source; shrimp and crab shells. Ecuador produced 600,000 tons of shrimp in 2017, as the food industry makes up around 3 billion dollars of its gross domestic product (Riofrio et al., 2021). This readily available source for chitosan could be utilized for potential profit. Starting with a manufacturing factory with a capacity to process 5,000 tons of chitosan, estimates of cost such as resources, manufacturing costs, waste removal, and infrastructure were calculated. Over a period of 10 years as the business model, the estimated return of cost was 3 years, after an initial investment of 1.5 million dollars. In other words, after around year 3, the factory would be making a profit and by the end of year 10, would have a valuation of 10 million (Riofrio et al., 2021).

Social Implications.

Humans generate 0.79 kg/person/day of waste per year (The World Bank Group, 2022). In developing countries, where recycling treatment centers are non-existent, over 90% of solid waste is eventually dumped in landfills or burned, adding to CO2 emissions and thus global warming. By generating profits from the potential production of chitosan, there could be an increased standard of living via job creation. Jobs would generate taxes, which in turn could fund municipal services such as waste management and health clinics. By taking a resource that would have ended up in a landfill (chitin), and turning it into a profitable exportable product, society would benefit.

Environmental Implications

The environmental impact of such an endeavor, even small scale as one factory, needs to be investigated. Production of chitosan from chitin involves using water, ethanol, and sodium hydroxide. Chitosan is derived from chitin through a process of demineralization and deacetylation of chitin (Riofrio et al., 2021) (Figure 8).

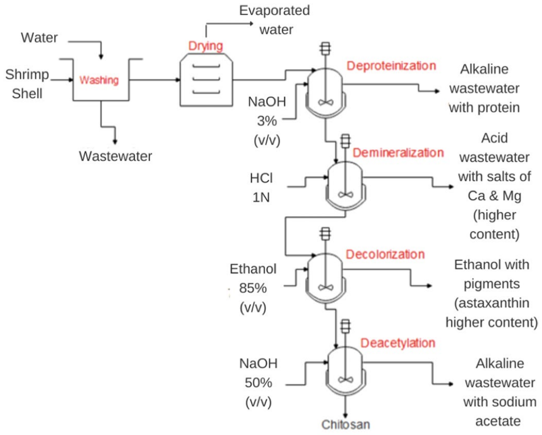

Figure 8. Flow chart of the proposed manufacturing steps of chitosan production from shrimp shells, based on established production steps in use currently. From Riofrio et al.(Riofrio et al., 2021)

Per Riofrio et al, the estimated global warming impact of shrimp exoskeleton is 39%, ethanol 27%, and sodium hydroxide 31% per 1 kg of chitosan produced. However, as shown in *Figure 9*, there are many other factors that impact the environment (Riofrio et al., 2021). Research into reusing sodium hydroxide and ethanol needs to be investigated to offset the environmental impacts of its production and use. Electricity and water are also needed to manufacture chitosan.

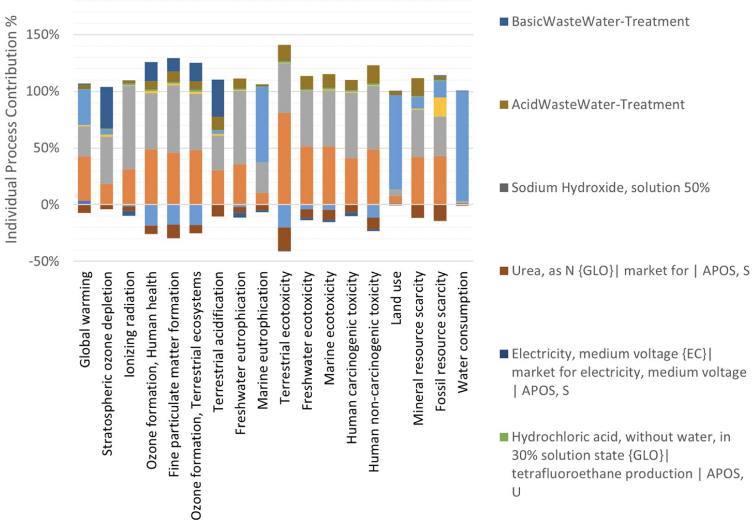

Figure 9. Bar graph of estimated environmental impact from specific materials/energy and processes used for chitosan manufacturing. By Riofrio et al. (Riofrio et al., 2021).

Challenges and Opportunities for Chitosan Nanofibers

An analysis of the estimated cost of a large scale nanotech facility varies. Small scale operations are a practical option, but still the cost would be in the millions. Costs associated with chitosan nanofibers include manufacturing, maintenance, and replenishing the chitosan nanofibers (as they will need to be replaced once used). Removal of saturated chitosan nanofiber mats would have the benefit of being biodegradable, as it is a naturally occurring polysaccharide. Chitosan end-of-life processes include compost and reusing or recycling the chitosan if possible. The nitrate contamination on the nanofibers would theoretically be minimal as the algal substrate utilizes the nitrate for growth.The algae substrate will need to be harvested from the chitosan, then made into biofuel. Despite the environmental advantages of chitosan nanofibers, this technology is still at the laboratory scale. Researchers should seek to scale the process to a mid sized factory, however, there will be maintenance costs. One such issue would be the dreaded biofilm or scaling that occurs. Measures would have to be taken so that is prevented and levels measured so it is within safe parameters. Zhang et al showed brushing a hydrophilic polymer layer over any membrane resisted biofilm and scaling (Alvarez et al., 2018). Sustainability of chitosan is dependent on its source and the environmental impact of aquatic shrimp farming. This would come with its own environmental issues of waste removal (CO2 emissions, production of waste, and waste removal), but it has the added benefit of a food source for humans. All said, the biggest factor is cost, which may be tackled with smaller treatment facilities and decentralized wastewater treatment structuring (Mauter et al., 2018a). More research needs to be implemented with the goal of providing clean drinking water and decontaminating wastewater for all life on Earth.

Conclusion

Removal of nitrate in drinking water is a both a local and global health issue. Elevated levels are proven to affect the blood oxygenation of infants and are associated with different types of cancers. Contamination of the environment with excess nitrate is increasing as the population increases. By 2050, the Earth's population is forecasted to be 9.8 billion people, a significant increase from 8 billion currently (*World Population Clock*, n.d.). With an increase in human numbers, there comes an increase in agricultural production, animal husbandry, and both human and animal waste. All of these contribute to excess nitrate in the environment, and humans are the cause. As these anthropogenic sources of nitrate are one of the main causes of nitrate excess in the environment, now is the time to invest in our environment. The use of nanotechnology, specifically chitosan nanofibers with an algal substrate, needs to be investigated more and scaled for production. The benefits of using sustainable, environmentally friendly materials for nitrate removal cannot be undervalued. The by-product of algal cell proliferation as a source of biofuel as a possible source of added revenue is of interest and will help with economic feasibility. As stated earlier, one of the WHO's tenets is clean water as a right to all humans, and using nanotechnology for nitrate remediation is a potential solution.

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