CO2 and NO Removal from Flue Gas

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Background

The combustion waste product known as flue gas is a pollutant of serious environmental concern with effects including acidification, eutrophication, and hazards to human health. The two main hazardous chemical are Carbon dioxide (CO2) and Nitric Oxide (NO), with sulfur dioxide (SO2) as a less prominent pollutant (Jin et al, 2005). CO2 from Steel Plant flue gas emissions is the largest single source of CO2 in the world (Chiu et al, 2011). Industrial emission such as this make stationary sources the major concern, even though combustion from motor vehicle engines also produce these harmful chemicals.

Pre-Combustion methods of reducing these emissions such as reducing the excess air in combustion would be very effective; however this would lower the efficiency of the combustion, making it not feasible. Post combustion, non- biological methods of treatment includes selective catalytic reduction, adsorption and scrubbing. Bioprocesses for the removal of these chemicals offer a less expensive and often simpler solution. Complications arise in optimizing the conditions for the organisms used, and overcoming unexpected reactions such as the inhibition of SO2 removal in the presence of NO2 (Jin et al, 2005).

Most research on this topic observed the effectiveness of microorganisms operating in mesophilic temperatures of around10-40 degrees Celsius. However after exiting a combustion chamber, flue gases are likely to be in the thermophilic temperature range of around 40-70degrees Celcius (Ono et al, 2007). Cooling the gases is possible, but the extra operation costs are unnecessary and make biological processes more unattractive. Because of this, thermophilic species of microorganisms with nitrogen fixing and CO2 mitigating abilities need to be isolated or engineered, and there performance in relation to other chemical present in flue gas needs to be quantified.

Project 1-Conjugating Nitrogen Reductase/Oxidase into Autotrophic Bacteria

Introduction

Biological methods of remediating various types of pollutants are of increasing interest as the understanding of molecular biology, and microbiology develops. Biological treatment can offer many advantages over abiotic processes, but two main factors to be considered are cost effectiveness, and how permanent the solution is. In general, microorganisms are cheaper to utilize than alternative catalysts, which must be constantly supplied. This is even more advantageous if they are indigenous and just have to be activated. Another key advantage of microorganisms is that they change the chemical composition of the pollutant, as opposed to just removing or containing it as dredging or capping does (Kamika and Momba, 2013). Many ways of remediating water sources polluted with heavy metals, hydrocarbons, other harmful chemicals have been described. However ideally, chemicals should be remediated before leaving a controlled area. This allows for any type of microorganism to be used, and provides indefinite containment.

The use of bioreactors and Photobioreactors is of current interest because of their low space usage and the ability to control all the growth conditions. They also contain all microorgansims involved, which is very important if genetic engineering is involved (Ford et al). They are especially useful in mitigating CO2 and other gases. Cyanobacteria have shown great potential in mitigating CO2. There have been multiple studies on the activity of thermophilic cyanobacteria, especially that of Chlorogleopsis, in bioreactor conditions (Ono and Cuello, 2007). These bacteria showed promising results in CO2 mitigation, light adaptability, and temperature tolerance. The same photobioreactor used in this study could also be used for NO reduction. However in this case only CO2 at elevated temperatures was used. For the usage of Chlorogleopsis to be feasible for remediation of flue gas, it would need to be at least resistant to NO, but ideally it would metabolize NO to a less harmful chemical such as NO3 or N2. Although separation of these gases is possible, it would greatly increase operation costs.

One possible solution to the inhibition potential of NO in flue gas is to transfer a resistance or reduction gene into the cyanobacteria Chlorogleopsis. Genetic engineering of cyanobacteria is uncommon, especially in subsection V, the subsection of Chlorogleopsis. This

subsection has the most complex species of cyanobacteria, which are filamentous and form heterocysts. Heterocysts are cells in which nitrogen fixation happens. Heterocysts are oxygen impermeable, which is interesting considering that denitrifying bacteria require anoxic conditions (Stucken et al, 2012).

Project Goals:

-Transfer a NO resistance gene (preferably one which reduces NO to N2 or oxidizes NO to NO3) into Chlorogleopsis.

-Quantify the ability of Chlorogleopsis to fix NO, and its resistance to NO pre and post gene transfer.

-Determine if the presence of NO severely lowers biomass or CO2 mitigation.

-Determine the effect of temperature on NO fixation, and optimal temperature range.

Gene Transfer:

Gene transferring in microorganisms is a naturally occurring process. These processes are replicated in a laboratory environment. In many cases, bacteria are genetically engineered to complete a metabolic pathway (Pieper and Reineke, 2000). However, nitrogenases have also been transferred into bacteria (Pelczar Reid and Chan MICROBIOLOGY TEXTBOOK). One gene of interest in NO resistance is that of Mycobacterium tuberculosis. This bacterium causes tuberculosis, and has developed a truncated hemoglobin as a resistance mechanism to NO production by macrophages in the body. NO produced by the body usually causes the bacterium to remain dormant, but because of such resistances, the bacterium may remain active. Ouellet et al. (2002) demonstrated the NO resistance activity of this gene by transferring it into another *Mycobacterium* in the species *bovis*. They found that this gene increased NO resistance up to 20 times over.

Transferring genes into cyanobacteria is a more difficult process in comparison to many other types of bacterial gen transfer. This is mainly due to their unusual abundance of restriction endonucleases. Restriction endonucleases act as guards for incoming DNA, which can be a major issue when transferring a gene into them. However, this complication can be overcome

using Cargo plasmids. In fact, conjugal gene transfer, which was the most reliable method, was only possible using a cargo plasmid (Karina et al. 2012).

Methods

Stucken et al. performed intensive research on three methods of gene transfer in Cyanobacteria subsection V and found that conjugation was the most reproducible. In their case both the bacteria and the plasmids were provided. Before genetic information can be transferred into a bacteria, it must first be connected to a plasmid.To cause this, first DNA helixes are disrupted using PCR (Polymerase Chain Reaction), which leaves unbounded DNA overhangs, available for incoming plasmids. Then bacteria will be sonicated, which will disrupt their polysaccharide layers, allowing DNA strands to penetrate and exit the cell membrane. Once the plasmids are hosting the foreign genes, a carrier plasmid must be employed, both to mobilize these host plasmids, and to protect them from the endonucleases. Since even the most reliable methods of DNA transfer leave some bacteria unaffected, the solution must be subjected to contact with NO and living bacteria have exhibited resistance and are usable.

NO Resistance and CO2 Mitigation

NO resistance can be tested simply by increasing the atmospheric concentration of NO very gradually. This will be done before bacteria are modified, and afterward. If bacterial mutants are determined resistant to NO, then a gas containing high concentrations of CO2 and NO at an elevated temperature (around 55 degrees Celsius) will be added to the reaction chamber. Amounts of CO2 and NO entering and exiting the flow meter can be measured using a flow meter. The difference in these amounts can be used to calculate the total reduction caused by the cyanobacteria. Some variables include: temperature, pH, addition of CO2 separate from NO or vice versa, and pulses of gas as opposed to a continuous flow. Pulses of gas was shown to dramatically increase growth of microorganisms, and efficiency of another bioreactor (Chiu et al 2011)

Growing Conditions

Bacteria will be grown on vertical membranes inside the bioreactor. Although it is unlikely that there will be a lack of carbon or nitrogen in the system, other nutrients and growth

factors may need to be added. One common growth factor used is bovine serum. There may also be a need for the addition of sulfur, however in a direct injection of flue gas from a factory into a bioreactor, sulfur would certainly be present. The temperature of the system will be kept as high as possible, with a reasonable cell growth present to represent a direct injection of flue gas. A light source would be provided by fluorescent lamps as was done by Ono and Cuello (2007)

Results

Most important is the reduction of CO2 and NO2 concentrations in the reactor, and the chemicals released as a result.

Biomass is critical in overcoming the toxicity of these chemicals, and can be measured simply by drying a culture of cells and weighing them.

Conclusion

CO2 and NO released from flue gas are to harmful pollutants which must be filtered from industrial emission. The cyanobacteria *Chlorogleopsis* has shown effective CO2 removal in thermophilic conditions, but would be much more useful if it simultaneously removed NO. This could be made possible by inserting a gene such as the truncated hemoglobins of *Mycobacterium tuberculosis*.

Project 2- Simultaneous CO2 mitigation and NO reduction by different microorganisms.

Background

The release of harmful chemicals such as Carbon dioxide (CO2) and Nitric Oxide (NO) has been increasing exponentially over the previous decades and the disposal of these greenhouse gasses is an international issue (Chiu et al. 2011). Often, reluctance to follow the most environmentally friendly methods of filtering these chemicals is based on financial strain. Biological methods of remediating waste gas from factories offers a more simple, and financially reasonable option as opposed to conventional abiotic methods (Jin et al. 2005). Such processes as

Selective catalytic reduction, adsorption and Scrubbing require either expensive catalysts or have high energy costs.

Introduction

For the most effective results, would be prevented before combustion by processes such as reducing excess air involved in the combustion. However, this often causes reduced combustion efficiency, negating its positive effects. Because of this, post combustion ways of cleansing flue gas are of interest. Bioreactors are currently one of the most promising strategies of biological gas emission control. These involve utilizing the activities of microorganisms which naturally carry out processes such as CO2 mitigation or NO reduction in a controlled environment called a bioreactor. Although most research on various microorganisms for use in a bioreactor involved mesophillic species, a thermophillic bacteria would be much more feasible, because it prevents the need for an expensive cooling stage before the gas enters the bioreactor. One thermophillic cyanobacteria, *Chlorogleopsis*, has been isolated, and shown to be extremely effective in removing CO2 from gases in temperatures around 50 degrees Celsius (Ono and Cuello, 2006). However for a bioreactor connected directly to a stream of flue gas from a factory to be effective, both CO2 and NO would have to be removed before allowing the gas to exit into the atmosphere. Previously I proposed a method to solve this issue by engineering a mutant strain of *Chlorogleopsis* to incorporate the NO detoxifying capabilities of a truncated hemoglobin into it. However, the reliability of such a procedure is very low, especially considering the restriction endonucleases present in cyanobacteria subsection V, and small knowledge base of cyanobacterial gene transfer in general (Stucken et al). Here a method of simultaneous NO and CO2 removal from gas in thermophillic conditions is proposed.

Methods

Ono and Cuello (2006) show an excellent model for testing bacteria in conditions representing those of flue gas. In their model, a CO2 gas cylinder releases gas into a four containers housing Chlorogleopsis. These containers include a water solution and are submerged in a heated water bath, which regulates the temperature of the containers. The inflow and outflow of gas from the containers are measured by a flow meter. The light source for the cyanobacteria is provided by Fluorescent lamps.

The biomass or population density of the solution will be measured by the dilution plate method. This involves taking the container from the model, and pouring one tenth of the solution into another container holding nine tenths of the original volume of water. This is then repeated and a sample from the container is placed on a petri dish. Individual cells will form visible cultures. The fraction of number of cultures/volume of water is multiplied by 10^times diluted, and the result is the original population density.

This model is simpler than most, and very replicable. However, because this must represent a situation where two different strains of bacteria are operating, some small variations must be made. Mainly, when the gas exits the container hosting the Chlorogleopsis, it must flow into the container hosting the denitrifyer, then exit through a flow meter.

As discussed, the bacteria used to mitigate the CO2 in the system will be Chlorogleopsis. The bacteria used to denitrify the gas will be *Bacillus stearothermophilus.* This is a thermophillic bacteria identified by Lebedeva and co-workers (1998). The bacteria has demonstrated 60-90% NO removal at 60 degrees Celsius. Thermophillic properties, and extremely high NO removal efficiencies make this bacteria an ideal candidate for a flue gas bioreactor.

Results

At a glance, a study such as this may seem to have predictable results. A CO2 mitigating bacteria, and a NO removing bacteria combined, would have CO2 and NO removing abilities. However, there are multiple variables, which could cause results to skew. A critical variable is the simultaneous addition of NO and CO2 to these bacteria, which could certainly cause halted growth of one or both the bacteria, or inhibition of their removal abilities. *Desulfovibrio desulfuricans* was shown to remove SO2 from a gas stream, but when NO was added to the system, it inhibited SO2 reduction (Dasu BN et al. 1993). Another important factor to measure is biomass, or the density and amount of cells present. Biomass is key to overcoming the toxicity of NO, and because the more cells present, the more cellular activity taking place, and the higher efficiency of the bioreactor.

Finally, the removal of NO by Chlorogleopsis alone will be interesting, considering that all cyanobacteria in subsection V have heterocysts, which are impermeable to oxygen and are

known for nitrogen fixation (Stucken et al, 2012). Nitrogen fixation is often accompanied by Nitric Oxide fixation.

Conclusion

The simultaneous removal of NO and CO2 in from flue gases is a pressing concern on environmental cleanliness. If these processes could be carried out in thermophilic conditions, it would greatly reduce operation cost and the feasibility of this system. Chlorogleopsis used for CO2 removal and *Bacillus stearothermophilus* used for NO removal would provide an excellent solution to this issue.

Project 3-Characterization of Isolates involved in the BioDeNOx process.

Introduction

Nitrogen Oxide (NO) is an air polluting chemicals released in almost all industrial processes. NO is a greenhouse gas and is involved in eurtrophication and acid rain. Besides these environmental concerns, nitric oxide is involved in the development of Parkinson's disease and asthma, due to its detrimental effect on neurons (Kumaraswamy et al, 2005). Because of its harmful effects, many methods of NO removal from these gases are under investigation. Many abiotic NO removal systems exist, including selective catalytic reduction, selective non-catalytic reduction, and adsorption. However, there are several major drawbacks to these conventional methods. Some major flaws in these systems is the unsustainable cost of treating large amounts of gas, and often subsequently generated wastes are also harmful. Biological methods of NO removal offer a promising alternative. One biological mean of NO removal is the BioDeNOx Process (Jin et al, 2005)

The BioDeNox process is based on four chemical reactions.

1. Fe(II)(EDTA) quickly absorbs NO.

2. The Fe(II) is oxidized to Fe(III) by oxygen present.

3. Denitrifying bacteria use ethanol as an electron donor to reduce NO to N2, which is non-toxic.

4. Bacteria reduce Fe(III) to Fe(II), again using ethanol as the electron donor.

This final step is critical because it Fe(II) is required to for bacteria to reduce NO to N2. It is also the limiting factor in the process because 90% of the electron flow was into Fe(III), and only 10% went into reducing NO (Jin et al, 2005) It is because of this that more characterization of the exact species present in the reaction chamber, and their possible electron donors (ethanol being the electron donor in this example) needs to be done.

Materials and Methods

Kumaraswamy et al (2005) took a sample from a BioDeNOx reactor to study the dynamics and species of the microbial colonies present. In this study they found that the limiting factor of the process was the reduction of Fe(III) to Fe(II), which competed with NO reduction for electrons. Two isolates made up the majority of the biomass. The NO reducing bacteria, *Bacillus Azotoformans* made up 15-20%, and the Fe(III) reducing bacteria *Deferribacter thermophilus* made up 65-70%. These species will be the focus of this study.

Bacteria will be hosted in an air tight reaction chamber. A gas flow meter will measure the inward flow and outward flow of NO, O2, CO2, and SO2. These are four common chemicals in flue gas. The difference in the concentrations of each chemical added to the chamber, and that which exits will be considered the amount reduced.

Rationale

The questions addressed here are:

What are environmental conditions are optimal for each species? Does *D. thermophilus* have an unknown advantage in this environment?

What conditions allow for *Bacillus azotoformans* and *Deferribacter thermophiles* to reduce the greatest amount of NO and Fe(III) with the greatest efficiency?

What substrate will make the highest amount of electrons available to the species?

Results

D. thermophiles made up a greater amount of the bacterial colony compared to *B. azotoformans*. D. thermophiles also used much more of the electron flow to reduce Fe(III) than B. azotoformans did to reduce NO. It would be interesting to discover if the larger population was based on the larger concentration of Fe(III), or if it was based on an unknown environmental factor. Because 90% of the electrons from ethanol in this study were used for iron reduction, it would be interesting to see how the kinetics of the reaction changed with a different substrate such as H2. If the addition, or replacement of a substrate such as this could create a quicker or more efficient reduction of Fe(II) to Fe(III), it would dramatically increase the productivity of the entire process.

Conclusion

In their study, Kumaraswamy et al (2005) used a bioreactor with various, unidentified species, all living in one environment. They were the first group to do any type of characterization of the BioDeNOx bacterial community. The isolation of these individual species, and characterization of their productivity, will provide new insights on how to select species for flue gas removal purposes.

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