The Microbial Conversion of Nitric Oxide*

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Abstract

The Microbial Conversion of Nitric Oxide. CRYSTAL M. SMITH (Texas A & M University – Corpus Christi, Corpus Christi, Texas 78412) K. THOMAS KLASSON (Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831).

A system for the biological removal of NO_x from the environment is essential. This removal was attempted with the facultative, bacterium *Paracoccus denitrificans.* When 5 mL of inoculum with *P. denitrificans* were placed into six serum bottles containing 50 mL of medium and various amounts of nitric oxide, the bacterium removed 100% of the nitric oxide. A spectrophotometer (Spectronic 20 Genesys) was set at the wavelength of 600 nm and used periodically by sampling 1.5 mL of liquid culture from each bottle by measuring the amount of light absorbed. A gas chromatograph (Hewlett Packard 5890 Series II) (GC) was used throughout the experiment by injecting a 100 µL gas sample from each bottle and correlated the area of the peak to calibration standards. These results were then collected and graphed. The project experiment proved to be challenging, in such that the bacterium is highly specialized and it cannot cope with subtle changes in its environment, thus the bacterium was hard to grow.

Category (circle one): Physical – Life - Engineering

TYPE ALL INFORMATION CORRECTLY AND COMPLETELY

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Introduction

For more than a century, atmospheric levels of nitrogen oxides (NO, NO_2, N_2O) have been increasing steadily, in which most is due to human activity (Muzio & Quartucy, 1997). In 1997, a record level of 3.13 trillion kilowatt-hours of electricity was generated in the United States. Coal-fired power generation accounted for 57% of this production producing an estimated 7.2 million tons of nitrogen oxide (NO_x) compounds. Generally, greater than 95% of the NO_x compounds in combustion gas streams are in the form of nitric oxide. The NO_x compound consists of nitric oxide (NO) and nitrogen dioxide (NO_2). Nitric oxide and NO_2 contribute to photochemical smog, acid rain, visibility degradation, and fine particulate in the atmosphere (Muzio & Quartucy, 1997). NO_x compounds issues tend to be local and regional, because of the relatively short lifetime in the atmosphere (Muzio & Quartucy, 1997). Nitrous oxide (N_2O) has a long life span in the atmosphere, it is a strong absorber of infrared radiation in the troposphere and is the major component of global warming, and breaking down of the stratosphere or ozone (Muzio & Quartucy, 1997). Since N_2O is stable in the troposphere, it is transported to the stratosphere. In the stratosphere, N_2O is the largest source of stratospheric NO, and the major natural chemical sink establishing the stratospheric ozone concentration; thus N_2O is a global issue (Muzio & Quartucy, 1997).

Recent scientific papers report daily increases in particle air pollution are associated with daily increases in adverse human health effects, primarily those among the elderly and children with increase of hospitalization with respiratory diseases (Ball, Hurley, Straccia, & Gierczak, 1999). Nitric oxide is considered a signaling molecule that acts primarily in the nervous and cardiovascular systems, and it diffuses freely across cell boundaries to activate nearby 'target' cells (Stepanov & Korpela, 1997). Moreover, NO has cytotoxic properties and is implicated in

the degeneration of neurons that is associated with Parkinson's disease, AIDS, dementia, and stroke (Stepanov & Korpela, 1997). Nitric oxide is produced by a variety of cell types, including neurons in the central and peripheral nervous systems, endothelial cells, platelets, and certain activated cells of the immune system (Stepanov & Korpela, 1997).

The 1990 Clean Air Act Amendment Titles I and IV state that an overall goal of reducing total NO_x emissions by two million tons below 1980 levels by the year 2000. One possible alternative for NO_x treatment is microbial denitrification. Denitrification, is the process in which nitrate is reduced to nitrogen through nitrite, nitric oxide, and nitrous oxide (Sakurai & Sakurai, 1997).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

The goal in this project was to use the bacterium *Paracoccus denitrificans* to carry out the last three steps in the denitrification process by using various amounts of NO.

Materials and Methods

The bacterium *P. denitrificans* was used in the conducted experiments. *P. denitrificans* reduce nitrate to nitrous oxides to molecular nitrogen under anaerobic conditions. The bacterium was grown in a solution with acetate and minerals. The medium used for the cultures contained (500 mL): $CH_3COONa{\cdot}3H_2O$, 2.0 g; K_2HPO_4, 0.4 g; KH_2PO_4, 0.15 g; NH_4Cl, 0.2 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; and 1 mL trace metal solution. The trace metal solution contained: EDTA, 5.0 g; $ZnSO_4{\cdot}7H_2O$, 0.22 g; $CaCl_2$, 0.55 g; $MnCl_2{\cdot}4H_2O$, 0.5 g; $FeSO_4{\cdot}7H_2O$, 0.5 g; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.11 g; $CuSO_4{\cdot}5H_2O$, 0.157 g; $CoCl_2{\cdot}6H_2O$, 0.161 g; and 500 mL distilled H_2O. The pH was brought to 8 by using KOH.

The 500 mL of medium was then divided into nine 157.5 mL serum bottles with 50 mL of medium in each. Each of the nine bottles were then flushed with helium for five minutes, then capped, before autoclaving at 121ºC for twenty minutes. After autoclaving, the bottles were taken out to cool down to room temperature (21ºC). Next, the bottles were flushed with helium again for five minutes, and 5 mL of culture was injected into six of the nine bottles. Then, various amounts of nitric oxide (2 mL, 3 mL, 4 mL, 5 mL, 6 mL, and 7 mL) were added to the bottles and the bottles were placed into a shaker operating at approximately 100 rpm and 37ºC.

Liquid and gas samples from each of the bottles were analyzed with the Spectronic 20 Genesys and Hewlett Packard 5890 Series II Gas Chromatograph (GC) after bottles had been in shaker for five minutes. The spectrophotometer was set at the wavelength of 600 nm and used to measure the light absorbed in 1.5 mL of liquid culture. The absorbance is a relative measurement of the cell concentration. The higher the absorbance the higher the cell concentration. The GC was used periodically throughout the day by injecting 100 µL of gas sample from each bottle and correlated the area of the peak to calibration standards. After each round of sampling was completed, the bottles were placed back into the shaker. A sample was taken from each bottle about every hour and half until the NO was depleted or the depletion had diminished.

Results and Discussion

The experimental project consisted of six bottles containing medium and bacterial culture. Each of the bottles were exposed to various amounts of nitric oxide: Bottle A, 2 mL; Bottle B, 3 mL; Bottle C, 4 mL; Bottle D, 5 mL; Bottle E, 6 mL; and Bottle F, 7 mL. The bacterium, *P. denitrificans*, consumed the NO and the consumption was measured using the GC.

Figure 1 depicts a polynomial curve of the NO calibration. This calibration was necessary in order to calculate the amount of NO present in the samples taken from the bottles. The calibration data was collected from the initial gas samples (0.1 mL) taken from each bottle. It was assumed that no NO had been consumed at this initial sample event. Since the amount of NO added to each bottle was known, the amount of NO in the 0.1-mL sample could be calculated. Their amounts were then compared with the response from the gas chromatographic areas.

Figure 2 indicates the depletion of NO with respect of time. Gas samples of 0.1 mL were collected from each bottle about every one and half-hour for the first day, and at a lesser frequency the following days until the bacterium depleted the nitric oxide. The gas samples were injected into the GC and the areas of oxygen (O_2), N_2, and NO were recorded onto a spreadsheet. The calibration information was then used to calculate the amount of NO present in each bottle at sampling time. As seen in Figure 2, NO was consumed faster in the bottles having less initial amount of NO than in bottles having a greater initial amount of NO.

In addition to collecting gas samples for NO analysis, liquid samples (1.5 mL) were also collected. The absorbance was measured using the spectrophotometer in these samples. The spectrophotometer measured the amount of light that was absorbed or passed through each of the samples. That data was recorded into a spreadsheet then complied onto a chart. In Figure 3 bottles A and B showed a small increase in their absorbency levels, while in comparison bottle F showed a great increase in absorbency. Overall, the larger amount of NO concentration the higher the final absorbance, since more NO was available for growth. The results in Figure 3 show a constant error for the data collected from Bottle E. There is no logical explanation for this, other than maybe a higher background absorbance in this bottle.

In Figure 4, the results indicate that N_2 levels increased very little in bottles with small initial amounts of NO. This is because there was a small amount of NO to consume and transform into N_2. On the other hand, bottles containing larger initial amounts of NO had much greater amounts of N_2 present at the end of the experiment.

Figure 5 displays the amount of N_2 produced, as a function of the amount of NO consumed throughout the experiment. As the amount of NO decreases the amount of N_2 rises. Theoretically, the production of N_2 should be half as much as the input of NO based on the reduction reaction of NO to N_2.

$$4e^- + 2NO + 4H^+ \rightarrow N_2 + 2H_2O$$

This experiment proved to be close to the theory, Bottle F had an input of about 300 µmol of NO and an output of about 120 µmol of N_2. For many of the other bottles, experimental error associated with gas sampling and accidental incorporation of air made it difficult to calculate the correct amount of N_2 in bottles.

Conclusion

The project experiment resulted in many trials with little success. The *P. denitrificans* are a highly specialized bacterium that cannot withstand large amounts of O_2. The bacterium must have a stable environment to live in, since they are unable to cope with drastic changes that may occur in the environment. About ten experiments were tried and only two out of the ten proved to be a success. The microbial conversion of NO can occur with the *P. denitrificans*, but the bacterium was hard to grow.

Acknowledgements

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Figures

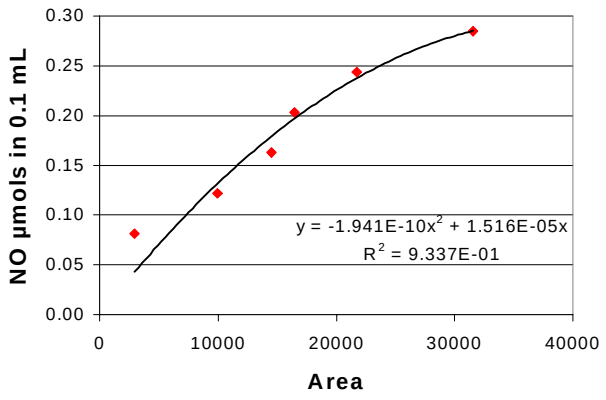

Figure 1. Calibration of NO on GC. Shows the NO µmols in the initial 0.1 mL samples taken from the bottles, as a function of GC peak area.

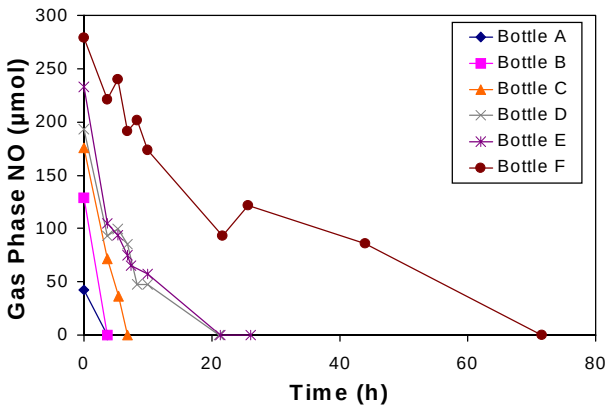

Figure 2. NO consumption profile. Shows the amount of NO (µmol) in the gas phase as a function of time.

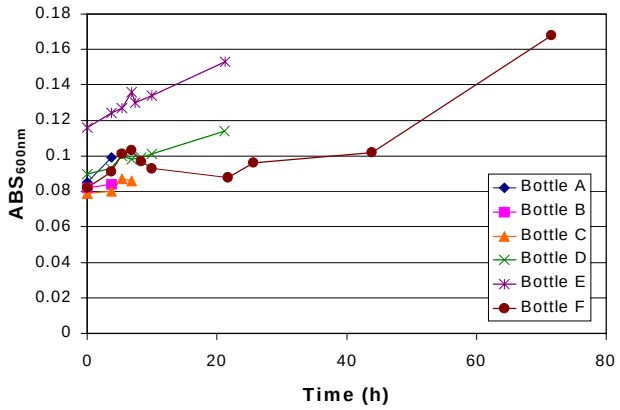

Figure 3. Absorbance profile. Shows the light absorbance levels as a function of time.

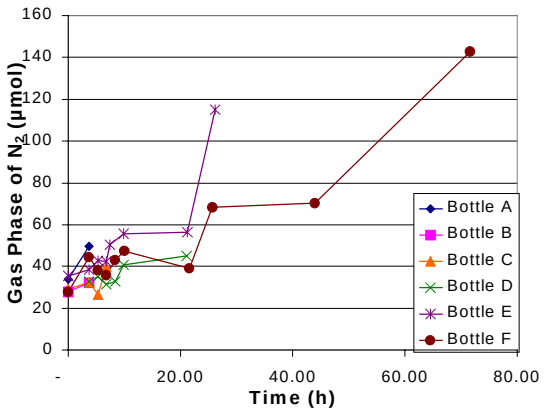

Figure 4. N_2 production profile. Shows the gas phase of N_2 (µmol) as a function of time.

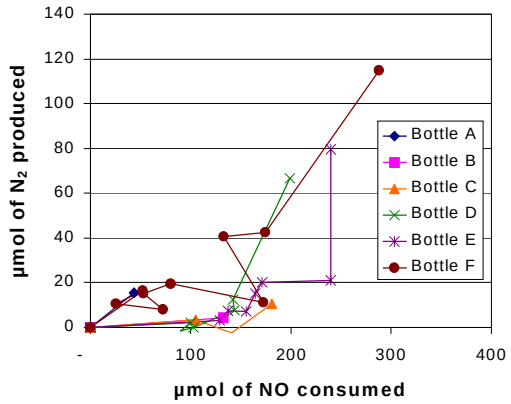

Figure 5. Amount of N_2 produced as a function of NO consumed for each bottle.