Nitrogen Oxide Reduction from Air Pollution Using Denitrifying and Nitrogen Fixing Bacteria

eConviction

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Abstract

The clean technology solution described in the following proposal aims to reduce nitrogen oxide levels in air through the use of nitrogen-fixing and denitrifying bacteria, and a reaction with lithium that creates a precipitate. The nitrogen-fixing bacteria *Bradyrhizobium japonicum* lives in the root nodules of the common clover, *Trifolium*, in a symbiotic relationship. It converts nitrogen (N_2) into ammonia (NH_3) useful to the plant. The denitrifying bacteria *Paracoccus denitrificans* lives in soil of both aerobic and anaerobic environments and decomposes gaseous nitrogen oxides (NO_x) into nitrogen (N_2) and water (H_2O). The device described grows clover plants in a soil mixture containing *Paracoccus denitrificans,* granular lithium and charcoal. The powdered lithium reacts with nitrogen from the denitrifying reaction to form lithium nitride (Li_3N), a visible red-violet precipitate that can be collected and disposed as waste. The charcoal absorbs excess moisture in the system to minimize reactions between lithium and water. The finished product consists of a transparent box containing the clover plant in a specialized soil mixture with one opening to the container for gas exchange and watering. The product is intended as a do-it-yourself solution to nitrogen oxide air pollution that will appeal to individual consumers and industrial consumers hoping to reduce emissions or to actively participate in environmental action. The solution is targeted for use in the United States and the United Kingdom where nitrogen oxide emissions are highest, and the ultimate objective is to reduce air pollution by nitrogen oxide through a clean biological solution.

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Introduction

The rapid growth of industry and metropolitan sprawl in the current century has led to a preeminent environmental crisis concerning man-made pollution. Air pollution is one of the more pernicious aspects of pollution, and is caused by greenhouse gas emissions from industrial centers and automobiles. Carbon dioxide, surface-level ozone, methane, and other hazardous gases have been regulated by national and international atmospheric standards for nearly a century, but maintaining and conforming to these standards is difficult for most nations and industries since they must also compete economically to retain market standards. While national emission caps struggle to maintain steady progress against the global warming crisis, environmental action groups and active individuals are an influential voice against ecological disaster and man-made pollution. Individuals and groups working against air pollution on the local level can take action that substantially impacts the movement for a clean planet. Nitrogen oxide reduction is one avenue to environmental improvement that can be addressed on a local level to improve air quality.

Pollution Background Information

Nitrogen oxides are prominent greenhouse gases that can be toxic to living species and hazardous to planet health in large quantities. While nitrogen alone (N_2) is nontoxic, it reacts with oxygen in the atmosphere to form compounds such as nitrogen monoxide (NO), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and nitrous oxide (N_2O). Many of these compounds are not only toxic but are also increasingly abundant in the atmosphere due to man-made pollution that disrupts the natural nitrogen cycle. Nitrous oxide, for example, has a global warming potential of 310 times as much as carbon dioxide, and it stays in the atmosphere for 120 years before being removed by natural processes[1]. Nitrogen oxide pollution results from cars, trucks, buses, power plants, and off-road equipment that burns gas. The most prominent source is the internal combustion engine which was invented in the late 1850s, giving rise to the automobile industry. Since then, nitrogen oxides in the atmosphere have augmented in quantity and recently became an eminent problem. Emissions of nitrogen oxides contribute to respiratory disease, the production of ground-level ozone, and particulate pollution due to reactions with other compounds.

Pollution Problems Assessment

Many health-pertinent and environmental problems arise as a result of nitrogen oxides in the atmosphere. Long exposure to nitrates can decrease the functionality of lungs and increase the chances of respiratory diseases such as acute bronchitis. Asthma is also more severe in nitrate-heavy environments. Nitrogen dioxide, in particular, acts as both an irritant to respiratory systems and as a greenhouse gas that contributes to the formation of ground-level ozone[2]. In 2010, the Environmental Protection Agency established a standard of 100 parts per billion of nitrogen dioxide allowable in the United States, averaged over one hour[3]. Nitrogen oxide pollution in the United States is consistently regulated, but it remains a problem in many regions. Overseas, nitrogen oxide pollution has become particularly severe in the United Kingdom, to the point that the European Union has decided to fine the nation for failing to keep a cap on nitrogen oxide emissions. According to the United Kingdom’s Environment Agency, many metropolitan areas have maintained nitrogen oxide levels between 100 to 1000 tonnes emitted per year[4]. A do-it-yourself, localized solution to nitrate reduction would be most effective in the largely metropolitan parts of the United Kingdom and the United States where nitrate pollution requires severe reduction. While the emissions of many greenhouse gases are strictly limited in most countries, the problem of nitrogen oxide pollution has remained unnoticed while carbon dioxide, methane, and other pollutants receive most public attention. The greatest difficulty in addressing this particular type of pollution is the fact that the chemical isolation of nitrogen requires extremely low temperatures and energy inefficient chemical processes. However, some organisms can reduce nitrogen oxides biologically through a process known as denitrification[5].

Scientific Approach

The solution detailed below is based on the hypothesis that if denitrifying bacteria can be used to decompose nitrogen oxides in the atmosphere, the resulting nitrogen can be reacted with lithium to produce a solid, storable precipitate, lithium nitride. *Paracoccus denitrificans*, a species of coccoid, rod-shaped denitrifying proteobacteria, can decompose nitrogen oxides to produce nitrogen gas and water[6]. The bacteria perform the following redox reaction:

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$$

Air naturally consists of many gaseous elements, including approximately 21% oxygen. Because of this, the nitrogen that is produced from the reaction above can easily react with the abundant oxygen to produce nitrogen trioxide. Lithium, which has a greater affinity for nitrogen than oxygen does, can be added as particulate powder to the soil in the system in order to isolate nitrogen, and to ultimately avoid the reverse reaction. The following reaction occurs between nitrogen and lithium:

$$6Li\ (s) + N_2\ (g) \rightarrow 2Li_3N\ (s)$$

Powdered, or colloidal, lithium dispersed throughout the soil can theoretically cause a reaction between lithium and the nitrogen released by the *Paracoccus denitrificans*, creating the precipitate lithium nitride. The resulting precipitate, lithium nitride (Li_3N), has a red-violet color that is easily visible and indicates the success of the process. The precipitate formed by the reaction between lithium and nitrogen can be disposed of as waste by periodically replacing the soil mixture. Disposal must take place with minimal contact to skin to prevent hazard, but using plastic gloves and precaution while handling the waste soil can ensure safety. The solution entailed harnesses the output of the decomposition reaction of nitrogen dioxide performed by *Paracoccus denitrificans* bacteria and converts the nitrogen to a solid, storable form - lithium nitride - with the second reaction.

The bacteria being used, *Paracoccus denitrificans*, is found in soil in aerobic and anaerobic environments. *Trifolium*, the common clover, contains a species of nitrogen-fixing bacteria known as *Bradyrhizobium japonicum* that lives in the root nodules. This bacteria converts nitrates in air to form ammonia for plant use as part of a symbiotic relationship through the following reaction:

$$N_2\ (g) + 3H_2\ (g) \rightarrow 2NH_3\ (g)$$

Meanwhile, other nitrogen oxides are reduced through the nitrogen-fixing reaction of *Bradyrhizobium japonicum*[7] in the root nodules of *Trifolium*, resulting in a net decrease in nitrogen oxides in a closed system containing both. One species produces a disposable product while the other converts nitrogen to a form that is useful for the plant. All reactions take place as a result of modifications--biological and chemical additions--to the soil.

Limitations to the product design arise due to the fact that both solid lithium and lithium nitride react with water, which can cause ignition in large quantities, or even with moisture in the air, through to the following reactions:

$$2Li\ (s) + 2H_2O\ (l) \rightarrow 2LiOH\ (aq) + H_2\ (g)$$

$$Li_3N\ (s) + 3H_2O\ (l) \rightarrow 3LiOH\ (aq) + NH_3\ (g)$$

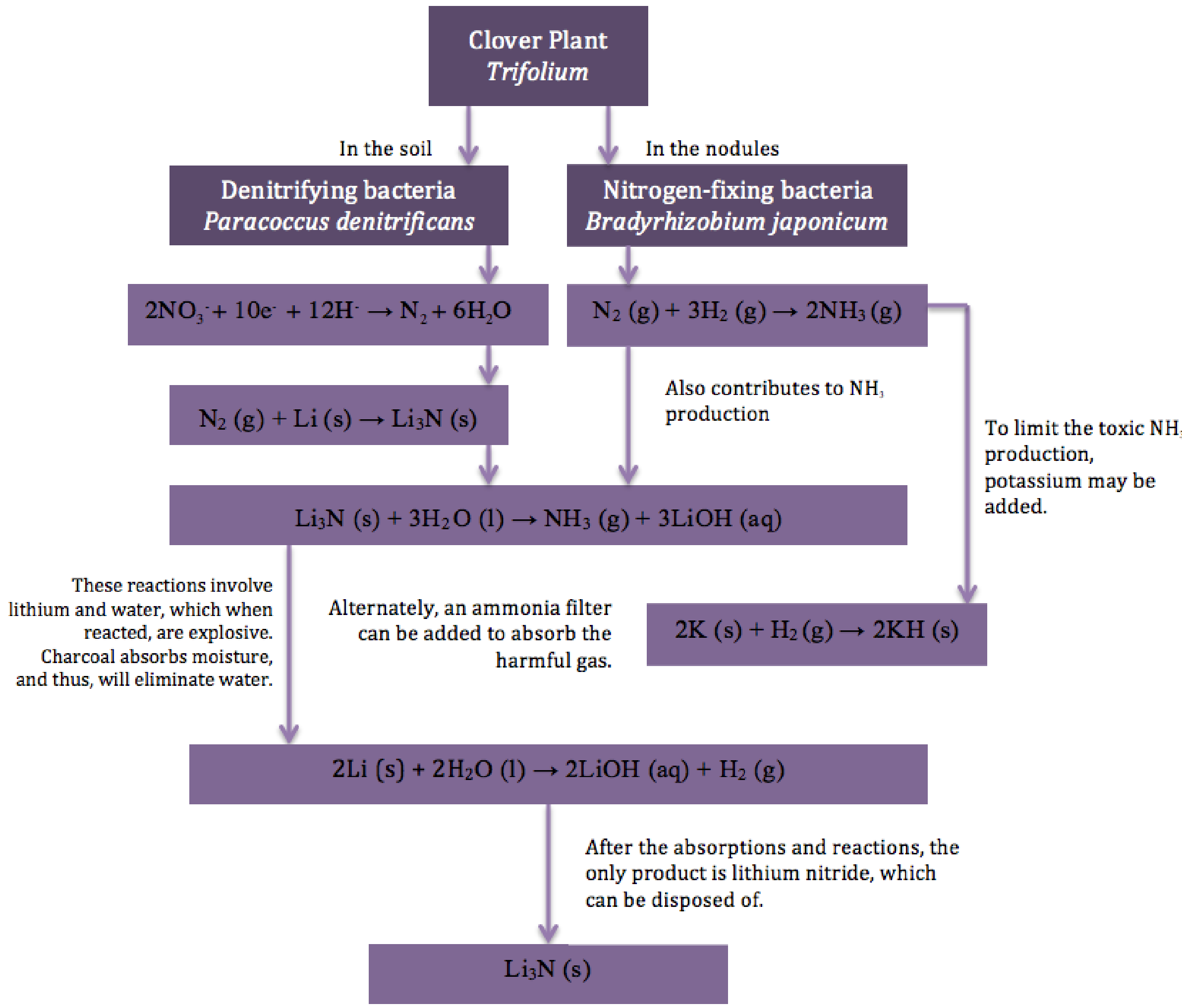
Ammonia (NH_3), one of the products, is a greenhouse gas and is thus harmful to the environment. However, it is vital for plant growth because plants need nitrogen to produce chlorophyll and for structural growth. Plants cannot use pure nitrogen gas, and therefore, have to convert it into ammonia, a more usable form. Much of the nitrogen produced by denitrifying bacteria in symbiosis with legume plants is used by the plant in this form, and *Bradyrhizobium japonicum* converts nitrogen oxides to this form specifically for plant use. However, to maintain plant growth, water must be added periodically. This may cause lithium nitride to ignite (if the amount used is large enough), which, in turn, may cause other nearby combustible materials to burn as well. For these reasons, either isolation or particulate dispersion of the lithium and lithium nitride is essential to the safety aspect of the air purifier. Isolation of the lithium is not ideal for the current design, so through experimentation, an optimal size for lithium particulates can be determined to minimize this reaction. Further design modifications may be able to accommodate full isolation of the lithium and lithium nitride from any moisture. To further minimize the risk of gaseous water molecules coming into contact with the lithium and lithium nitride, charcoal powder will also be dispersed throughout the soil to absorb moisture not needed by the plant.

Bradyrhizobium japonicum are a related species of nitrogen-fixing bacteria known as *Rhizobium* that grow in the nodules of *Trifolium* and other legume plants. *Rhizobium* grows faster than *Bradyrhizobium*, and both exist in legume nodules[8], but the latter are specifically known to live in the common clover and are therefore a basis of our experimentation, along with *Paracoccus denitrificans* which live in the soil. In later experiments, the bacteria growing in the nodules can be classified to maximize the growth of the most efficient species of *Rhizobium* and *Bradyrhizobium* growing in symbiosis with *Trifolium*.

The ammonia produced by *Bradyrhizobium* will not be an entirely negative product because it will be useful to the *Trifolium*. Ammonia, and a resulting ion ammonium, can be harmful to humans in large quantities. In small quantities, they are mostly harmless, but in large quantities, they can burn skin and irritate the eyes and throat. If further experimentation reveals high ammonia or ammonium levels, a granular ammonium filter or an ammonia filtration sheet will be placed inside the container to restrict dispersion into the atmosphere.

The complete combined reaction process will produce an output of solid lithium nitride, which can be disposed of with proper precaution. Most ammonia will be used by the plant, and the water produced from the denitrification reaction will be used by the plant or absorbed by the charcoal. Through testing and redesign, we will be able to make any further modifications that will maximize the efficiency and effectiveness of the process and reduce safety concerns.

Experimentation, detailed in *Testing and Redesign*, will measure nitrogen oxide levels in a closed system through a series of time periods as a dependent variable against changes in particulate size and concentration per unit volume of lithium and charcoal, the number of nodules on a plant, population growth of *Paracoccus* and *Rhizobium*, and various living conditions for the plant. Further experimentation will gather data on how to minimize the risk of extraneous products produced in the chain reaction through further chemical reactions or natural biological processes that can convert dangerous products to harmless forms. The do-it-yourself aspect of our solution calls for a product that is not only effective, but also safe and easy to make. The flowchart below provides a general outline of the processes described:



Technological Elements

Our technology does not rely on mechanical energy, which allows it to be implemented in a variety of conditions. The air purification capabilities provided by denitrifying bacteria can be used to purify air leaving smokestacks in factories and air leaving car tailpipes, addressing the problem of nitrogen pollution directly at its greatest man-made sources. Companies that generate air pollution will find our solution ideal for environmentally clean landscaping and low cost pollution prevention. When the product is ready for large-scale implementation, the plant growth of clovers will promote wildlife population growth in rabbit and gopher species among others.

The product uses chemical energy, optimizing the output of naturally occurring chemical reactions through the use of tested lithium and bacteria concentrations, among other variables. Since the product does not require electrical energy or fuel, it will produce no emissions of its own that can not be collected and rejected as solid waste. When the objective of optimal output is obtained, the product will become an ideal air purifier that can be produced and maintained locally by individuals and environmental protection groups taking action on a local scale.

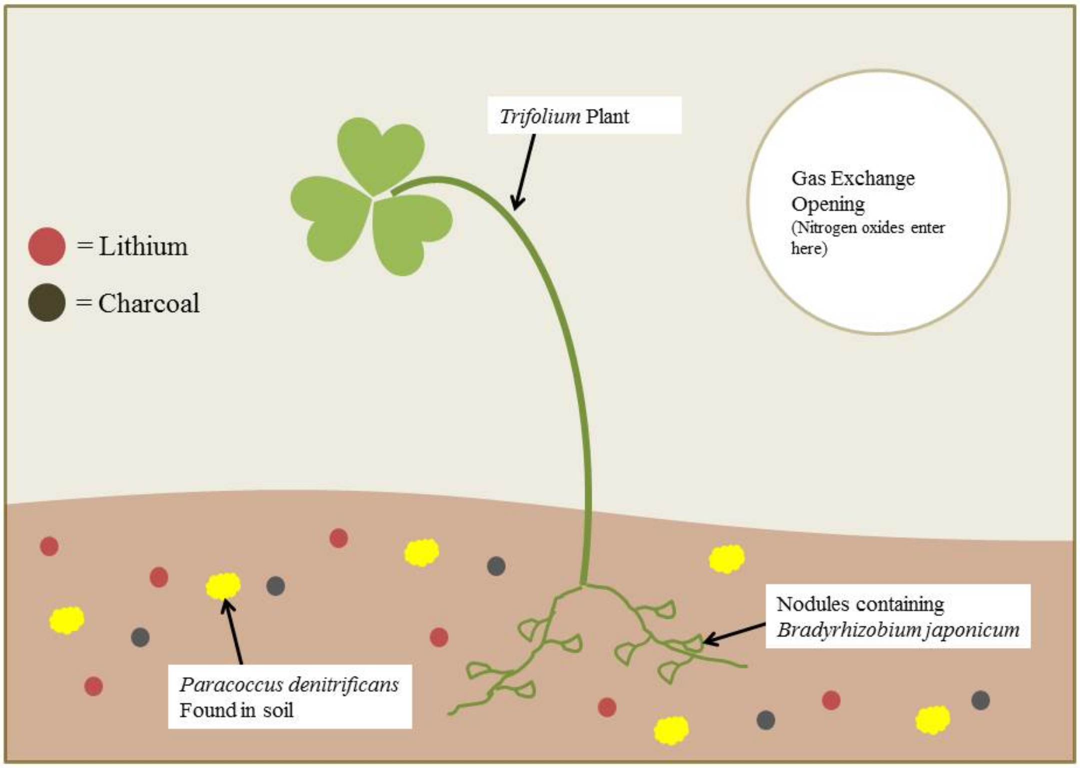
The finished product will be a do-it-yourself kit, accessible to the consumer. The product will require low maintenance involving only the periodic replacement and disposal of the soil, and it will promote environmental awareness and responsibility while teaching basic gardening skills. The directions for how to create the product will also be made public so that aspiring groups can create larger models of the product for wide-scale use.

In the future, denitrification and nitrogen fixation can be used to harness nitrogen oxides from their greatest man-made sources: automobiles and industrial factories. A denitrifier using common clovers could eventually be attached to a car tailpipe, to the tailgate of a car or semi-truck, or to the opening valve of a smokestack to harness nitrates before they reach the atmosphere.

Engineering Design

The current design of the prototype consists of a transparent container containing the *Trifolium* plant and potting soil mixture. The container will be fully enclosed with the exception of one opening, a measured hole in one side, for controlled gas exchange to allow polluted air to pass through. Since the container itself will be transparent, sunlight will be able to enter, enabling plant growth. Water for the plant will be inserted through the opening for gas exchange. Nitrogen-fixing bacteria growing in the plant's nodules will react pure nitrogen with hydrogen gas to produce ammonia for the plants, while the denitrifying bacteria in the soil convert nitrogen oxides to elemental nitrogen gas for the lithium reaction. The lithium and charcoal in the soil will control these reactions as mentioned in *Scientific Approach*.

A granular ammonia filter or filtration sheet will absorb excess ammonia if experimentation reveals a necessity for this precaution so that it does not leave the system. Theoretically, lithium nitride will be the primary output of the system, and any extraneous products, such as ammonium and water, will have been minimized. The lithium nitride can be disposed of as a waste product when the soil is replaced. Wrapping the soil in plastic or aluminum foil and using gardening gloves when handling soil with high precipitate concentrations will minimize the risks associated with lithium nitride. Waste handling on a larger scale is an aspect of redesign that will be addressed once the reaction chain has been optimized through experimentation. The basic design of the finished product is shown below:



The limitations of the current design include the fact that the growth and survival of the *Trifolium* and the fact that the proliferation of the bacteria living within it and within the soil must be controlled and maintained in order for denitrification and nitrogen fixation to occur continuously. Since a high concentration of bacteria theoretically provides maximum output, the growth of bacteria may need to be accelerated periodically to maintain a population that is optimal for *Trifolium* survival and for symbiosis with denitrifying bacteria.

Through testing and redesign, the most advantageous size and quantity of Trifolium, and the concentrations per unit volume of lithium and charcoal will be calculated. Water levels in soil will be measured by draining samples with different quantities and concentrations of charcoal to identify charcoal saturation or necessity. Lithium nitride levels will be measured using a quantized color scale, and nitrogen oxide reduction will be measured with a nitrogen detector. If the reaction time is too long or restrictive to effectiveness, a catalyst can be introduced to increase the reaction speed of the lithium nitride forming reaction.

Testing and Redesign

To test the proposition, a prototype using *Trifolium* and soil containing charcoal and lithium will be designed. A nitrogen detector will be placed inside the prototype during testing to measure levels of nitrogen in various forms. While nitrogen oxide reduction is the ultimate goal, our null hypothesis assumes no decrease in nitrogen oxide levels. A nitrogen sensor that can sense a variety of nitrogen oxides will allow a more diverse range of measurable results.

Beginning with the null hypothesis that a *Trifolium* plant with nodules exposed to lithium will not have any effect on nitrogen oxide content in the air, experiments will be conducted to test the validity of various aspects of the product design. By varying plant growing conditions such as sunlight, water, soil brand, and air content of nitrogen oxides, the correlation of bacteria population to *Trifolium* growth can be quantized and analyzed. As mentioned previously, charcoal and lithium concentrations and quantities will be varied in soil samples to test for optimal values for use in the final product.

Initial and final nitrogen quantities in the air will be recorded to determine the extent to which the bacteria species decomposed nitrogen oxides, and lithium nitride concentrations will be compared to measure the amounts of nitrogen distributed to plant intake and product output. The presence of nitrogen-fixing bacteria can be easily tested by quantifying the amount of root nodules growing on a *Trifolium* plant. The *Bradyrhizobium japonicum* bacteria are housed in the nodules of the legume plant as part of the symbiotic relationship. The nodules can be counted manually because they are easily visible. The concentration of *Paracoccus* in the soil can be determined using phenotype or a biological stain during experimentation. Both bacteria maintain population size based on the environment in which the plant and soil are placed, but initial populations will be placed in the final product before use. A hygrometer can be used to measure the amount of moisture inside the container to prevent the ignition of used components.

Observing whether or not a visible, colored precipitate forms will reflect whether or not lithium nitride formed. Since lithium nitride has a distinct red-violet color, we will use a quantified color scale to measure the amount of lithium nitride that is produced. Color, saturation, and brightness will be relative to lithium nitride concentrations in soil samples.

Since all of these tests entail quantified values, statistical analysis will determine possible correlations between lithium nitride production and variables pertaining to the plant and apparatus design, such as the number of *Trifolium* plants, the number of nodules, the amount of bacteria involved, the growth conditions of the plant, and the symbiosis of the plant and bacteria.

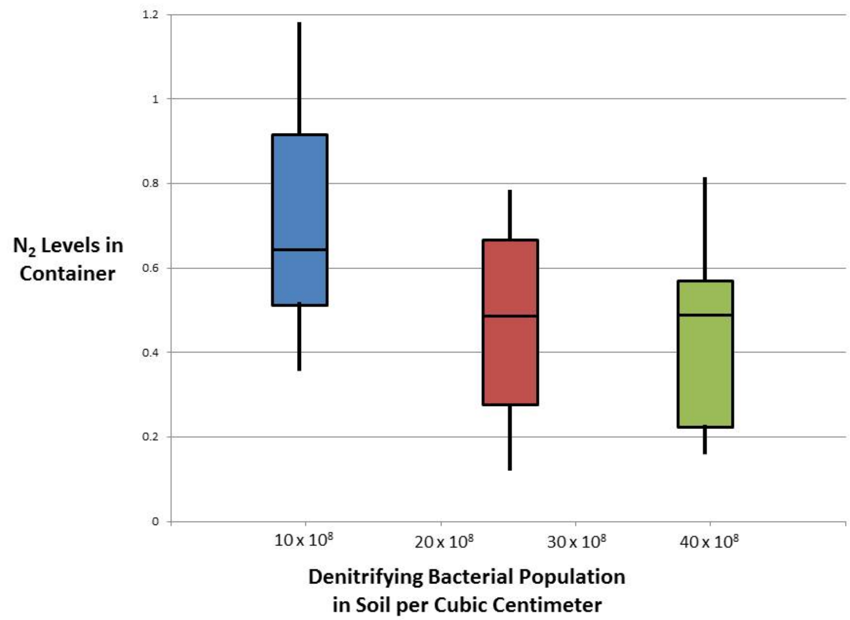
There are many aspects of the system that may be redesigned in order to maximize efficiency. We may place air filters within the container in order to eliminate particulate matter and gases that theoretically should not interfere with the reactions. In addition, after evaluating the proliferation rate of the *Bradyrhizobium japonicum* and *Paracoccus denitrificans,* the growth of either bacteria can be accelerated through the use of added growth mediums. A more effective denitrifying species can also be determined by classifying the bacteria in the nodules by phenotype if the *Bradyrhizobium* is ineffective. Potassium may also be added to the soil to further minimize extraneous ammonia production. The objective of testing different conditions for the reaction and for plant growth is to attain an optimal product such that maintaining the survival of the *Trifolium* plant becomes the only form of maintenance required to keep the denitrifier active and efficient.

In the future, we hope to make this product more portable to make the process more economical and the device itself a "do-it-yourself" accessible kit. The denitrification chain can occur without a Trifolium plant, so soil with only *Paracoccus* is one portable form that can be expanded and improved as a subproduct. The kit can be marketed to individual consumers and to industrial consumers as an attractive, easy to use, low cost solution to pollution problems. In order to create the product, we plan to first ensure that it functions properly and then find ways to make it more efficient. Other testing may be necessary as other variables become apparent.

Results and Conclusion

The reduction of nitrogen oxide levels in surrounding air is the ultimate objective and theoretical possibility of all experimentation. The results obtained from the testing of plant growth conditions and reaction conditions can be analyzed to determine graphical relations between nitrogen reduction and conditional variables, as well as to obtain quantifiable data to measure nitride reduction with a given air quality. In order for the product to be efficient and useful, nitrogen reduction percentages need to be high enough to produce a substantial decrease in air nitrogen levels if tested in a natural atmosphere. We will measure nitrogen oxide levels with a nitrogen detector or nitrogen oxide specific detector with and without the use of the apparatus within a closed system.

The quantities of many nitrogen oxides will be tested to determine the effect of the apparatus. The following chart describes the prospective results of measuring pure nitrogen levels against changing population of *Paracoccus denitrificans*:



Similar results will be obtained for other nitrogen oxides and for changes in other variables, such as Bradyrhizobium japonicum population, nodule quantity, sunlight, fertilizer, lithium concentration, charcoal concentration, and others. The levels of nitrous oxides may change in continuous relation to change in the independent variable, but the expected results as of now are that levels of pollutants converted in the reactions, such as NO_2 and NO_3, will plateau after a certain level of lithium or a certain population of bacteria at which the quantities of reactants are imbalanced so that some reactants remain unreacted.

Once the apparatus has attained a maximum capacity of nitrogen oxide reduction, we will also measure the efficiency of the product to determine how much time passes before soil replacement is necessary and how long each reaction takes. Based on the data from these experiments, observations can determine if a catalyst or growth medium is necessary to maintain efficiency or bacterial population among other factors.

The ultimate goal of the denitrifying apparatus is to reduce nitrogen oxide levels in surrounding air. The finished product uses denitrifying bacteria with a lithium reaction along with nitrogen fixing bacteria in order to reduce nitrogen oxide levels. The objective of all experimentation detailed above is to create an optimal soil mixture that will maximize nitrogen oxide reduction. The solution detailed in the pages above uses the potential of a biologically based solution to fight pollution on a local level, and the nature of the product is such that it can be improved and expanded to counter nitrogen oxide pollution on a wide scale.

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