TREATMENT OF NITROGEN OXIDES BY *CHLORELLA VULGARIS* ALGAE IN PHOTOBIOREACTORS

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ABSTRACT

Treatment of Nitrogen Oxides by *Chlorella Vulgaris* Algae in Photobioreactors

Steven Shihady

The effectiveness of algae to treat NO_2 and NO in simulated flue gas was tested using *Chlorella vulgaris* in photobioreactors (PBRs) using NO_x concentrations between 30 ppm to 780 ppm. NO_x dissolved and reacted in water to form NO_3^- and NO_2^- in the PBR growth medium, providing a nitrogen source that the algae readily assimilated for cell synthesis. Three 20-L photobioreactors were inoculated with a pure culture of *C. vulgaris* prepared in Bristol growth medium and algae were grown in the PBRs at 25°C and pH of 7.0 in a modified Bristol medium that did not contain nitrogen compounds. The *C. vulgaris* grew substantially using NO_3^-/NO_2^- as its nitrogen source for cell synthesis. The NO_3^- and NO_2^- were formed through the dissolution and oxidation/reduction of NO_x from the simulated flue gas. Algal growth by assimilation of NO_3^- and/or NO_2^- allowed for continual dissolution of NO_x, resulting in NO_x removal rates from the gas phase of up to 97%, with residual nitrogen of up to 7 mg-N/L in solution. Algae grew from an initial cell density of 3.1×10^5 cells/L to cell densities of up to 1.85×10^7 cells/mL and dry weights of up to 243 mg/L. Cell nitrogen content varied from 4-8%. PBR to treatment of gaseous NO_x was analyzed in terms of mass transfer rates, chemical kinetics, and biological growth.

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1. INTRODUCTION

The combustion of fossil fuels produces byproducts, such as carbon dioxide and other greenhouse gases, sulfur dioxide, nitrogen oxides, volatile organic compounds (VOCs), and heavy metals, which are harmful to both the environment and living beings. While it is important to reduce all of these harmful byproducts, the research in this thesis focuses on reducing nitrogen oxides (NO_x) through the use of living cultures of algae in an attempt to assimilate the nitrogen from NO_x into their cell mass.

The two major components that make up NO_x are nitric oxide (NO) and nitrogen dioxide (NO_2). These compounds are very toxic when inhaled and contribute to various environmental hazards (EPA, 2013b). Nitrogen oxides are formed through the reaction of N_2 with O_2 during combustion at high temperatures and through the conversion of nitrogen bound to a combustion fuel (EPA, 1995). The NO_x in flue gas directly out of a stack is typically composed of 90-95% NO and 5-10% NO_2 (MacDonald, 2003).

NO_x emissions from automobiles are primarily treated using catalytic converters to reduce the compounds to nitrogen and water. This method, known as selective catalytic reduction (SCR) is also widely used to treat NO_x from stationary sources by reducing the NO_x to N_2. While effective, SCR is rather expensive when applied to large scale power plants (Skalska et al., 2010). Another way to treat NO_x from stationary sources is to use scrubbers to transfer the gaseous NO_x into an aqueous medium. Although effective in removing NO_x from the air, unfortunately, this method only transfers the risk into an aqueous solution, which still must be treated or disposed of (Blaszczak & Cox, 1999). Thus, a push to develop an inexpensive, effective, and environmentally friendly process to treat NO_x pollution exists. One promising biochemical approach is the cultivation of algae to take up dissolved NO_x from a scrubber

as a nitrogen source, releasing only oxygen as a byproduct. This concept has been previously researched to determine that, given the right algal strains and conditions, algae can indeed assimilate nitrogen from dissolved NO_x (Nagase et al., 1997). Although there are certain inhibitory effects of growing algae using flue gas (i.e. acidic environments), under the right conditions the algae can thrive and effectively treat NO_x from stationary emission sources (Matsumoto et al., 1997). The purpose of the research presented in this thesis was to demonstrate algal NO_x treatment using a pure culture of *Chlorella*, which could potentially be developed as either a food source or biofuels source.

NO_2 has a relatively high solubility in water, allowing it to readily react with water to dissociate into compounds easily assimilated by algae. The dissociation of NO_2 is represented in Reaction 1.

$$NO_2(g) \leftrightarrow NO_2(aq) \leftrightarrow NO_3^-(aq) + NO_2^-(aq) \quad (1)$$

Algae are readily able to use aqueous nitrate or nitrite as a nitrogen source for cell synthesis (Mulholland & Lomas, 2008). The uptake of nitrate and/or nitrite pushes the equilibrium of Reaction 1 to the right, and decreases the concentration of dissolved NO_2. This increases the NO_2 concentration gradient between air and liquid, and theoretically increases the mass transfer rate. Ideally, this process will stabilize to develop a sustainable treatment method for NO_2, as long as algal growth occurs.

In addition to removing dissolved nitrate from the flue gas and enhancing total NO_x removal rates, specific strains of algae could potentially be harvested and sold as a food source for animals and/or humans. This adds economic benefit to the algae-based NO_x treatment technique. The algal species chosen for this research was *Chlorella vulgaris* because of its historical use as a human food source (Belasco, 1997).

Chlorella is a spherical, single-celled, green freshwater alga. It has been widely researched and used as a food since the late 1940's, after fears of population boom and food shortages occurred after World War II. NASA space programs and health food companies began funding research into developing algae as a source of sustenance. Chlorella was chosen because of its potential nutritional value (Belasco, 1997). It contains a combination of more than 50% protein, 20% fat, 20% carbohydrates, and includes essential amino acids, minerals, and vitamins as well as potent flavonoids (Sandoval, 2007). Chlorella is also especially adept at producing chlorophyll, allowing it to rapidly and efficiently harness energy from sunlight. Chlorella supplements are becoming increasingly popular in Japan and the United States (Sandoval, 2007).

The current research utilized these concepts to test the effectiveness of treating NO_x from simulated flue gas using *Chlorella vulgaris* in a photobioreactor (PBR). Three mechanisms affecting the potential productivity of such a system were examined. First, mass transfer of NO_x from the gas to liquid phases must provide sufficient amounts of dissolved nitrogen (NO_3^-/NO_2^-) for the algae to assimilate. Second, the dissolved NO_2 and NO must react to form dissolved nitrogen compounds available for the algae to assimilate. The low solubility of NO becomes an important limitation to the system. Third, biological conditions must be met for the uptake of the nitrate and/or nitrite by the algae. After examining these mechanisms, potential enhancements can be considered. For instance, the bioreactor can be designed to optimize mass transfer. Further, because biological growth is dependent on the health and activity of the algae and their enzymes, environmental conditions can be optimized for algal growth. This is very important, because small changes in operating conditions can mean the difference between a lively,

active culture and a decaying one. Conditions affecting algal growth include light, water temperature, pH, and growth medium (Barsanti & Gualtieri, 2006).

Three separate experiments were conducted to determine the optimal growth conditions and the ability of the algae to effectively remove the NO_x from simulated flue gas with varying NO_x loading rates. This simulated flue gas consisted of a mixture of NO_2, NO, and CO_2 in air. In each experiment, three different NO_x concentrations were supplied to three separate, but identical, PBRs inoculated with *C. vulgaris*. The growth medium in the reactors was depleted of nitrate to prove the algae were able to grow using dissolved NO_x as their sole source of nitrogen. The two most important parameters determined through each run were NO_x removal efficiencies and algal cell growth for each PBR.

2. BACKGROUND

This background section first provides an understanding of the reasons NO_x requires treatment. Then the conventional methods used for treatment are described, along with reasons for developing an unconventional method. The underlying mechanisms of how nitrogen oxides react in gas and aqueous phases are then described, since understanding these reactions is vital to understanding the proposed treatment method with algae. Finally, a review of previous research and experimentation in using algae for NO_x uptake is given. This background section explores each of these topics as they pertain to researching and testing the treatment of nitrogen oxides using *C. vulgaris* algae.

2.1 NO_x Hazards and Government Regulations

NO is a colorless, nonflammable, poisonous, oxidizing gas at room temperature and can be very toxic if inhaled, even at very low concentrations (Airgas, 2013a). NO_2 is red-brown colored gas above 20°C that is also nonflammable and oxidizing. It has a pungent, acrid odor and is very toxic and corrosive on skin, eyes, and major organs if inhaled or ingested (Airgas, 2013b).

The Occupational Safety and Health Administration (OSHA) has set a permissible exposure limit (PEL) of NO at 25 ppm (Airgas, 2013a). The PEL for NO_2 set by OSHA is 5 ppm (Airgas, 2013b). NO and NO_2 may both cause target organ damage and possibly death if inhaled. NO is severely irritating to eyes and skin while NO_2 can result in severe respiratory tract burns and is severely corrosive to the eyes and skin (Airgas, 2013a; Airgas, 2013b). The PELs for these two NO_x components are an indication that they can be toxic even at very low concentrations.

Together NO and NO_2 contribute to the "formation of fine particles...and ground-level ozone...[which] are associated with thousands of premature deaths and illnesses each year" (EPA, 2012). Fine particles are formed when NO_x reacts with ammonia, water, and other compounds. These small particles are able to enter deep into the lungs causing respiratory disease and aggravating existing heart disease. Ground-level ozone is formed through a series complex reactions involving NO_x and volatile organic compounds (VOCs) under heat and sunlight (Pudasainee et al., 2006). Ozone also has adverse effects on the respiratory system, particularly in children and elderly individuals (EPA, 2013b).

The U.S. Environmental Protection Agency (EPA) Clean Air Act (CAA), implemented in 1971, established primary and secondary national ambient air quality standards (NAAQS) for NO_2 at 52 ppb, averaged annually. In 2010, the EPA established an additional NAAQS primary standard of 100 ppb averaged over one hour to further protect public health (EPA, 2013b). In 2005, the EPA also finalized the Clean Air Interstate Rule (CAIR) to further reduce nitrogen oxide levels. The rule establishes a cap-and-trade approach to reduce NO_x emissions from power plants by 2 million tons in 27 eastern states by 2015 (EPA, 2012).

2.2 NO_x Production from Stationary and Mobile Sources

Automobiles are the largest contributor of NO_x emissions in the United States, followed by stationary fuel combustion. In 2012, the transportation sector contributed 56% of total NO_x nationwide, while stationary fuel combustion contributed 32% (NEI, 2012). Because of the mobile and compact nature of automobile combustion

engines, NO_x reduction technology for this source is greatly limited. Therefore, a focus can be made to reduce the nitrogen oxides emitted through stationary sources, particularly power plants. Of the 32% of total NO_x emissions produced through stationary fuel combustion in 2012, nearly half was caused by fuel combustion for electricity generation (NEI, 2012).

2.3 Conventional Post-Combustion NO_x Treatment Methods

2.3.1 Selective Catalytic Reduction

Currently, the most widely used post-combustion treatment of NO_x for stationary sources is selective catalytic reduction (SCR) (Skalska et al., 2010). This method uses either a noble metal catalyst, metal oxide catalyst, or metal ion exchange zeolite along with ammonia to reduce NO to N_2 based on the following reaction:

$$4NO + 4NH_3 \rightarrow 4N_2 + 6H_2 \quad (2)$$

The main benefit of SCR is that the catalyst allows the reaction to occur at lower temperatures, between 300-800 K.

Precautions must be taken when using this technology, because of the use of ammonia. Unreacted NH_3 may slip into the exhaust gas and enter the atmosphere as an air pollutant. Spent catalyst must be replaced or regenerated, resulting in both a capital expenditure and an expensive maintenance cost. Overall, the process ordinarily removes approximately 60%-85% of NO and results in concentrations from 1-5 ppm NH_3 in the exhaust stream. To reduce the amount of reduction catalyst required, an oxidation catalyst may be implemented upstream to convert NO to NO_2. Another effective method is to inject ozone upstream to achieve the same purpose. To avoid ammonia slip, as well

as difficulties involved in transporting and storing ammonia, certain hydrocarbons are also effective reactants for the process (Skalska et al., 2010).

2.3.2 Selective Noncatalytic Reduction

Selective noncatalytic reduction (SNCR) utilizes the same concepts as SCR, but without the use of a catalyst. This means that the reactions must take place at higher temperatures. SNCR uses ammonia, urea, or cyanuric acid to reduce NO to N_2. This process is less efficient than SCR, achieving between 30%-70% removal of NO (Skalska et al., 2010). Because of the lower efficiencies, this method must be coupled with combustion process modifications or other post-combustion treatment techniques to meet regulatory requirements. The process may also produce byproducts of N_2O and CO, which are themselves harmful to the environment (Skalska et al., 2010).

2.3.3 Adsorption and Absorption

Both absorption and adsorption can be used to collect gaseous NO_x. Dry, powdered limestone can be sprayed into flue gas through a scrubber to react with both sulfuric acid and nitric acid formed through the reaction of SO_x and NO_x with water. Another approach is to spray a slurry of dry limestone and aqueous ammonia. The limestone will react preferentially with SO_x, while the ammonia will react with NO_x, creating an aqueous solution that can be further treated or disposed of. Dry sorbents can also be injected in-duct to form ammonium nitrate that can be used to make explosives or fertilizers. Activated carbon can be used to finish the capture of NO_x, and then collected through existing particulate removal methods (Blaszczak & Cox, 1999).

2.3.4 Nitrification

Nitrification is a process in which bacteria oxidize ammonium to nitrate through a series of intermediate steps. Two types of bacteria are required to complete the process, bacteria that oxidize ammonium to nitrite, and those that oxidize nitrite to nitrate. Using nitrifying bacteria to degrade NO_x into nitrate has promise as a NO_x treatment method, because NO is a known intermediate in the nitrification steps. Unfortunately, most nitrifying bacteria would not survive in very high temperatures, so flue gas would need to be cooled drastically for this method to prove effective. Studies have found that NO inhibits biomass and biofilm growth, increasing the required residence time of nitrification systems. Even with an established biofilm, the poor solubility of NO does not allow for practical residence times when using nitrification to treat NO_x (Jin et al. 2005).

2.3.5 Denitrification

Denitrification is the reduction of nitrate to nitrogen gas. Bacteria are capable of reducing nitrogen oxides when oxygen is limited or unavailable. As with nitrification, NO is an intermediate in the denitrification process. Evidence suggests that all, or most, denitrifying bacteria share a common ability to reduce NO, making the process a promising method for treating NO_x (Jin et al., 2005). Unfortunately, as with nitrification, the majority of successful research has been conducted in mesophilic conditions. This again raises the question of whether it is cost effective to cool emitted flue gas to appropriate temperatures to treat NO_x with bacteria (Jin et al. 2005).

2.4 Chemistry and Kinetics of Nitrogen Oxides

The basis of the current research depends on the dissolution of gaseous NO_x in water, followed by reactions to form NO_3^- and NO_2^-. Therefore, an important aspect of this study is the solution chemistry and kinetics of NO_x. The solubility of NO_2 in water is 213 g/L, while the solubility of NO in water is only 0.032 g/L (more than 6,000 times less soluble than NO_2) (Van Den Hende et al. 2012).

2.4.1 NO_2

NO_2 exists in equilibrium with N_2O_4 in both the gaseous and aqueous phases. The aqueous phase reaction, shown in Reaction 3, has an equilibrium constant of $6.54 \times 10^4\ M^{-1}$ at 20°C (Schwartz & White, 1981) and a second-order rate constant for the formation of N_2O_4 of $4.5 \times 10^8\ M^{-1}s^{-1}$ at 25°C (Gebicka & Stawowska, 2012).

$$2NO_2 \leftrightarrow N_2O_4 \quad (3)$$

The formation of N_2O_4 is the intermediary step in the conversion from dissolved NO_2 to NO_3^- and NO_2^- as shown in Reaction 4.

$$N_2O_4 + H_2O \rightarrow NO_2^- + NO_3^- \quad (4)$$

The first-order rate constant for Reaction 4 is $1 \times 10^3\ s^{-1}$ at 25°C (Gebicka & Stawowska, 2012).

According to Cheung et al. (2000), the high solubility of the N_2O_4 dimer results in complications when trying to measure and understand NO_2 solubility. This is especially important at high NO_2 (g) concentrations, when N_2O_4 is present as a significant fraction of the total NO_x concentration. Cheung et al. overcame the problems with previous studies by using a horizontal bubble train flow reactor with precise control of the gas-

liquid interaction time. The study assumed that gas-phase diffusion and mass accommodation had a negligible effect on the uptake of gaseous NO_2 into the aqueous phase. Mass accommodation can be defined as the fraction of molecules that contact the aqueous phase that are absorbed to the aqueous phase (Julin et al., 2013). Cheung et al. used Equation 5 for uptake flux presented by Danckwerts (1970) for irreversible reactions in bulk liquid to determine the kinetic parameters of NO_2:

$$J = n_g HRT \left[\left(\frac{D_1}{\pi t} \right)^{1/2} e^{-kt} + (D_1 k)^{1/2} \mathrm{erf}(kt)^{1/2} \right] \quad (5)$$

where n_g is the gas-phase density of NO_2, H is the Henry's law constant, R is the universal gas constant, T is the temperature, D_1 is the diffusion coefficient of NO_2 in the liquid, k is the pseudo first-order reaction rate of NO_2 in the liquid, and t is the gas-liquid interaction time. The parameters H and k are determined as described below.

The solubility of NO_2(g) is governed by Henry's law. Taking into consideration the tendency for NO_2 and N_2O_4 to be in equilibrium with one another in both the gaseous and aqueous forms, Cheung et al. (2000) established an equation for an effective Henry's law constant at low NO_2 concentrations defined as:

$$H_{eff} = H_{NO_2}(1 + 2K_{aq} H_{NO_2} p_{NO_2}) \quad (6)$$

where H_{NO2} is the Henry's constant of NO_2, p_{NO2} is the partial pressure of NO_2, and K_{aq} is the equilibrium constant of NO_2 and N_2O_4 in the aqueous phase. This effective Henry's law coefficient represents the solubility of the nitrogen species in the oxidation state IV. Equation 6 allows for the value of H in Equation 5 to be determined, and respectively, the flux of NO_2(g) into solution.

The other variable to be determined in Equation 5 is the value of *k*. Cheung et al. used Reactions 3 and 4 to derive the rate of disappearance of NO_2(aq), $d[NO_2(aq)]/dt$, into HNO_2 and HNO_3 as a second-order equation as follows:

$$-\frac{d[NO_2(aq)]}{dt} = 2k_2[NO_2(aq)]^2 \quad (7)$$

where k_2 is the second-order rate coefficient. Because Equation 7 is only valid for first-order reaction rates, the value of *k* can be determined by Equation 8 below:

$$k = \frac{2}{n+1} k_F [N(IV)_{aq}]_0^{(n-1)} \quad (8)$$

where *n* is the order of the rate equation, $[N(IV)_{aq}]_0$ is the concentration of nitrogen species in the oxidation state IV (NO_2 and N_2O_4) at the liquid surface, and k_F is defined as:

$$k_F = \frac{2k_2[NO_2(aq)]^2}{([NO_2(aq)]+2[N_2O_4(aq)])^n} \quad (9)$$

Through experimentation, Cheung et al. concluded that the value of H_{NO2} is 1.4×10^{-2} M/atm and the value of k_2 is 3.0×10^7 M/s, both at 293 K.

Lee and Schwartz (1981) used a completely different approach to describe the kinetics of NO_2 dissolution. They focused their efforts primarily on the formation of nitrate rather than the dissolution of NO_2. They established that the rate-limiting step in the formation of NO_3^- was the reaction of NO_2 with water, as demonstrated in Reactions 1 and 2 above. As mentioned earlier, the rate of reaction from gaseous NO_2 to NO_3^- and NO_2^- in solution is dependent on the solubility of the gas, the rate of mass transfer, and the aqueous phase reaction kinetics. Therefore, Lee and Schwartz theorized that the rate and mechanism of the reaction is dependent on the mass-transfer regime in which the reactions are taking place. They proposed three distinct regimes: molecular diffusion

controlled, convective mass-transfer controlled, and phase mixed. The conditions for each regime were established based on the characteristic times of reaction, mixing, and molecular diffusion. Their studies determined the following reaction rate equations for the three regimes based on the partial pressures of NO_2:

a) Phase-mixed

$$R_1 = k_1 {H_{NO_2}}^2 {p_{NO_2}}^2 \qquad p_{NO_2} \leq 8 \times 10^{-8}\text{atm} \tag{10}$$

b) Convective mass-transport limited

$$R_1 = \frac{1}{2} k_m H_{NO_2} p_{NO_2} \qquad 8.5 \times 10^{-6}\text{atm} \leq p_{NO_2} \leq 1.1 \times 10^{-4}\text{atm} \tag{11}$$

c) Diffusive mass-transport limited

$$R_1 = a \left(\frac{Dk_1}{3}\right)^{1/2} \left(H_{NO_2} p_{NO_2}\right)^{3/2} \quad p_{NO_2} \geq 1.9 \times 10^{-3}\text{atm} \tag{12}$$

where R_1 is the reaction rate, k_1 is the rate coefficient for aqueous phase reaction, H_{NO2} = 7.0 x 10^{-3} mol/L-atm, p_{NO2} is the partial pressure of NO_2, k_m is the convective mass transfer constant (typically denoted $k_L a$), a is the interfacial area per unit liquid volume, and D is the aqueous-phase diffusion coefficient of dissolved NO_2, determined to be 2.0 x 10^{-5} $cm^2 s^{-1}$ using the semi-empirical correlation of Wilke and Chang (1955) (Lee & Schwartz, 1981).

2.4.2 NO

Aqueous NO reacts with oxygen in water to form NO_2^- by Reaction 13:

$$4\text{NO} + \text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + 4{\text{NO}_2}^- \tag{13}$$

Many previous authors have confirmed that the rate of reaction of Reaction 13 is second-order in NO and first-order in O_2, following Equation 14 (Lewis & Deen, 1994).

$$-\frac{d[NO_2^-]}{dt} = k_1[NO]^2[O_2] \quad (14)$$

Pires et al. (1994) and Kharitonov et al. (1993) both determined k_1 to be approximately 6.3 x 10^6 $M^{-2}s^{-1}$ at 20°C.

Lewis and Deen (1994) presented a more detailed kinetic scheme for Reaction 13, taking into account intermediates of NO_2 and N_2O_3 as presented in Reaction 15:

$$2NO + O_2 \rightarrow 2NO_2 \quad (15a)$$

$$NO + NO_2 \leftrightarrow N_2O_3 \quad (15b)$$

$$N_2O_3 + H_2O \rightarrow 2H^+ + 2NO_2^- \quad (15c)$$

They determined, however, that NO_2 and N_2O_3 were present only in very small amounts, leading them to Equation 14 with a k_1 of 2.1 x 10^6 $M^{-2}s^{-1}$ at 23°C. Lewis and Deen also noted that there was no detectable formation of NO_3^- at any point during the reaction, as demonstrated in Reaction 15. Therefore, all of the NO_3^- that is formed when treating NO_x in a photobioreactor is through Reaction 4.

To determine the diffusion coefficient of NO (D_{NO}), Zacharia and Deen (2005) used Reaction 15 in conjunction with the reaction rate equation (Equation 16) and conservation equation (Equation 17):

$$R_{NO} = -4k_1 {C_{NO}}^2 C_{O_2} \quad (16)$$

$$\frac{\partial C_{NO}}{\partial t} = D_{NO}\frac{\partial^2 C_{NO}}{\partial x^2} + R_{NO} \quad (17)$$

where R_{NO} is the aqueous reaction rate, C_{NO} is the aqueous concentration of NO, and C_{O2} is the aqueous concentration of O_2. Using chemiluminescence to measure transient and steady fluxes of NO across aqueous films, they determined D_{NO} to be 2.21 x 10^{-5} cm^2s^{-1}. Comparing this to the diffusion coefficient of NO_2 in water found by Lee and

Schwartz (1981) (2.0 x 10^{-5} cm^2s^{-1}), the diffusion of NO and NO_2 in water are nearly identical.

2.5 Algae Nitrogen Assimilation

Microalgae have the ability to assimilate nitrogen from a multitude of compounds, including ammonium, nitrate, nitrite, urea, organic compounds, and sometimes amino acids and purines (Mulholland & Lomas, 2008). Uptake through passive diffusion through a cell membrane is considered minimal, and therefore it is important to look at active membrane transport systems to determine how and why certain sources are utilized preferentially. NH_4^+ appears to be the most preferred nitrogen source for algae as it is the most energetically efficient form for the algae to take up (Mulholland & Lomas, 2008). Assimilation of NO_3^- and/or NO_2^- requires added energy and reductants necessary to reduce these forms. To use nitrogen, the cells must first take up the dissolved NO_3^- or NO_2^-, reduce it to ammonium, and then assimilate it into amino acids. Therefore, if NH_4^+ is present, little additional energy is required as further reduction is not necessary, and it becomes the preferred source (Mulholland & Lomas, 2008).

When NH_4^+, NO_3^-, and NO_2^- are all present in a culture medium, the NH_4^+ is thought to have an inhibitory effect on the uptake of NO_3^- and NO_2^-. Algal cells grown initially on ammonium have no ability to take up NO_3^- and NO_2^- unless completely deprived of nitrogen first (Syrett, 1988). NO_3^- transporters are thought to only be induced under nitrogen starvation or growth on NO_3^- enriched medium. It appears that NO_2^- is more readily assimilated than NO_3^- (Mulholland & Lomas, 2008). The rate-limiting step in NO_3^- assimilation is thought to be the reduction of NO_3^- to NO_2^-, not the reduction of

NO_2^- to NH_4^+. Also, because nitrite reductase is required in the assimilation of NO_3^-, organisms which are able to take up NO_3^- are also able to take up NO_2^- (Mulholland & Lomas, 2008). However, higher concentrations of nitrite may inhibit algal growth (Syrett, 1962).

When algae are grown under nitrogen-deficient conditions, the products of photosynthesis change from proteins to carbohydrates and lipids (Syrett, 1962). These cells also develop a carbohydrate reserve not found in normal algal cells. This results in a decrease in nitrogen content of the cells from 8-10% to about 2%. The amount of chlorophyll also decreases, as well as the overall rate of photosynthesis (Syrett, 1962).

The reduction and uptake of nitrate may be stimulated by light for a variety of reasons (Syrett, 1962). One theory is that light increases permeability of cells to NO_3^-. Another is that a photochemically produced reductant or a product of photosynthesis may be readily available as an electron source for the reduction of NO_3^- and/or NO_2^-. Photo-phosphorylation may also stimulates NO_2^- reduction (Syrett, 1962).

2.6 Previous Research on Growing Algae with Flue Gas

The majority of literature on growing algae using flue gas is directed at determining the algae's ability to grow under high concentrations of CO_2 and whether SO_x and/or NO_x inhibit algal growth. Little research has been completed specifically on growing algae using NO_x as a nitrogen source. However, the pioneers of growing algae in simulated flue gas did determine that it would be possible to achieve such a feat. Their research focused mainly on testing with NO rather than NO_2. The details of their research are described below.

To first determine whether it would even be possible to grow algae under the high CO_2 concentrations in a flue gas, Negoro et al. (1991) tested ten strains of algae to determine which strains were able to survive with CO_2 concentrations of up to 15% by volume. It was found that half of the strains exhibited very poor growth while the other half grew after extended lag phases and with decreased growth rates. This was attributed to the drop in pH caused by the high CO_2 concentrations. The three species that grew the best were further tested under various pH conditions. It was found that certain species of algae grew just as well under slightly acidic conditions (pH of 6.1) as they did in a neutral pH medium. Taking this to the next step, the three strains were further tested to determine the effects of NO_x and SO_x at up to 300 ppm and 400 ppm, respectively. The drastic drop in pH with 400 ppm SO_x, as well as the accumulation of sulfate, completely inhibited the growth of algae after 20 hours. With 300 ppm NO feed, one strain was completely inhibited, despite little change in pH, while the other grew after a prolonged lag phase. However, nitrite was detected in both culture broth and cell-free medium, suggesting that NO can be converted to nitrite abiotically. This information, coupled with the fact that an increase in nitrite concentration slowed after the start of growth, suggests that some nitrogen oxides were assimilated by the cells. While effluent NO_x concentration was not measured to determine removal, because both aqueous nitrite was detected and algal growth occurred, the overall conclusion was that nitrogen oxides from flue gas are a feasible nitrogen source for certain algal strains (Negoro et al. 1991).

As a continuation of the above study, the ability of algae to eliminate both CO_2 and NO from flue gas was investigated. A model flue gas of 15% CO_2 and 100-300 ppm NO was fed to a culture of marine microalga NOA-113. After the initial pH drop from

the 15% CO_2, no further drop in pH was observed from the addition of NO. However, the addition of NO completely suppressed growth when initial cell concentrations were low (1.0 g ash-free cell dry weight/L). The cells were then cultivated without NO until halfway through their linear growth phase (approximately 4 days), and no inhibition was found. This resulted in almost 50% elimination of NO gas, proving the ability of algae to assimilate nitrogen oxides. The study also showed that the exposure of NO to the cells in the dark inhibited growth due to the lack of photosynthetic oxygen, which was found to play an important role in NO elimination (Yoshihara et al. 1996).

Further studies by Maeda et al. (1995) were conducted to determine the effects of NO_x on growth rate and the plausibility of growing algae on flue gas. NO fed at concentrations of up to 60 ppm with 15% CO_2 had no effect on pH, and no effect on algal growth. However, at NO concentrations above 150 ppm, pH dropped below 3 and growth was inhibited. When a buffer was added to control pH, no inhibition was detected. This held true for both simulated and actual (13% CO_2, 10 ppm SO_x, 150 ppm NO_x) flue gas (Maeda et al. 1995).

Another study, focusing on CO_2 uptake, determined the algal strain *Monoraphidium minutum* was able to tolerate 200 ppm SO_x and 150 ppm NO_x (Brown, 1996). In this study, algae was grown in a medium that contained excess $NaNO_3$. It was determined that the small amount of nitrogen provided by the flue gas, along with the poor solubility of NO, provided no measurable stimulation of growth as an added nitrogen source. However, the concentration of NO_2^- in flue gas treated cultures were higher than control cultures, showing that some NO may be dissolving to form nitrite, and in turn is available as a nitrogen source for the algae. The NO_3^-

concentrations over time were quite similar in both the flue gas treated cultures and the control, although slightly higher in flue gas treated cultures (Brown 1996). While the authors attributed this to the flue gas retarding NO_3^- utilization, it is possible that NO and/or NO_2^- chemically reacted to form an accumulation of NO_3^-. This is supported with the observation that there was no measurable difference in growth rate between the two cultures.

Hauck et al. (1996) determined that the inhibitory effects of SO_x went beyond simply lowering the pH of the medium. SO_2 at 200 ppm was added to two strains of algae: *Chlorella vulgaris* because of its use in previous research, and *Cyanidium caldarium* because of its alleged ability to thrive in acidic conditions and slightly elevated temperatures. Both strains experienced growth inhibition by dissolved SO_2 or an aqueous oxidation product of SO_2. *C. vulgaris* was completely unable to grow, while *C. caldarium* showed some growth at first but then crashed shortly thereafter. *C. vulgaris* crashed even in a buffered medium. This shows that the inhibition due to SO_x may be due to an accumulation of dissolved SO_2 or an aqueous oxidation product of the gas (Hauck et al., 1996). Therefore, in support of the conclusions made by Negoro et al. (1991), to grow algae using NO_x, the flue gas must be conditioned to exclude high concentrations of SO_x.

Matsumoto et al. (1997) later studied the effects of high concentrations of CO_2 as well as 400 ppm SO_x and 70 ppm NO_x on *Nannochloropsis salina* and *Phaeodactylum tricornutum*. As with previous research, it was found that algae could indeed grow with CO_2 concentrations of up to 20% by volume. After adding 400 ppm SO_2, a drop in pH was also observed. In contrast to earlier studies, no inhibition of growth was observed

when the medium was buffered with NaOH and the pH was increased to 8. Therefore, Matsumoto et al. were able to conclude that the effect of SO_x on the inhibition of algal growth was indeed due solely to the resulting acidic conditions. NO was also observed to dissolve and oxidize to NO_2^- under high concentrations of O_2. The accumulation of NO_2^- was found to slightly decrease algae production initially, but significantly increased growth rates later on. To substantiate this result, $NaNO_3$ was replaced with $NaNO_2$ in the culture medium, and the same result was observed, further verifying previous conclusions that NO_2^- can in fact be used as a nitrogen source for algal growth (Matsumoto et al. 1997).

Perhaps the first to try to use algae exclusively as a means of NO_x removal from flue gas were Nagase et al. in 1997. The primary experiment involved feeding a *Dunaliella tertiolecta* culture with a simulated flue gas containing 100 ppm NO and 15% CO_2 in N_2 at 25°C with white fluorescent lamp illumination in a long tubular photobioreactor. The reactor was cycled through alternating light and dark phases. It was found that the removal rate of NO increased sharply during the light phase, but leveled off around 60% removal. Removal during the dark phase decreased by about 20% when 2% O_2 was added to the influent gas. When no O_2 was added in the model flue gas, removal during the dark phase essentially stopped. Therefore, to obtain continued NO removal in light and dark phases, O_2 needs to be added during the dark phase to supplement the lack of O_2 produced photosynthetically. This further substantiates previous research that shows "NO removal...occurs by means of some mechanism related to light and O_2 dissolved in water." Because the NO removal rate leveled off between 60% and 65% in media with and without cells and in both light and

dark phases, a rate-limiting step independent of cell concentration and light was involved. By varying the column height, and therefore gas-liquid contact time, it was found that "the dissolution of NO in water obeys first-order kinetics with respect to the inlet NO concentration." This was suggested to be the rate-limiting step in the removal of NO. The authors substantiate this suggestion with the lack of NO removal in the absence of O_2 and the subsequent suppression of cell growth. It was concluded that the removal of NO can be explained in three steps. First, the NO is dissolved in the aqueous phase. Then, the NO is oxidized by algal cells via reaction with O_2. Finally, oxidized NO is assimilated by the algae as a nitrogen source. It was suggested that the most effective way to enhance NO removal would be to increase the gas-liquid contact area by reducing the bubble size (Nagase et al. 1997)

The effect of reducing the bubble size was later researched by the same team that accomplished the above study. A decrease in bubble size resulted in a higher volumetric mass-transfer coefficient and consequently higher removal rates. Unfortunately, when the bubbles were too small, the algal cells became concentrated at the top of the reactor due to froth floatation (dissolved air floatation) and sustained NO removal was not achieved. After also trying multiple reactor types, it was found that a counter-flow airlift reactor achieved the best NO removal. This type of reactor allowed for proper suspension of the cells and decreased the rising rate of the bubbles, allowing an 83% removal rate. To further enhance removal, air was used instead of nitrogen as the make-up gas to provide O_2 to the system. Greater than 90% removal efficiency was achieved for this model flue gas with 15% CO_2, 85% air, and 100 ppm NO in a counter-flow airlift reactor with a mean bubble diameter of 0.26-mm (Nagase et al. 1998). This result

validates previous conclusions about the necessity of O_2 and the importance of optimizing mass transfer rates of NO_x gas into the aqueous phase.

3. METHODS

The following experiments tested the ability of *C. vulgaris* to grow in a 20-L photobioreactor utilizing dissolved nitrogen from a simulated flue gas inlet stream containing between 30 ppm and 450 ppm gaseous nitrogen oxides at 3.0 – 3.6 L/min. The efficiency of NO_x removal in the gaseous stream was measured to determine whether the use of photobioreactors to treat NO_x is a viable and sustainable treatment method.

3.1 PBR Design

All experiments were run in photobioreactors set up using three, 20-L Nalgene® Clearboy® round polycarbonate carboys operated under a fume hood. Each carboy was sealed with a threaded cap with three ports. One port was used to connect influent gas tubing, another for effluent gas tubing, and the third was used for liquid sampling. Three holes were drilled into the top of each carboy to allow ports for chemical addition, a pH probe, and an aquarium heater. Figure 1 shows the design of the typical PBR used for all experiments. PBR system design and operation varied for each of the three experiments. The details of each experiment are described in the sections below.

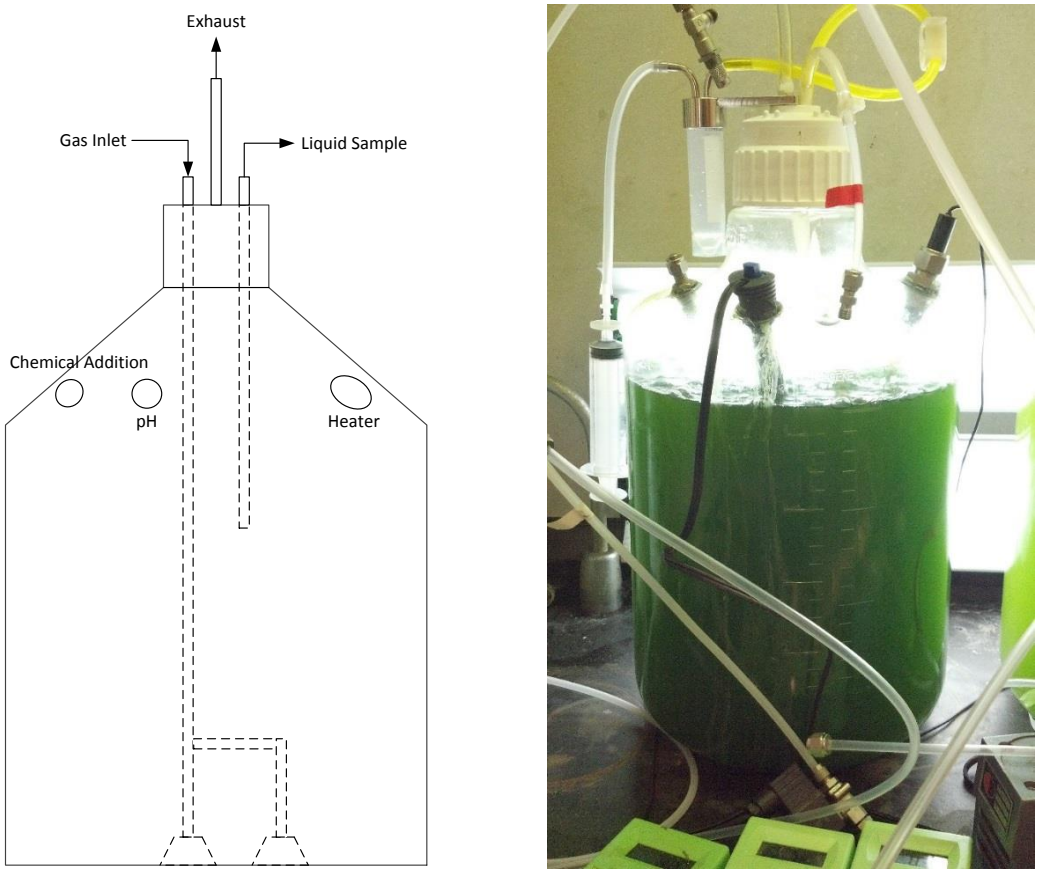


Figure 1. PBR system for lab experiments

3.2 Growth Medium

Modified Bristol growth media were used for inoculum and algae-growth experimentation. To ensure that all nitrogen assimilated by the algal cells was provided through influent gaseous NO_x, components with nitrate were not included in the modified form of Bristol used in the PBRs. Table 1 shows the recipe for the modified Bristol medium used in all the PBRs for experiments. The original Bristol medium used to grow the inoculum included 2.94 mM $NaNO_3$

Table 1. Modified Bristol growth medium (UTEX)

Component	Concentration (mM)
$CaCl_2 \cdot 2H_2O$	0.17
$MgSO_4 \cdot 7H_2O$	0.3
K_2HPO_4	0.43
KH_2PO_4	1.29
NaCl	0.43

3.3 Chlorella Culture

All three PBRs were inoculated with 660-mL of pure-culture *C. vulgaris* grown in Bristol medium (with $NaNO_3$) at 25°C and a pH of 7.0. The *C. vulgaris* strain was a "bacteria-free" culture slant on solid medium obtained from Carolina Biological Supply (catalog #15-2075). The inoculum was grown to a cell density of 1.03×10^7 cells/mL, resulting in an initial PBR cell density of 3.1×10^5 cells/mL. Any carryover of NO_3^- from the inoculum was carefully monitored as described below.

3.4 PBR Set-up and Operation

Gas feed systems and other design features were modified for each progressive experiment. Operating conditions for each of the three runs are described below.

3.4.1 Run 1 - Pure NO_2 Feed Source

The first run was set up using pure NO_2 gas (Praxair) diluted with ambient air as the simulated flue gas. The boiling point of pure NO_2 is approximately 20°C at atmospheric pressure; therefore the NO_2 was initially released as a liquid in the tubing when the gas cylinder was opened. To overcome this, an empty glass container was set up before pumping to the PBRs to allow the liquid NO_2 to vaporize before entering the

PBRs (Figure 1). NO was produced passively through natural chemical reaction before entering the PBR, and no additional NO was fed into the system. Therefore, no attempt was made to control the production or concentration of NO. The NO_2 was blended with 3.6 L/min of air through a series of peristaltic pumps in an attempt to achieve target NO_x influent concentrations of 150 ppm, 300 ppm, and 450 ppm in PBRs 1A, 1B, and 1C respectively.

The blended gas entered each PBR through a single 15-cm aquarium air stone placed flush with the bottom of each reactor. Carbon dioxide feed was regulated by separate solenoid valves to each PBR controlled based on the pH inside the reactor. The solenoid valves would open to allow CO_2 to flow if the pH raised above 8.0. A process flow diagram of the PBR configuration in Run 1 is shown in Figure 3. All connections were made with 6.35-mm Teflon tubing with stainless steel or brass connectors and fittings. The system was run for four days to assess NO_x removal efficiencies.

No attempts were made to optimize algal growth conditions in Run 1, with the exception of pH. The PBRs were illuminated with four 1.2-m, 3000-K fluorescent light bulbs placed behind the reactors, and two fluorescent light bulbs placed above them (Figure 2). No temperature controls were installed for this first experiment. Table 2 summarizes the experimental conditions of Run 1.

Table 2. Run 1 experimental set points

Setting	PBR 1A	PBR 1B	PBR 1C
Influent NO_x (g) Target	150 ppm	300 ppm	450 ppm
Influent Gas Flow Rate	3.6 L/min	3.6 L/min	3.6 L/min
Influent CO_2	Bled non-continuously to adjust pH		
pH	7.5-8.0	7.5-8.0	7.5-8.0
Temperature	20°C	20°C	20°C

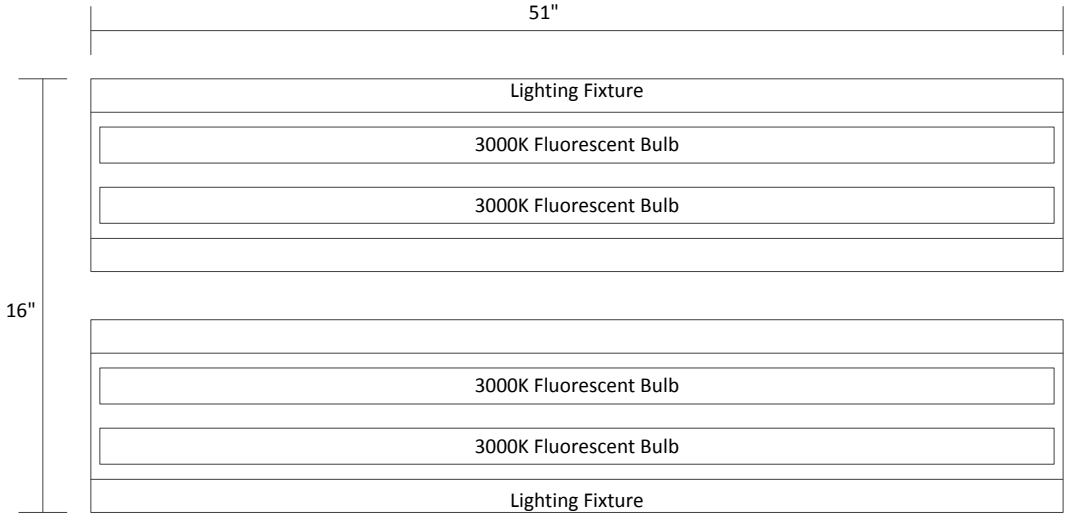


Figure 2. Diagram of lighting behind PBRs

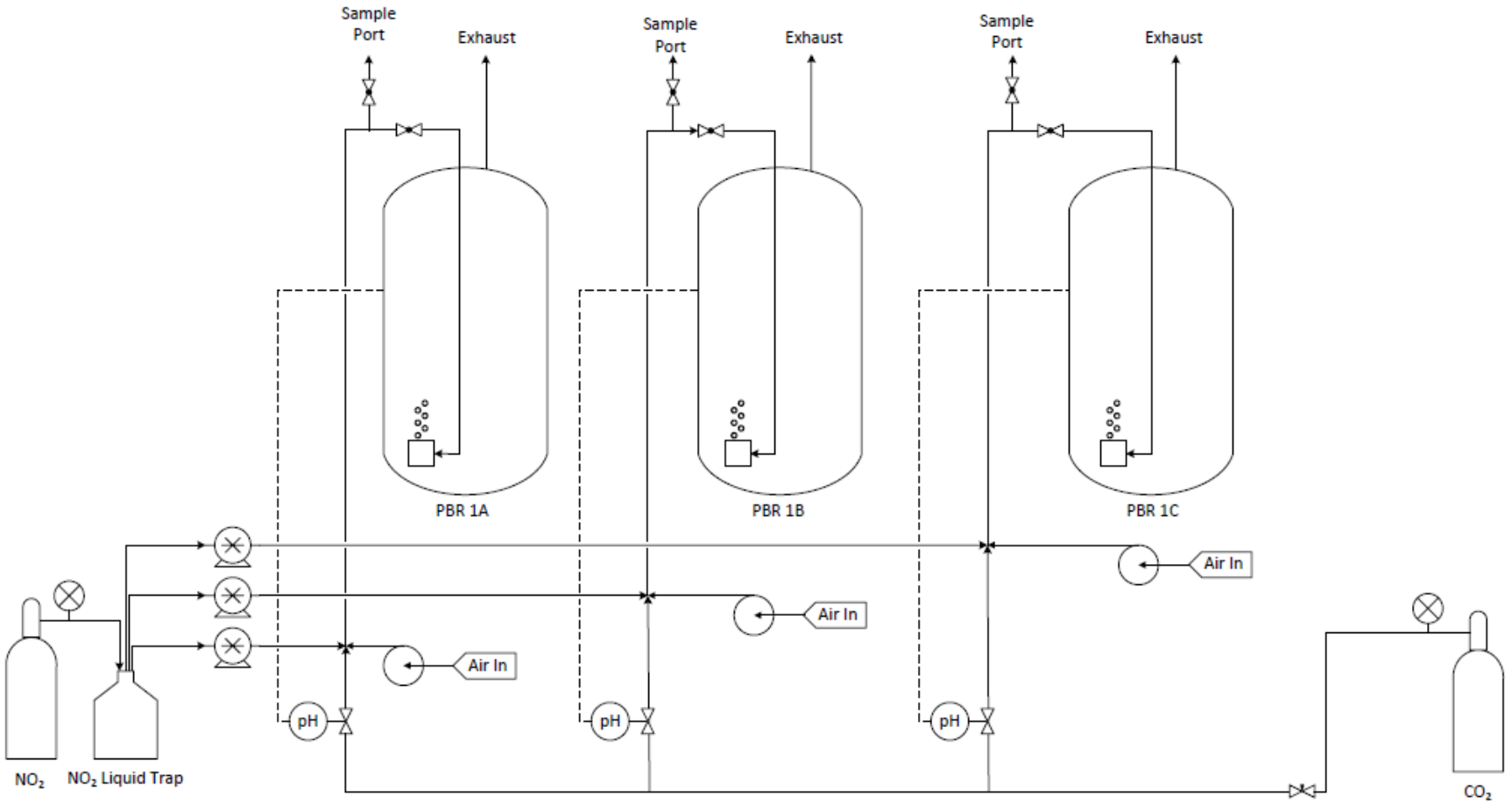


Figure 3. Process flow diagram of Run 1

3.4.2 Run 2 - Pure NO2 Feed Source with Gas Recycle

The second run had a set-up similar to the first, but included the addition of a second 15-cm aquarium air stone in each PBR to increase mass transfer. A recycle stream was also added to increase gas residence time. Additionally, CO_2 was bled in through a single peristaltic pump at a constant concentration of 400 ppm and was only shut off if the pH dropped below 7.0. If the pH dropped below 7.0, a 1.0 g/L solution of potassium bicarbonate ($KHCO_3$) was added slowly through the chemical addition port to increase the pH, and the CO_2 was allowed to flow again. Figure 4 shows the process flow schematic for this run. Again, all connections were made with 6.35-mm Teflon tubing with stainless steel and brass fittings.

Target NO_x loading concentrations for Run 2 were 60 ppm, 90 ppm, and 180 ppm for PBRs 2A, 2B, and 2C respectively. The system was again run for four days. The target pH was 7.5-8.0, and no temperature controls were implemented. The same six standard 1.2-m, 3000-K fluorescent light bulbs used for illumination in Run 1 were also used for Run 2. Table 3 summarizes the experimental conditions of Run 2.

Table 3. Run 2 experimental set points

Setting	PBR 2A	PBR 2B	PBR 2C
Influent NO_x Target	60 ppm	90 ppm	180 ppm
Influent Gas Flow Rate	3.6 L/min	3.6 L/min	3.6 L/min
Influent CO_2	400 ppm	400 ppm	400 ppm
pH	7.5-8.0	7.5-8.0	7.5-8.0
Temperature	20°C	20°C	20°C

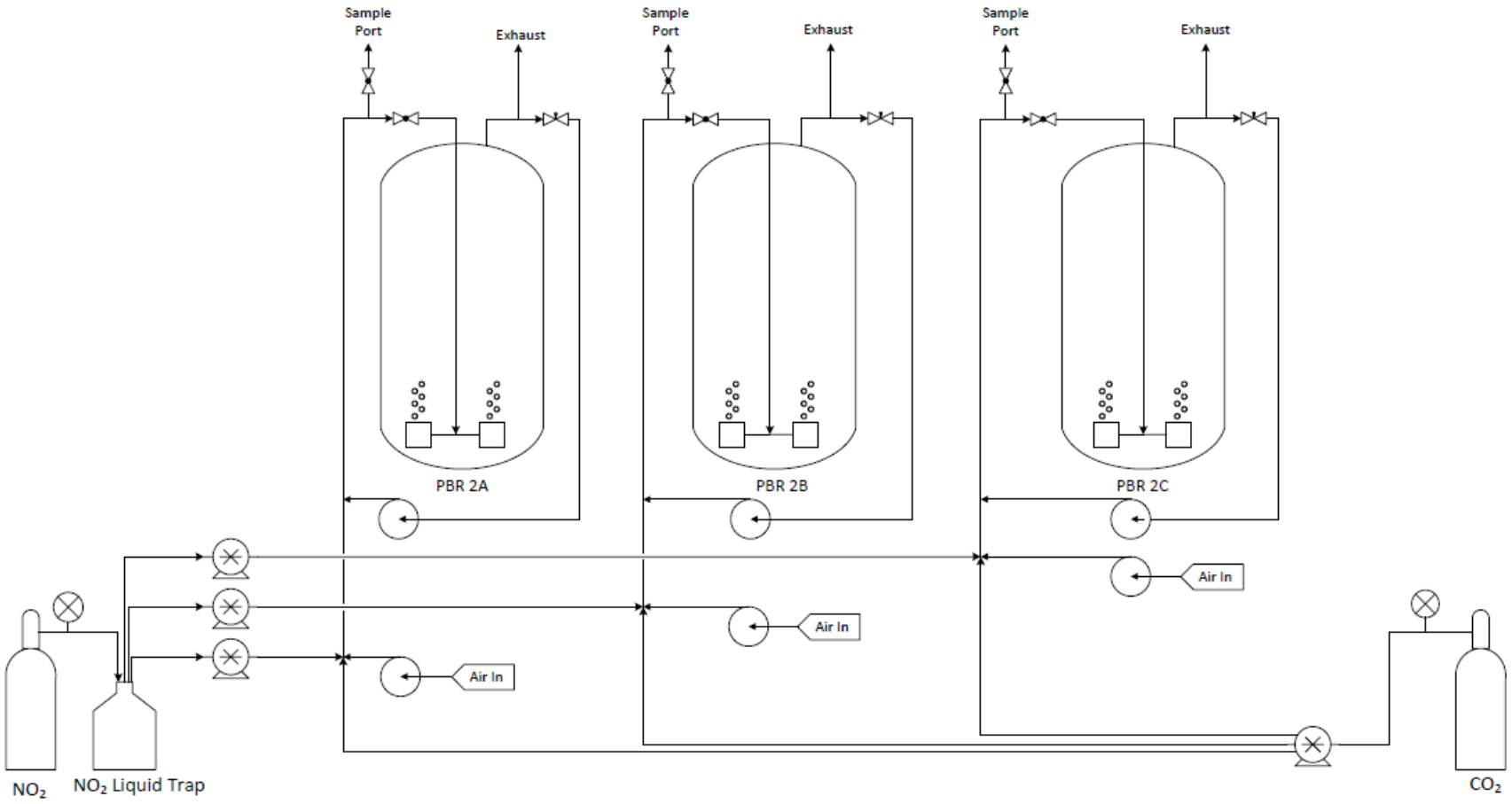


Figure 4. Process flow diagram of Run 2

3.4.3 Run 3 - Calibration Gas Feed Source

For the third run, NO_x was supplied from NO_2 calibration gas cylinders of 5,800 ppm and 10,000 ppm concentrations (Praxair), eliminating the need for the liquid NO_2 trap and peristaltic pumps and, allowing for more consistent and controllable inlet concentrations. The gas cylinders were connected to the system through three separate capillary tubes with varying diameters depending on the desired flow rate to each PBR, and controlled with needle valves. The NO_2 was blended with air at 3.0 L/min to achieve target influent concentrations of 30 ppm, 60 ppm, and 150 ppm respectively. These concentrations were chosen to simulate realistic ranges produced through power plants. The recycle stream was eliminated for this final run to keep the gas/liquid concentration gradient as high as possible to maximize mass transfer rates. Two 15-cm aquarium stones were used to diffuse gas into the PBRs. Figure 5 provides a diagram of the PBR configuration for the third run. Once again, all connections were made with 6.35-mm Teflon tubing. For the third run, all brass connections and fittings were replaced with stainless steel to prevent any reactions between the constituents and equipment.

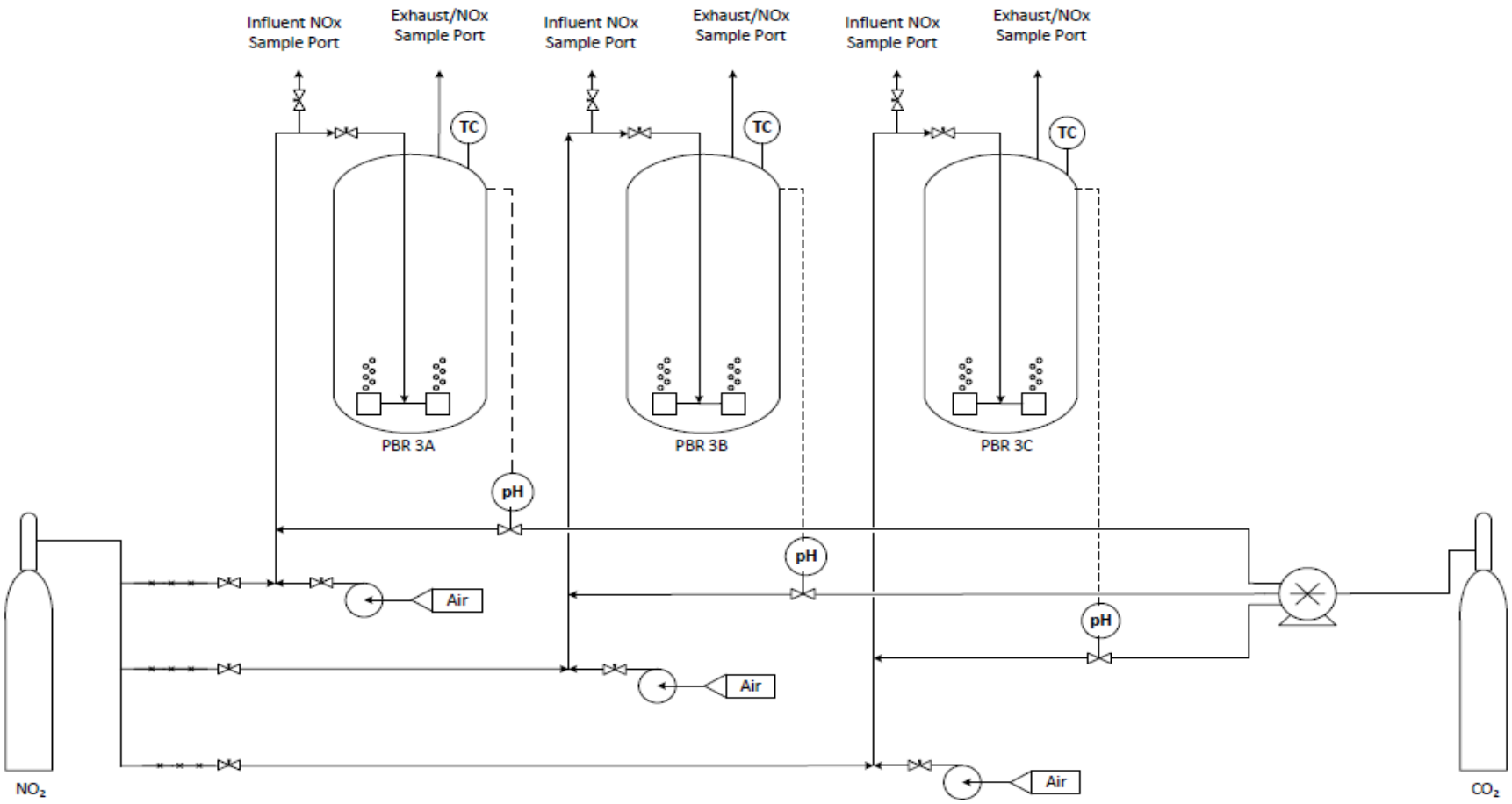


Figure 5. Process flow diagram of Run 3

Temperature control was added during the third run to provide a more optimal environment for the algae. A 50-W aquarium heater was added to each PBR and set to 25ºC. The four standard fluorescent bulbs behind the PBRs were replaced with four 1.2-m, 5600-K "Natural Light" fluorescent bulbs to provide better quality light to the PBRs. The two fluorescent bulbs above the PBRs were unchanged from the previous runs. CO_2 was bled into the system, as with Run 2, at a constant 400 ppm. The pH was adjusted manually with either a basic solution of 1.0 g/L $KHCO_3$, or an acidic solution of 0.5 M HCl to maintain the target pH of 8.0 to 8.5. Table 4 provides a summary of the target experimental conditions for Run 3.

Table 4. Run 3 experimental set points

Setting	PBR 3A	PBR 3B	PBR 3C
Influent NO_x Target	30 ppm	60 ppm	150 ppm
Influent Gas Flow Rate	3.0 L/min	3.0 L/min	3.0 L/min
Influent CO_2	400 ppm	400 ppm	400 ppm
pH	8.0-8.5	8.0-8.5	8.0-8.5
Temperature	25°C	25°C	25°C

To prevent culture crashes and allow the algae to acclimate to PBR conditions, the PBRs were inoculated 48 hours prior to the start of NO_x loading. During this 48-hour period, only ambient air was run through the system, and the algae grew on residual nitrate from the inoculation solution. After the 48-hour acclimation period, the algae had begun their exponential growth phase, and NO_x loading was started. The system was run for an additional 144 hours to ensure the algae were able to achieve peak growth.

3.5 Sampling and Testing Procedures

Sampling and testing procedures are summarized in Table 5 and described in the subsequent sections.

Table 5. Sample and analysis procedure summary

Metric	Sampling/Analysis Method	Frequency	Analytical Method
Temperature	Infrared Thermometer	Several times/day	Infrared Thermometer
Influent/Effluent Flow Rates	Calibrated Flow Meter	Several times/day	Calibrated Flow Meter
pH	pH Probe	Several times/day	pH Meter
Influent/Effluent NO_x	Tedlar Bags	4x/day	Chemiluminescence
Aqueous Nitrate/Nitrite	25-mL Filtered	2x/day	Spectrophotometer
Organic Nitrogen	50-mL	Initial and Final	Total Kjeldahl Method
Cell Count	Aqueous Sampler	2x/day	Hemocytometer
Algal Weight	100-mL Filtered	Initial and Final	Total Suspended Solids Method

3.5.1 Gas Sampling

Influent and effluent gas samples were taken four times daily, approximately three hours apart, between the hours of 7am and 7pm. NO and NO_2 concentrations were measured using an Advanced Pollution Instrumentation Model 200AH chemiluminescence analyzer. The influent sampling port consisted of two valves positioned immediately prior to the inlet port to each PBR, as depicted in Figures 3-5.

During sampling, the valve to the PBR inlet was closed and the valve to the sample port was opened. In this way the NO_x stream was directed to either a Tedlar bag or directly to the analyzer. When a Tedlar bag was used to collect the sample, NO_x concentration measurements were measured discretely. Effluent samples were collected similarly, although no valves were used to direct flow. Effluent samples were taken directly off the exhaust stream of the PBR, either through collection into Tedlar bags or diverted directly into the analyzer. The process flow diagrams in Figures 3-5 show a visual representation of the sampling streams.

The NO_x analyzer was calibrated every morning prior to collecting samples using EPA Protocol 1 NO gas concentrations of 40.3 ppm, 198 ppm, and 441 ppm. The analyzer was also checked periodically throughout the day using the calibration gases to ensure the accuracy of the data collected. The analyzer was recalibrated if measured concentrations deviated from the EPA Protocol 1 calibration gas concentrations by greater than 3%.

3.5.2 Algae Cell Counts

Cell counts were measured twice daily using a hemocytometer. A liquid sample was taken from each PBR twice daily, and approximately 7µL of each sample was pipetted into a counting chamber. The central 1-mm square of each chamber was viewed at 200x magnification using bright field microscopy. The number of suspended cells were counted from each of the four corners and the middle 0.25 mm square. The final cell count in each chamber was then determined by multiplying the total number of

suspended cells counted by 250,000. Four counting chambers per sample were analyzed and averaged to obtain the cell density of each PBR in cells/mL.

3.5.3 Dry Weight

Dry weight samples were taken at the beginning and end of each run and analyzed for the total suspended solids (TSS) concentration of each sample. A gravimetric analysis was used following the American Public Health Association (APHA) Standard Methods for the Examination of Water and Wastewater Method 2540 (Rice & Bridgewater, 2012). A 100-mL liquid sample from each PBR was filtered using a standard Fisherbrand grade G4, glass-fiber filter. The filters were conditioned prior to TSS testing by washing with 50-mL of filtered, deionized water while applying a vacuum. Conditioned filters were placed in aluminum weighing dishes, dried for one hour at 105^{o}C, and then placed in a desiccator. At the time of sampling, the conditioned filters were removed from the desiccator and weighed on aluminum weighing dishes to obtain the initial weight. The 100-mL liquid samples from each PBR were filtered through the conditioned filters and then dried at 105^{o}C. Three samples were filtered for each PBR. The final weights of each filter were recorded and an average TSS was calculated for each PBR per Equation 18.

$$TSS = \frac{(weight\ after\ drying - weight\ of\ conditioned\ filter)}{sample\ volume} \quad (18)$$

3.5.4 Nitrate/Nitrite

Liquid samples were analyzed for nitrate and nitrite concentrations for Run 3 only. Approximately 25-mL samples were taken from each PBR twice daily, and filtered

using the Fisherbrand grade G4, glass-fiber filters. The filtrates were analyzed using a Hach® DR 3800™ UV-Vis Spectrophotometer and Nitrate and Nitrate TNTplus, using LR pre-coded vials. Methods TNT 835 and TNT 839 were followed to determine nitrate and nitrite concentrations, respectively. The Hach® chemicals were added to the vials, mixed and allowed to react per each method, and then placed in the spectrophotometer. The spectrophotometer automatically scanned the barcode of each vial and ran the correct program, analyzing each sample instantaneously.

3.5.5 Organic Nitrogen

Also exclusive to Run 3 was the determination of the total organic nitrogen content of the algal cells at the beginning and end of the run. This was done using the Total Kjeldahl Nitrogen (TKN) method adapted from the APHA Standard Methods for the Examination of Water and Wastewater 4500-NorgB and 4500-NH3C (Rice & Bridgewater, 2012). Duplicate initial and final samples for each PBR were collected and digested with strong sulfuric acid at 189^{o}C, oxidizing the organic material and liberating the organic nitrogen as ammonium. The solution was then cooled and neutralized with sodium hydroxide to convert ammonium to ammonia gas. The ammonia gas was then trapped in a solution of boric acid to neutralize the ammonia, and then titrated with 0.02 N sulfuric acid using a mixed pH indicator that changes from purple to green under basic conditions.

3.6 Laboratory Safety

As previously discussed, nitrogen oxides are highly toxic and harmful to both living beings and the environment. Great care and consideration must be taken with regard to laboratory safety and proper handling of the nitrogen oxides gases. All experiments were conducted in a fume hood to ensure proper ventilation and to limit contamination of ambient laboratory air with nitrogen oxides. Passive continuous monitoring of ambient nitrogen oxide concentrations was conducted using the chemiluminescence analyzer. All experimentation was conducted in a laboratory with an emergency shower and eyewash station, particularly in case of contact with the highly corrosive liquid NO_2. Nitrile gloves were worn when handling the PBRs and associated equipment, and during all sampling and testing. All gas cylinders were properly secured and chained to limit movement during a potential seismic event. Any and all adverse effects from potential exposure to NO_x were treated with utmost seriousness and consultation with a medical professional.

4. RESULTS

4.1 NO_x Removal Efficiency and Cell Growth

Table 6 summarizes the removal efficiencies of the PBRs in all three runs and the varying loading ratios (NO_2:NO). More detailed results are presented in the subsequent sections.

Table 6. Influent and effluent NO_x concentrations

PBR	Loading Ratio (NO2:NO)	Influent NOx (g) Concentration (ppm)*	Effluent NOx (g) Concentration (ppm)*	Average Percent Removal (Concentration)
1A	2.1	181 ± 89	95 ± 33	48%
1B	6.5	30 ± 26	15 ± 12	50%
1C	2.8	62 ± 48	9 ± 7	86%
2A	0.2	783 ± 758	61 ± 54	92%
2B	0.1	521 ± 672	31 ± 25	94%
2C	3.2	41 ± 34	25 ± 19	39%
3A	26	33 ± 6	17 ± 4	49%
3B	28	72 ± 19	2 ± 9	97%
3C	23	149 ± 29	7 ± 13	96%

*± indicates standard deviation

4.1.1 Run 1 - Pure NO_2 Feed Source

The PBRs in Run 1 were fed with pure NO_2 diluted with air through a series of peristaltic pumps and a liquid NO_2 trap. Target loading rates for Run 1 were 150 ppm, 300 ppm, and 450 ppm for PBRs 1A, 1B, and 1C respectively. NO_2 flow rates to achieve such low concentrations using pure NO_2 gas were extraordinarily low. Due to difficulties with controlling the peristaltic pumps at such low flow rates, actual average influent

concentrations for PBRs 1A, 1B, and 1C were 181 ppm, 30 ppm, and 62 ppm respectively (Table 6).

Table 7 presents the NO_x removal data for the PBRs of Run 1. Influent and effluent NO_x in Table 7 is the sum of measured NO and NO_2 concentrations in the influent and effluent streams. The research focuses on the effectiveness of overall NO_x removal and the fate of total nitrogen through the system, not any particular component of NO_x. Therefore, only percent removal of total NO_x is considered in the analysis. PBR 1A achieved an average NO_x removal of 48%, PBR 1B achieved an average 50% NO_x removal, and PBR 1C was able to remove an average of 86% of influent NO_x

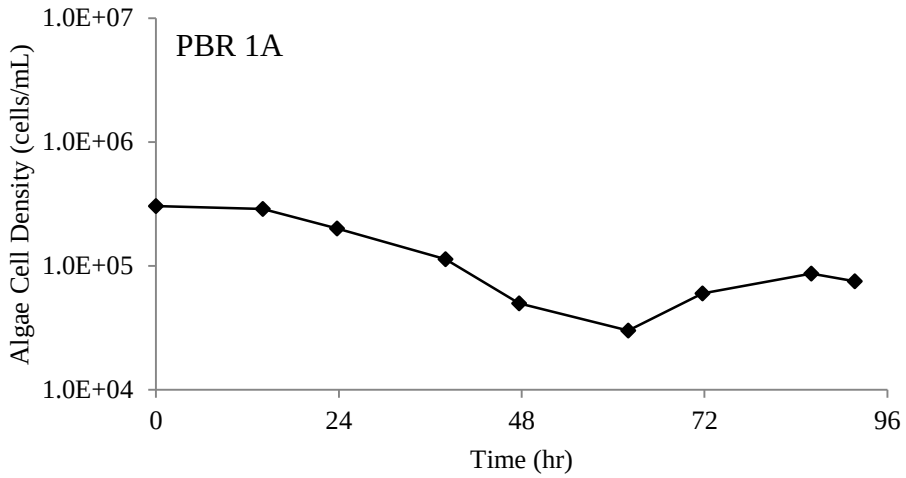
Table 7. NO_x removal data for PBRs 1A, 1B, and 1C for Run 1

	PBR 1A			PBR 1B			PBR 1C		
Time Elapsed (hrs)	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal
0.0	53	40	25%	27	13	52%	107	20	81%
14.0	133	74	44%	40	31	23%	128	16	88%
16.0	98	80	18%	42	26	38%	167	24	86%
19.5	128	81	37%	40	21	48%	89	10	89%
24.0	160	94	41%	37	20	46%	44	5	89%
37.5	106	79	25%	20	12	40%	15	3	80%
40.0	128	87	32%	27	13	52%	13	3	77%
44.0	397	145	63%	12	5	58%	13	3	77%
46.5	320	153	52%	7	4	43%	10	2	80%
61.5	217	145	33%	20	8	60%	21	-	-
63.5	270	142	47%	27	7	74%	35	9	74%
66.5	176	65	63%	116	49	58%	127	10	92%
69.5	245	90	63%	11	6	45%	74	12	84%
85.5	141	77	45%	3	1	67%	22	2	91%
88.0	139	69	50%	45	22	51%	74	6	92%
92.0	-	-	-	11	5	55%	50	3	92%
Average	181	95	48%	30	15	50%	62	9	86%

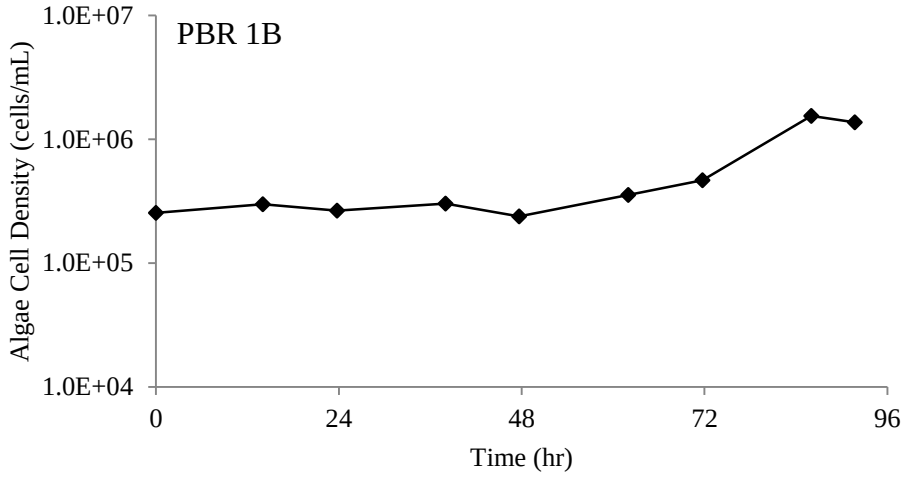
Figure 4 shows the growth curves of algae in PBRs 1A, 1B, and 1C during the 96-hour run. All three PBRs began with 3×10^5 cells/mL. The maximum cell densities for PBRs 1B and 1C were 1.54×10^6 cells/mL and 1.58×10^6 cells/mL respectively. As shown in Figure 4a, the culture in PBR 1A began crashing sometime during the first 24 hours, so the algal cells never reached a density greater than the initial 3×10^5 cells/mL. A slight revival in the last 30 hours of Run 1 in PBR 1A may have contributed to enhanced removal rates for the same period of time. As determined through Table 6, the average NO_x removal efficiency for the first 65 hours was 38%, while the average removal for the last 25 hours was 53%. As the algal culture was crashing, all NO_x removal could be attributed to the dissolution of NO_2 into the medium. PBRs 1B and 1C exhibited modest growth following an extended lag phase, as shown in the growth curves in Figure 6. The run was ended after 96 hours, and the algae in PBRs 1B and 1C had not yet truly experienced exponential growth.

The culture in Run 1 began with a faint tint of green from the addition of the algal inoculum culture. At the end of the run, PBR 1A was colorless due to the culture crash, while PBRs 1B and 1C exhibited a light yellow-green color with some murkiness.

a)



b)



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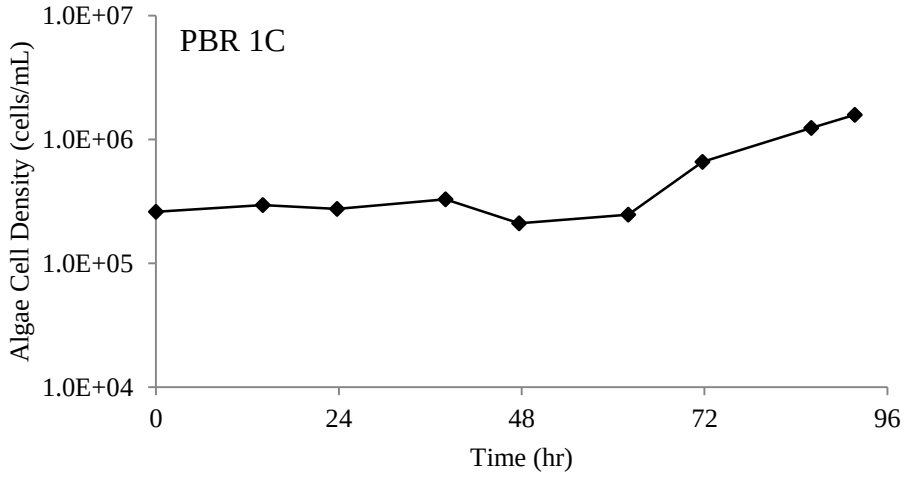


Figure 6. Run 1 growth curves; a) PBR 1A. b) PBR 1B. c) PBR 1C.

4.1.2 Run 2 - Pure NO_2 Feed Source with Gas Recycle

The PBRs in Run 2 were also fed with pure NO_2 diluted with air through peristaltic pumps and a liquid NO_2 trap. Target influent NO_x concentrations were 60 ppm, 90 ppm, and 180 ppm for PBRs 2A, 2B, and 2C respectively. NO_2 flow rates to achieve these concentrations using pure NO_2 gas were still too difficult to accurately control. Again, because of flow rate control issues with the peristaltic pumps, actual average influent concentrations for PBRs 2A, 2B, and 2C were 783 ppm, 521 ppm, and 41 ppm respectively (Table 8).

Table 8 presents the NO_x removal data for the PBRs of Run 2. As with Run 1, influent and effluent NO_x in Table 8 is the sum of measured NO and NO_2 concentrations in the influent and effluent streams. Only percent removal of total NO_x is considered in the analysis. PBR 2A achieved an average NO_x removal of 92%, PBR 2B achieved an average 94% NO_x removal, and PBR 2C was able to remove an average of 39% of influent NO_x. Due to the high variability in loading, Run 2 data was not analyzed, and is presented for continuity purposes only.

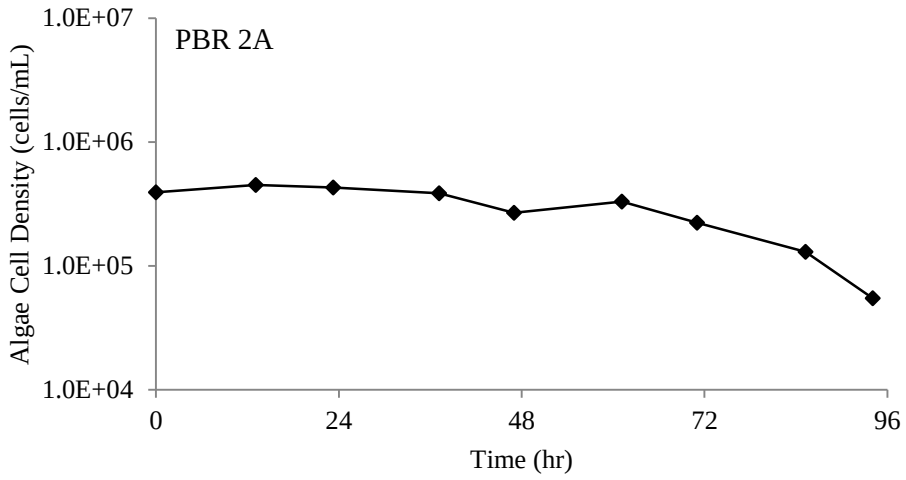
Table 8. NO_x removal data for PBRS 2A, 2B, and 2C for Run 2

	PBR 2A			PBR 2B			PBR 2C		
Time elapsed (hrs)	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal
0.0	85	51	40%	157	63	60%	123	61	50%
13.5	78	43	45%	64	38	41%	45	1.6	96%
16.0	24	31	-29%	124	44	65%	1.5	1.5	0%
18.5	170	87	49%	153	62.5	59%	2.9	1.5	48%
19.0	164	85	48%	136	57.9	57%	3.4	1.8	47%
20.5	205	88	57%	123	55	55%	94	51	46%
37.5	1712	29	98%	1.1	0.8	27%	34	24	29%
39.0	1374	17	99%	-	-	-	31	26	16%
43.0	208	170	18%	99	36.1	64%	58	39.2	32%
45.5	360	172	52%	64	37.9	41%	41	26.7	35%
61.5	1473	2.3	100%	1375	3.9	100%	29.8	24.4	18%
64.0	1822	9.1	100%	1821	2.9	100%	37.6	34.7	8%
67.0	1833	34.5	98%	1548	1.7	100%	44.3	33.5	24%
70.0	1448	29	98%	1110	4.5	100%	24.7	22.6	9%
Average	783	61	92%	521	31	94%	41	25	39%

Figure 7 represents the growth curves of PBRs 2A, 2B, and 2C during the 70-hour run. All three PBRs began with 3×10^5 cells/mL. The maximum cell densities for PBRs 2A, 2B, and 2C were 4.50×10^5 cells/mL, 1.09×10^6 cells/mL, and 5.80×10^5 cells/mL respectively. Figure 7 shows that the algae did not crash immediately, but little growth was observed, and two of the PBRs went into early death phases. This may have been due to highly variable loading rates and high NO_x concentrations, which may have stressed the algae. Again, exponential growth was never observed. The growth medium in Run 2 began with a faint tint of green from the addition of the algal inoculum culture. At the end of the run, the culture in the PBRs had a brown tint, a possible result of very high NO_x concentrations.

After approximately 62 hours, the peristaltic pumps began to malfunction and influent NO_x to PBRs 2B and 2C far exceeded concentrations of 1000 ppm for the remainder of the run. While this caused a crash in PBR 2C, slight growth was observed in PBR 2B (Figure 7). Removal during the last 30 hours was measured to be essentially 100% for both reactors. Run 2 was terminated early because of the equipment malfunction. Due to the uncontrolled experimental conditions and inexplicable nature of the results, Run 2 data were used solely for experimental understanding, rather than analysis.

a)



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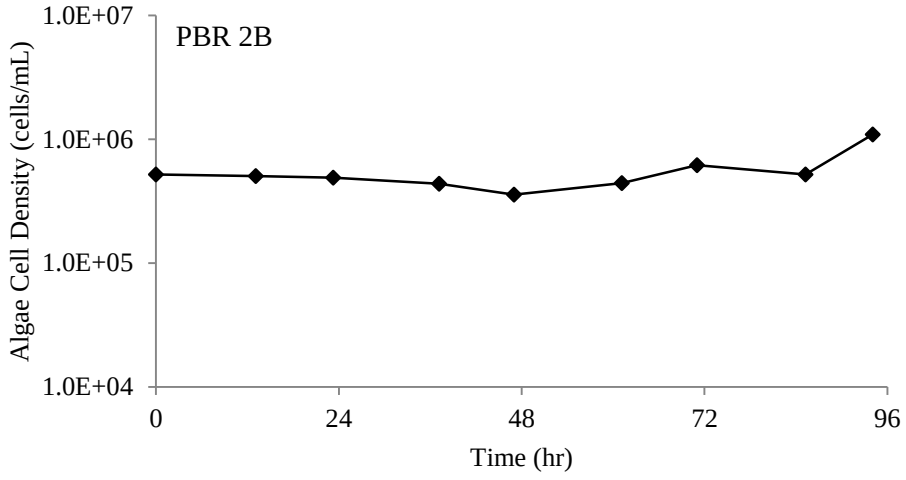

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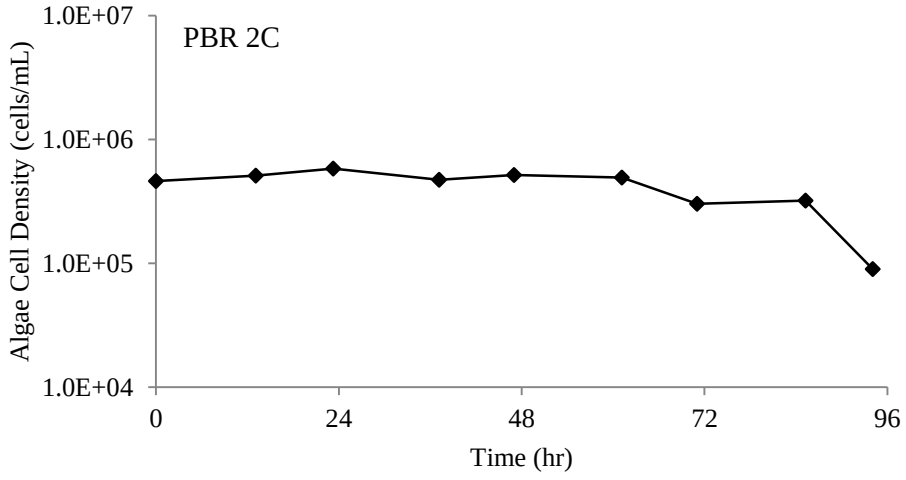

Figure 7. Run 2 growth curves; a) PBR 2A. b) PBR 2B. c) PBR 2C.

4.1.3 Run 3 - Calibration Gas Feed Source

To eliminate some of the problems that occurred from using pure NO_2 gas in Runs 1 and 2, NO_2 calibration gases of 5,800 ppm and 10,000 ppm NO_2 were used to supply NO_x for Run 3. This allowed for more accurate and precise NO_x loading concentrations. Also, to reduce any potential stress of NO_x on the algae, the culture was allowed to acclimate to PBR conditions and reach substantial growth rates before loading NO_x. Target loading rates for Run 3 were 30 ppm, 60 ppm, and 150 ppm for PBRs 3A, 3B, and 3C respectively. The calibration gases allowed for easier control, leading to actual average influent NO_x concentrations in PBRs 3A, 3B, and 3C of 33 ppm, 72 ppm, and 149 ppm respectively.

Table 9 presents the NO_x removal data for the PBRs of Run 3. Influent and effluent NO_x in Table 9 is the sum of measured NO and NO_2 concentrations in the influent and effluent streams. As with the previous runs, only percent removal of total NO_x is considered in the analysis. PBR 3A achieved an average NO_x removal of 49%, PBR 3B achieved an average 97% NO_x removal, and PBR 3C was able to remove an average of 96% of influent NO_x.

Table 9. NO_x removal data for PBRS 3A, 3B, and 3C for Run 3

	PBR 3A			PBR 3B			PBR 3C		
Time elapsed (hrs)	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal	Influent NO_x Concentration (ppm)	Effluent NO_x Concentration (ppm)	Percent Removal
0.0	30	13	57%	60	0	100%	146	0	100%
1.0	33	14	57%	74	0	100%	147	0	100%
5.0	44	19	57%	46	0	100%	159	34	78%
18.5	28	9	68%	66	43	35%	181	50	72%
24.0	32	13	58%	67	0	100%	145	0	100%
26.5	34	13	62%	64	0	100%	150	1	99%
41.0	45	26	43%	126	6	95%	235	0	100%
44.0	27	17	38%	69	0	100%	113	0	100%
47.5	24	17	30%	88	0	100%	132	0	100%
51.5	25	15	39%	95	1	99%	154	20	87%
63.5	25	13	47%	19	0	100%	163	21	87%
67.5	30	16	46%	89	0	100%	129	9	93%
71.5	31	17	45%	75	0	100%	122	7	95%
74.5	31	17	45%	75	0	100%	169	6	96%
87.5	36	19	48%	68	0	100%	159	3	98%
91.5	32	18	45%	80	0	100%	87	0	100%
95.5	38	20	47%	68	0	100%	109	0	100%
99.5	33	18	46%	67	0	100%	141	0	100%
103.5	32	18	43%	47	0	100%	146	0	100%
112.5	38	20	47%	81	0	100%	158	0	100%
116.0	41	23	45%	79	0	100%	162	0	100%
124.5	31	17	47%	80	0	100%	168	0	100%
136.5	46	23	51%	75	0	100%	144	0	100%
140.5	28	14	50%	66	0	100%	149	7	96%
Average	33	17	49%	72	2	97%	149	7	96%

Growth curves for PBRs 3A-3C are shown in Figure 8. All three PBRs began with 3 x 10^5 cells/mL. The maximum cell densities for PBRs 3A, 3B, and 3C were 1.26 x 10^7 cells/mL, 1.46 x 10^7 cells/mL, and 1.85 x 10^7 cells/mL respectively. With delayed NO_x loading, the lag phase was reduced to less than 24 hours, and exponential growth was observed between 24 and 48 hours. NO_x loading began at 49 hours, at which point all three PBRs showed continued growth, but a significant decline in growth rate (Figure 8). Each PBR seemed to experience a secondary lag phase for greater than 24 hours after NO_x was introduced to the system. This lag phase was more prominent with increasing influent NO_x concentrations (Figure 8). PBR 3C, which had the greatest NO_x loading concentration at 149 ppm, even underwent a brief decline in cell density before continuing growth.

Runs 1 and 3 both demonstrated a phenomenon in which higher NO_x loading concentrations resulted in higher percent removal efficiencies. At influent NO_x concentrations of approximately 30 ppm, only about 50% of the pollutant was taken up by the PBRs. In contrast, 85-97% removal was achieved for inlet NO_x concentrations above 64 ppm (Table 9).

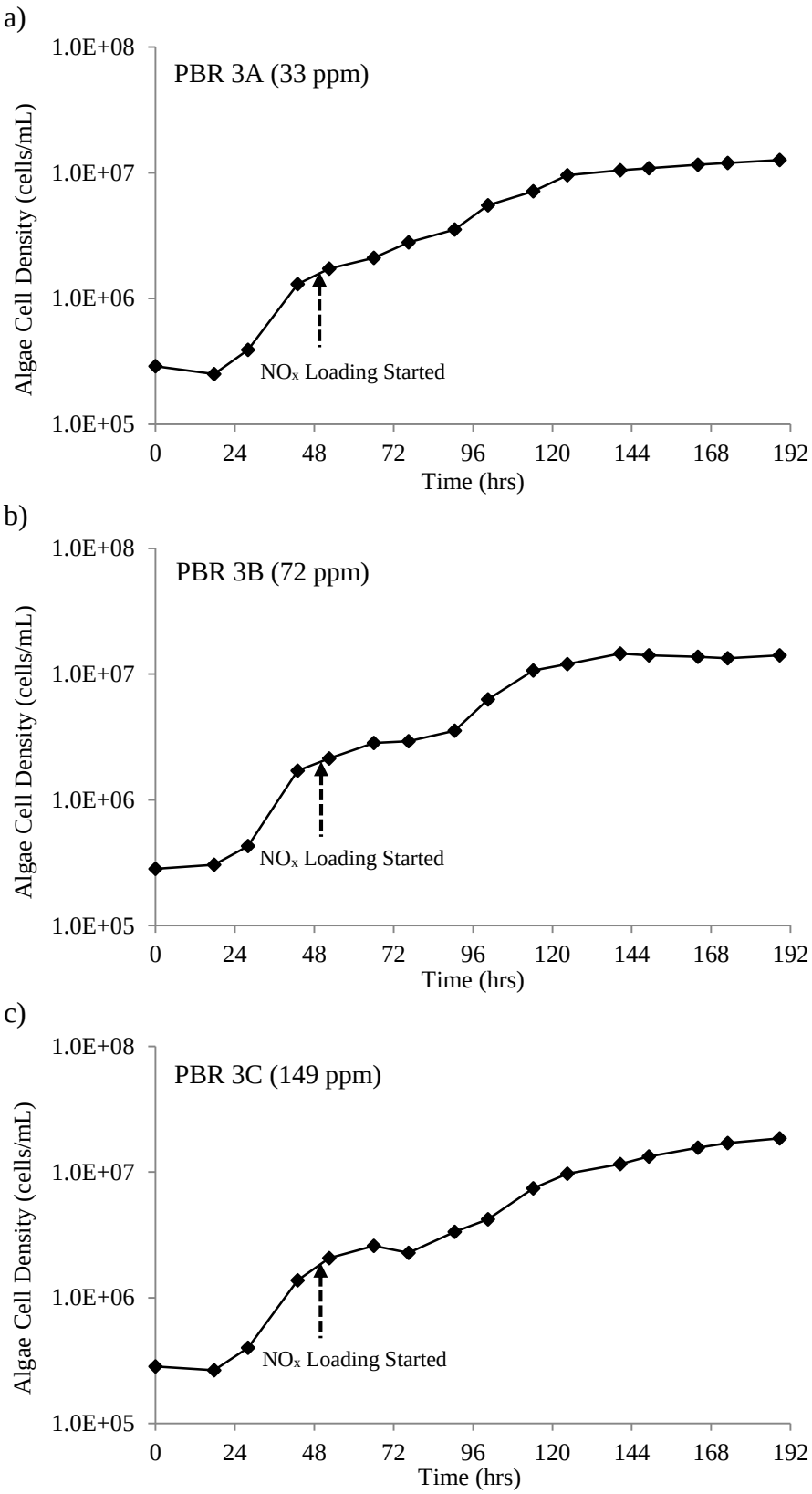

Figure 8. Run 3 growth curves; a) PBR 3A. b) PBR 3B. c) PBR 3C.

The culture in PBR 3B appeared to stop growing after approximately 140 hours, while PBRs 3A and 3C continued growing through the entire length of the experiment. Also, after introducing NO_x to the system, growth rates in all three PBRs never achieved the same magnitude that was demonstrated just before NO_x loading began. The biological acclimation period and extended length of the experiment allowed the algae to reach much higher cell densities than in previous runs. In addition to improved cell counts, the media in all three PBRs were deep green and completely opaque. These are all signs of a very healthy culture.

Initial and final total suspended solids (TSS) samples were taken to quantify algal growth and assess the nitrogen content of the cells. The results are summarized in Table 10. TSS results indicate a 37-fold average mass growth over the 192-hour run. All three PBRs exhibited similar final cell mass.

Table 10. Total suspended solids of Run 3 PBRs

PBR	TSS Initial (mg/L)	TSS Final (mg/L)
3A	6.0	210
3B	5.7	243
3C	6.3	222

4.2 Nitrate/Nitrite

Liquid samples from Run 3 were analyzed for nitrate and nitrite concentrations, and the results are shown in Figure 9. Nitrate was completely depleted in all three PBRs before NO_x was introduced to the system at 49 hours. No nitrite was observed in solution prior to NO_x loading. This demonstrates that the only nitrogen source available for the algae growth after that point was from dissolved NO_2.

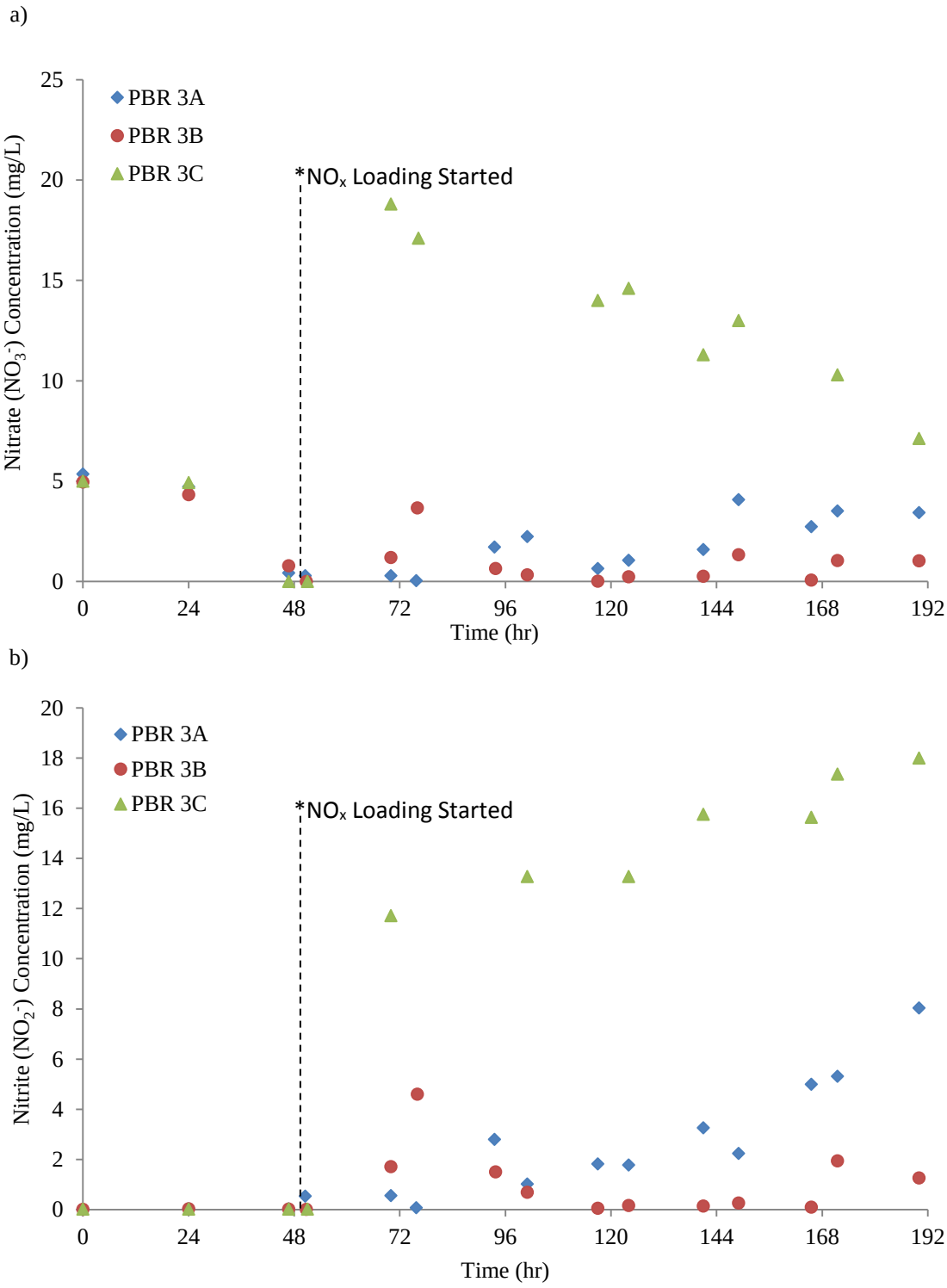

Figure 9. a) Nitrate concentrations in Run 3 over time. b) Nitrite concentrations in Run 3 over time.

The high NO_x loading in PBR 3C caused a drastic and sudden increase in nitrate immediately, but the algae were able to take up this excess nitrate at a very steady rate throughout the remainder of the run (Figure 9). As NO_3^- concentrations decreased in a linear trend in PBR 3C, NO_2^- concentrations increased at essentially the same rate. Based on a linear regression, NO_3^- was consumed and/or chemically converted at a rate of approximately 0.02 mg $N\text{-}NO_3^-/L\cdot hr$, and NO_2^- was accumulated at a similar 0.02 mg $N\text{-}NO_2^-/L\cdot hr$ in PBR 3C.

Nitrate and nitrite concentrations were much lower for PBRs 3A and 3B with lower NO_x loading rates. Initially, nitrate concentrations were higher for PBR 3B as expected from the higher NO_x loading concentration compared to PBR 3A. However, after approximately two days, the nitrate concentration in PBR 3B dropped below that of PBR 3A, even though the influent NO_x concentration was more than double in PBR 3B. While the nitrate concentration remained essentially constant in PBR 3B after this point, it appeared to slowly, yet steadily, increase in PBR 3A. Nitrite concentration was also lower in PBR 3B than PBR 3A, even though PBR 3B was loaded with more NO_x.

4.3 Organic Nitrogen Content and Mass Balance

Initial and final total organic nitrogen content of the algal cultures was used to determine algal uptake of nitrogen and assess the nitrogen content of the cells. Table 11 summarizes the results of these analyses. The cells in PBRs 3A, 3B, and 3C were found to contain 6.7%, 4.2%, and 8.1% nitrogen respectively.

Table 11. Total organic nitrogen content of algal cultures in Run 3

PBR	TKN Initial (mg N/L)	TKN Final (mg N/L)
3A	0	14.1
3B	0.5	10.2
3C	0	17.9

Using the above results, a mass balance on nitrogen was conducted to quantify the fate of NO_x through the system (Table 12). In PBR 3A, the mass balance accounted for 120% of the observed NO_x removal, as slightly more nitrogen was found in the cells and growth medium than had entered the system. However, for PBRs 3B and 3C, only 20% and 25%, respectively, of the nitrogen that entered the system was found in the cells and growth medium.

Table 12. Run 3 nitrogen mass balance data and results

	Calculated N (mg)		
	PBR 3A	PBR 3B	PBR 3C
NO_x Input	477	1041	2198
NO_x Output	182	22	70
N Consumed from NO_x gas phase	295	1019	2128
Initial N in growth medium	27	25	25
Final NO_3^-	17	5	35
Final NO_2^-	54	8	121
Net Accumulation of NO_3^- plus NO_2^-	*44*	*-12*	*131*
Initial Organic N	0	11	0
Final Organic N	310	224	394
N Accumulated in Algal Cells	*310*	*213*	*394*
Total N Accumulated	354	201	525
Mass Balance (% NO_x uptake accounted for)	120%	20%	25%

5. DISCUSSION

5.1 NO_x Feed System

Loading NO_x at a consistent concentration proved to be very difficult for the first two runs, as can be seen by the large standard deviations in Table 6. For the first two runs, NO_x was supplied from a cylinder of pure NO_2, and then the vapors from this liquid were pumped into the PBRs using peristaltic pumps. This method was unreliable due to the large deviations in concentration, so Run 3 was operated using calibration gases. The loading ratio (NO_2:NO) was low and varied largely for the first two runs. Considering no NO was purposely included in the influent gas stream, all the NO that appeared was due to natural chemical reactions, which made the ratio uncontrollable. Much more NO was created when using pure NO_2 as the feed gas than was created when using dilute NO_2 calibration gas as shown by the large loading ratios for PBRs 3A, 3B, and 3C in Table 6. Once calibration gases were used, the influent NO_2 and NO concentration were fairly consistent, with NO comprising about 4% of the NO_x stream. Because of the NO_x feed issues with Runs 1 and 2, removal data analysis will only be considered for Run 3.

5.2 NO_x Removal

Run 3 resulted in NO_x removal rates of 96-97% for PBRs 3B and 3C, which had the highest NO_x loading rates. In contrast, only 49% NO_x removal was achieved in PBR 3A, which had the lowest NO_x loading rate. The only difference in the three PBRs during Run 3 were the NO_x loading rates. With everything else identical, it can be hypothesized that varying mass transfer rates cause better removal efficiencies. The smaller concentration gradient between the inlet gas and the aqueous solution may have reduced mass transfer rates, effectively reducing NO_x removal. This is difficult to

rationalize, however, because one would expect the algae to more easily uptake smaller amounts of NO_3^-. The large discrepancies in the mass balances for PBRs 3B and 3C may also demonstrate a possible lab anomaly. Nonetheless, no definitive explanation has been found to support the outcome of higher loading concentration resulting in higher removal rates. These results will require more data to substantiate the mass transfer rates and their dependency on concentration gradient.

The conditions in each PBR, including light, temperature, and pH, all showed equal variability and were all well within range of each other. In addition, Figure 8 shows comparable algal growth in all three reactors. However, the nitrogen uptake by the algae in PBR 3A appears to have been limited, which might explain the low NO_x removal observed. Figure 9 shows that aqueous nitrate and nitrite concentrations in PBR 3A were higher than those measured in PBR 3B, even though more NO_x was loaded to PBR 3B. Something appears to have limited the ability for the algae to take up nitrogen in PBR 3A, which will be explored further in later sections.

5.3 Cell Growth

The results of PBR 1A showed that an increase in algal cell growth was associated with increased NO_x removal rates, supporting the hypothesis that algal growth facilitates NO_x removal. Algal growth consumes dissolved NO_3^- as a nitrogen source. Reduced concentrations of dissolved NO_3^- allows reaction rates of NO_2 (aq) to NO_3^- (aq) to increase based on Reaction 1.

$$NO_2(g) \leftrightarrow NO_2(aq) \leftrightarrow NO_3^-(aq) + NO_2^-(aq) \qquad (1)$$

This would result in a decreased concentration of NO_2 (aq) which would increase the mass transfer of NO_2 from the gas to liquid phase, effectively increasing removal of gaseous NO_x.

NO_x loading had numerous effects on the observed growth of algae. The biological crash that was observed in PBR 1A can be blamed on the sudden drop in pH caused by high NO_x loading rates. After 13.5 hours, the pH in this PBR had dropped to 3.8, and ultimately reached a minimum pH of 2.9, until experimental adjustments were made. According to Rachlin and Grosso (1991), growth of *Chlorella vulgaris* is substantially retarded at such low pH. After approximately 65 hours, sodium bicarbonate was used to adjust the pH in PBR 1A up to 6.7 to examine the effect of pH on cell growth and NO_x removal. Adjusting the pH to more neutral conditions stimulated some growth in the PBR, as shown in Figure 6a, and increased NO_x removal. The average removal rate for the first 65 hours of operation was 38%. After pH adjustments were made, average NO_x removal for the final 25 hours was 53%.

Allowing the algae to acclimate to the PBR environment and conditions in Run 3, and allowing the culture to reach a substantial cell density before exposing it to potentially toxic NO_x loading, resulted in higher initial growth rates. Consequently, the average specific growth rate for the first 48 hours in PBRs 3A, 3B, and 3C was 0.035 hr^{-1}, 0.039 hr^{-1}, and 0.036 hr^{-1} respectively. In contrast, the average specific growth rates for the same period in PBRs 1B and 1C were both negative, signifying an overall decay in cells. This suggests that the algae are initially shocked by the introduction of NO_x. The cells will eventually adapt to NO_x as a food source and continue to grow, but this comes after an extended lag phase as shown in the growth curves of Figure 6.

The initial shock of NO_x loading is further represented by the growth curves of Run 3 in Figure 8. Although the algae continued to grow, a decline in growth rate was observed immediately after introducing NO_x, even after allowing the algae to properly acclimate. After introducing NO_x, growth rates began to decline in all three PBRs and never achieved the same magnitude as before NO_x loading began. The extent of decline is proportional to the concentration of NO_x entering the system. In other words, higher NO_x concentrations resulted in lower growth of the algae initially. This effect lasted for approximately 48 hours after NO_x loading, after which the inlet concentration of NO_x appeared to have no effect on algal growth. Therefore, it took approximately 48 hours for the algae to adapt to their new nitrogen source. Once adapted, the concentration of NO_x was no longer a growth inhibitor.

In addition to environmental acclimation, other factors were adjusted in Run 3 to enhance algal growth. Unlike the first two runs, the pH never became acidic in any of the three PBRs in Run 3, and in general, pH remained in the optimum range of 7.5-8.0. The installation of heaters for Run 3 also ensured the temperatures were much warmer for the algae than in previous runs. Light was also enhanced, providing more photosynthetic energy for the algae to grow. Since all changes were made at once, it is impossible to determine which change had the greatest effect on algae growth. At this point it can only be concluded that consistent and non-extreme conditions, as well as sufficient cell density before NO_x loading, are favorable to avoid culture crashes. Further experiments should be conducted, changing one variable at a time, to determine precise optimal conditions and how to accurately maintain them.

5.4 Nitrate/Nitrite

The algae were able to take up all nitrate in solution before NO_x was introduced into the PBRs. The only source of nitrogen available to the algae after approximately 48 hours was from NO_x. The fact that the algae continued to grow for the last 144 hours of Run 3 is proof that the algae was able to grow on NO_x as a nitrogen source. The steady decline in NO_3^- concentrations and accumulation of NO_2^- in PBR 3C suggests that the algae preferred NO_3^- as its nitrogen source over NO_2^-. PBRs 3A and 3B do not show this as clearly. Although all three PBRs in Run 3 showed an accumulation of NO_2^-, PBR 3A also showed a slight accumulation in NO_3^-, and PBR 3B showed a relatively steady NO_3^- concentration. The NO_2^- may have accumulated because the algae were only taking up NO_3^- as a nitrogen source. Another possible explanation may be, as noted in Section 2.5, that only NO_2 is capable of forming NO_3^-, while both NO_2 and NO can react with water to form NO_2^-, resulting in excess nitrite. Because of the differing nitrate and nitrite concentrations in each of the PBRs, and the chemistry of aqueous nitrogen oxides, it cannot be conclusively determined whether the algae used NO_3^-, NO_2^-, or both as their nitrogen sources.

Substantial and steady growth in all three PBRs in Run 3 demonstrates neither NO_3^- nor NO_2^- were ever a limiting constituent. Also, with steady removal efficiencies in PBRs 3B and 3C, the PBRs never reached their saturation limit of aqueous NO_2. The higher NO_3^- concentrations in PBR 3C theoretically decreased the reaction rate of aqueous NO_2 to NO_3^- and caused an accumulation of dissolved NO_2. If saturation were achieved, further dissolution of NO_2 would have been unfavorable, and gaseous NO_2 would have passed through the system and into the exhaust, decreasing removal rates.

5.5 Nitrogen Mass Balance

The mass balance on nitrogen for the treatment system was relatively accurate for PBR 3A, but most of the nitrogen could not be accounted for in PBRs 3B and 3C. The mass balance for PBRs 3B and 3C (with high observed NO_x removals) only accounted for 20% and 25% of the nitrogen consumed from gaseous NO_x. Numerous potential explanations for the poor mass balance results for PBRs 3B and 3C were explored. The following are the most likely explanations:

- Gas leaks

One possible source of error is potential gas leaks on both the inlet and outlet ports. Although inlet flow rate was sampled at regular intervals and leak tests were administered periodically, a leak between the sample port and PBR inlet is possible. Exit flow rates were measured at approximately 27% lower than their respective inlet flow rates. If this leak occurred in the short stretch of tubing between the sample port and PBR inlet, 27% less NO_x would have been entering the measurement system than was measured, therefore inflating removal percentages. However, if inlet flow rates were adjusted to the measured outlet flow rates to account for leaks, the 27% difference in flow corresponds to a 14% difference in nitrogen removal in PBR 3A, and only a 1-2% difference in NO_x removal rates for PBRs 3B and 3C.

The drastic differences in the amount of organic nitrogen accumulated in the biomass and in solution and the difference in organic nitrogen in the influent and effluent gas concentrations was not likely due to inaccurate flow measurements and potential leaks. The differences are too severe to be accounted for, as shown in the analyses above. Other possible explanations for the poor mass balances are described below.

- Total Kjeldahl Nitrogen errors

The complex procedures followed to determine Total Kjeldahl Nitrogen may have been a source of significant error in calculating the nitrogen balance for the PBRs. In addition, the APHA Standard Method 4500-NorgB suggests that if an immediate analysis is not possible, the sample should be preserved by acidifying and storing at 4°C (APHA, 1995). While the samples were acidified appropriately, they were not immediately refrigerated. It is thus possible that the inaccurate mass balance was due to experimental error in the TKN analysis.

- Other forms of nitrogen not accounted for

Another likely explanation for the poor mass balances is other forms of nitrogen in the system that were not accounted for in measuring nitrogen compounds. This could include either aqueous nitrogen compounds in solution or gaseous forms of nitrogen oxides other than NO_2 and NO in the effluent streams, among others. Some likely compounds could include aqueous NH_4^+ in solution or gaseous N_2O_4 in the effluent gas stream. Neither of these nitrogen compounds were measured during experimentation, but are potential intermediates in the known reaction chemistries of NO and NO_2. The measured forms of NO_x have rather complex chemistries in both the aqueous and gaseous phases, so large concentrations of alternate forms of nitrogen could explain the large variability in the mass balances of PBRs 3B and 3C.

5.6 Cell Nitrogen Content and Residual Aqueous Nitrogen

Table 13 relates the average nitrogen content of the cells to cell mass, and compares these values to the final NO_3^- and NO_2^- concentrations.

Table 13. Comparison of cell growth to nitrogen content and uptake.

PBR	Final Cell Count (cells/mL)	TKN (mg/L)	TSS (mg/L)	Nitrogen Content (%)	Cell Weight (pg/cell)	Final Nitrate (mg/L)
3A	$1.26x10^7$	14.1	219	6.7	16.7	3.52
3B	$1.41x10^7$	10.2	243	4.2	17.2	1.04
3C	$1.85x10^7$	17.8	222	8.1	12.0	10.30

Nitrogen content was lower in all cases than the 12% typical value reported for *C. vulgaris* (Lourenço et al., 2004). Taking the typical protein content of *C. vulgaris* to be approximately 55% (Becker, 1994), total Kjeldahl nitrogen content should be approximately 12%. As shown in Table 13, the nitrogen content of the algal cells in all three PBRs of Run 3 were substantially below this assumed value. As mentioned in Section 2.6, according to Syrett (1962), this may mean the algae were nitrogen starved and began producing more carbohydrates and lipids rather than protein, without affecting overall growth. This would also explain why the algae in PBR 3B were a lighter shade of green, as these cells had the lowest cell nitrogen content and nitrogen starved algae produce less chlorophyll (Syrett, 1962).

Table 13 also shows that although cell density increases with increasing NO_x loading rates, nitrogen content does not necessarily share the same relationship. Nitrogen content appears to correlate better with residual nitrate/nitrite concentrations; higher dissolved nitrogen compounds resulted in greater cell nitrogen content, as shown in Figure 10.

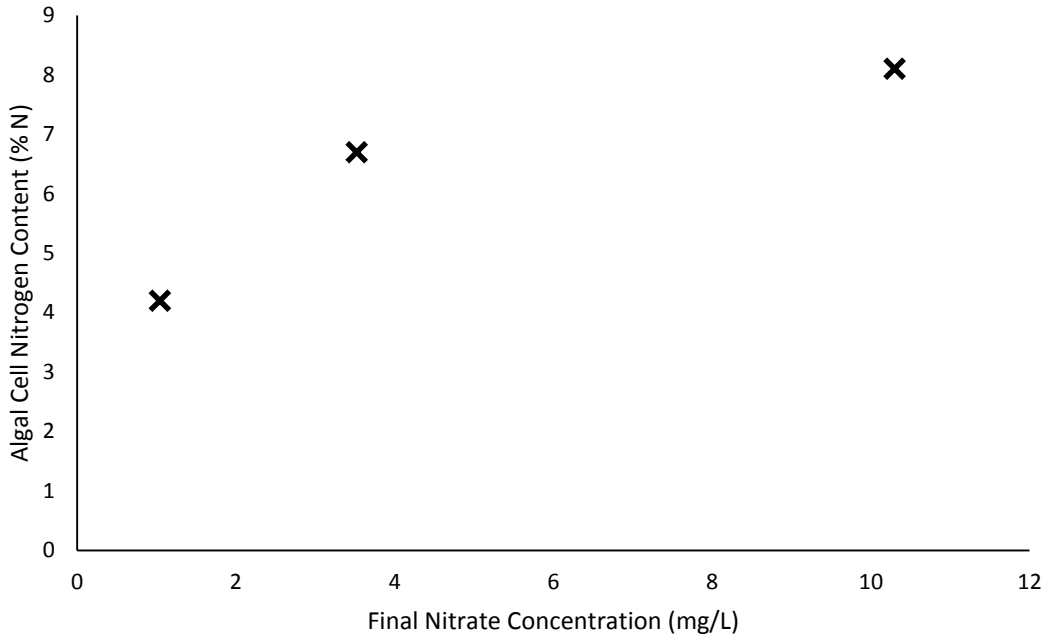

Figure 10. Correlation between final dissolved nitrate concentration and N content in biomass.

Excess nitrogen in solution appeared at high NO_x loading. This resulted in the highest nitrogen consumption, with nitrogen content at the highest measured (8%). This also resulted in the largest cells (by mass), although the added mass did not achieve better NO_x removal.

Despite the remarkable removal efficiency and algal growth of PBR 3C, the results are not ideal. The steady accumulation of nitrate and nitrite observed would not be sustainable for long periods of time because of the dissolved nitrogen accumulation. To counteract this, a larger volume of PBR can be implemented for higher influent NO_x concentrations to allow for more dissolved nitrogen capacity and greater algal growth. The results of PBR 3B show that this middle NO_x loading is the most efficient and sustainable of the three in terms of nitrate/nitrite levels. The low nitrate concentrations may indicate that the solution was nitrogen starved, yet sizeable algal growth and 97%

NO_x removal demonstrates that this did not affect growth and efficiency. Instead, PBR 3B appeared to take up nitrogen most efficiently, assimilating nitrate at the same rate NO_2 was dissolving and dissociating into the system. Based on nitrate concentrations in solution, the cells were able to assimilate the nitrogen most effectively while maintaining the greatest mass density.

5.7 Removal Mechanisms

To determine possible explanations as to why lower NO_x loading concentration resulted in lower removal rates, the data from Run 3 were analyzed in more detail. Run 3 proved to be the most consistent in terms of both experimental conditions and data obtained. Again, three possible mechanisms were explored, including mass transfer, chemical reactions to form nitrate, and biological uptake.

The limitations do not appear to be chemical in nature, as it can be assumed that the saturation point of aqueous NO_2 was never reached in any of the three PBRs, and Reaction 1 progressed in the forward direction to produce nitrate.

$$NO_2(g) \leftrightarrow NO_2(aq) \leftrightarrow NO_3^-(aq) + NO_2^-(aq) \quad (1)$$

If saturation had been reached, a decrease in removal rates toward the end of the run would have been witnessed, as undissolved NO_2 would have passed directly through the system and into the exhaust. This would have been particularly true in PBR 3B, which had the greatest overall accumulation of nitrate and nitrite. The high nitrate/nitrite concentrations would have prevented the equilibrium of Reaction 1 to proceed in the forward direction, and dissolved NO_2 would have accumulated and saturated the solution. Because no such decrease in removal rate was seen in PBR 3C, chemical saturation

and/or the inability for aqueous NO_2 to oxidize/reduce, could not have been the limiting factor in NO_x removal.

Based on the algal growth results of Run 3, it appears that the limitation is not entirely from biological growth, but possibly some other biological factor. All three PBRs grew comparably well no matter the conditions or loading NO_x concentrations. Biological growth was more dependent on environmental conditions (such as pH, temperature, and acclimation) than the amount of NO_x loaded into the system. This also eliminates the possibility of NO_x toxicity. If NO_x was indeed toxic, higher concentrations would have resulted in poorer overall growth rates. While NO_x loading did initially cause a decline in cell growth, this may be attributed to environmental acclimation. The fact that the algae grew just as well, if not slightly better, with higher loading concentrations, shows that the limitations to the system are not biological, in terms of pure algal growth. While algal growth was not directly a factor in differing removal rates, various other biological effects may have prevented the algae from efficiently assimilating nitrogen, indirectly affecting NO_x removal.

Mass transfer limitations seem to be the most likely cause of the large discrepancy in NO_x removal between the PBR 3A and the other two PBRs in Run 3. No enhancements were made to increase mass transfer for this study. The dissolution of NO_2 into solution is partially dependent on the concentration gradient between the inlet gas stream and aqueous nitrogen oxide. This difference was least in PBR 3A. Further studies should optimize mass transfer to validate the effect of concentration gradient on NO_x removal.

Based on the results of these preliminary experiments, it can be concluded that the limiting factors in NO_x removal are a combination of biological nitrate uptake and/or mass transfer rates. The slower uptake of nitrate by the algae caused an increase in nitrate concentration, effectively decreasing the driving force for mass transfer of NO_2 from the gaseous to aqueous phases.

6. CONCLUSIONS

One of the primary purposes of this study was to test the hypothesis that *C. vulgaris* can grow on nitrogen from dissolved NO_2 as its sole nitrogen source for cell synthesis. *C. vulgaris* grew substantially using only nitrate and/or nitrite generated by NO_x dissolution for cell synthesis, formed through Reaction 1, reaching a maximum cell density of 1.85 x 10^7 cells/mL (a 60-fold increase in cell density).

$$NO_2(g) \leftrightarrow NO_2(aq) \leftrightarrow NO_3^-(aq) + NO_2^-(aq) \quad (1)$$

This growth and assimilation of nitrogen allowed continual dissolution of NO_2 into solution, resulting in NO_x removal rates of up to 97%.

Although a major accomplishment, this pilot study simply shows a proof of concept. An anomaly emerged in the study in which one PBR with lower NO_2 loading concentrations resulted in lower NO_x removal rates. For this PBR, nitrate was not assimilated as efficiently as a similar PBR with greater NO_x loading. For the third run of the study, conditions in all three photobioreactors (i.e. light, temperature, pH, etc.) were comparable with reasonable precision. Light was identical, all reactors had an average temperature of approximately 22°C, and average pH ranged from 8.0-8.5. The only observable and substantial difference between the reactors was the concentration of influent NO_x.

In Run 3, cell growth by mass was between 200 mg/L to 240 mg/L. Nitrogen was removed from gaseous NO_x at a rate of 0.07 – 0.49 mg N/mg cell growth. Assuming a 600-MW natural gas fired power plant can produce up to 1,600,000 m^3/hr of flue gas with approximately 40 ppm NO_x concentrations (Mimura, 1997), growth of a minimum of 75 kg algal cells/hr would be required to effectively treat this stream. One power plant

could therefore produce about 660,000-kg of *C. vulgaris* per year treating its NO_x emissions. This could provide a substantial revenue stream if food-grade algae is produced in the treatment.

Further studies should be completed to determine the true limitations to NO_x removal by *C. vulgaris* and identify optimal conditions. This should include optimization of reactor geometry, enhanced environmental conditions for algal growth, and testing of large scale reproducibility. Any further study should focus on eliminating as many variables as possible and optimizing the system for NO_x removal.

Enhancing the geometry of the PBR will allow for the optimization of the mass transfer rates of NO_x from the gaseous to aqueous phases. This can also be accomplished through adjusting the sparger's bubble size and increasing bubble residence time. By ensuring the maximum amount of NO_x is dissolving in the system, other limitations of the treatment method can be better determined.

In addition, optimization of algal growth conditions can further help narrow the scope of possible limitations. By ensuring light, pH, and temperature conditions are optimal for the particular strain of algae used, any biological limitations can be reduced. If environmental conditions allow the algae to grow to their maximum potential, then the only effect on algal growth will theoretically be from outside sources, such as possible NO_x toxicity or inability to assimilate a particular nitrogen source. Sufficient cell density should exist before any NO_x loading is introduced. Future studies should take this a step further to determine the minimum cell density required to overcome any growth inhibition caused by NO_x. This will minimize the time and resources necessary to achieve effective removal.

To determine the actual feasibility of using algae to treat nitrogen oxides, actual conditioned flue gas should be used in future experiments. This will determine whether any additional components in flue gas have an inhibitory effect or otherwise prevent the inlet stream from being conditioned (e.g. SO_x). Using flue gas with steady NO_x concentrations and NO_2:NO ratios will prevent problems with loading fluctuations as witnessed in this study. Adding NO to the system may make the gas more difficult to treat because of the poor solubility of NO. A pre-treatment oxidation process may help increase removal efficiencies by oxidizing the NO to the more soluble NO_2.

If true flue gas cannot be obtained, it is advised that NO be loaded in addition to NO_2 to more accurately simulate real world conditions. In this case, to avoid fluctuations, a larger system with higher flow rates should be tested. With higher flow rates, the low NO_x concentrations required will be much easier to obtain without the use of peristaltic pumps or highly sensitive valves that proved significant obstacles in the above experiments.

As also shown in this study, it may be difficult to control the ratio of NO_2 to NO. NO appeared in the inlet gas in varying concentrations even though no NO was intentionally loaded into the system. Due to the unstable nature of both NO and NO_2, the two constituents are constantly converting back and forth to and from one another. This would be true no matter what source of NO_x is used. The instability of NO_x may therefore present an insurmountable limitation to this treatment method, largely due to the insoluble nature of NO. If NO dominates over NO_2, removal rates will decrease no matter how optimized the system may be. Therefore, an appropriate and reliable conditioning method, as well as a delivery system completely isolated from the

environment with constant temperature and pressure, will be needed to ensure that NO_x ratios always favor NO_2.

Beyond optimization of these factors, the next step should include a scale-up of the model and a cost analysis to determine whether a full scale system is economically feasible. The actual size of a system used in real world power-plant applications would require extremely large volumes, depending on the flue gas production. In this research, nitrogen was removed at a rate of between 15 – 106 mg N/L in the 20-L PBR. Assuming a 1,600,000 m^3/hr stream of flue gas with approximately 40 ppm NO_x concentration (Mimura, 1997) and similar removal efficiencies with a scale up, a full scale system to treat nitrogen oxides from a 600-MW natural gas fired power plant using algae would require 50,000-m^3 of photobioreactors. For this scale-up, algae would need to be harvested every 140 hours, as with the experimentation presented above. The ability to scale these parameters depends on various factors, including the ability to grow and harvest the algae, mass transfer rates, geometry of the photobioreactors, cell densities, and many other factors. Future studies would require testing these variables to determine the minimum volume capable of treating an actual flue gas stream to minimize the footprint and reduce the cost of this treatment method.

To maximize its effectiveness, this method of NO_x treatment should be optimized and implemented as soon as possible for power plants to utilize the technology to stay in compliance with more stringent future emission standards. Overall, with appropriate environmental conditions and land area, the treatment of NO_x in photobioreactors using *C. vulgaris* could provide an effective and possibly inexpensive method of meeting NO_x emission standards.

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