

**Impact of Organic Substrate on NO Oxidation in Biofilters**

**Final Report**

**ARB Contract #00-311**

**Submitted by**

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## Glossary of Terms and Acronyms

### Acronyms

**ATCC** - American Type Culture Collection

**EBCT** - empty-bed contact time calculated as bed media volume divided by flow rate

**EISG** - Energy Innovations Small Grant program

**COD** – chemical oxygen demand - quantity of oxygen required to oxidize compounds through a standard chemical oxidation process)

**FISH** - Fluorescent *In Situ* Hybridization

**NO** – nitric oxide

**PPI** – pores per inch in reference to pore dimensions of carbon foam

**RBC** – rotation biological contactor

### Terms Associated with Nitrification

**Autotrophic nitrification** - The oxidation of ammonium,  $\text{NH}_4^+$ , to nitrate,  $\text{NO}_3^-$ , via nitrite,  $\text{NO}_2^-$ , is an aerobic process called autotrophic nitrification. Autotrophic refers to organisms that utilize inorganic carbon dioxide as a carbon source for cell growth. Two groups of chemolithotrophic bacteria, microorganisms that use inorganic compounds for energy, are responsible for nitrification, ammonia oxidizers typified by the genus *Nitrosomonas*, and nitrite oxidizers, typified by the genus *Nitrobacter*.

**Heterotrophic nitrification** - Heterotrophic nitrification refers to the oxidation of chemically reduced nitrogen compounds such as ammonium, hydroxylamine, hydroxamic acids, amino nitrogen, and nitrite, to products such as nitrite, nitrate, and other nitrogenous compounds. Heterotrophic nitrifiers are aerobic fungi, actinomycetes, or bacteria requiring an organic carbon source. Energy production or growth has not been associated with heterotrophic nitrification and the process is believed to be endogenous or secondary metabolism.

**Denitrification** - The process by which oxidized forms of nitrogen are reduced to di-nitrogen gas,  $\text{N}_2$ . Denitrification is conducted by denitrifying bacteria under anaerobic conditions and is coupled to electron transport phosphorylation. Denitrifiers are typically facultative organotrophs, which utilize organic carbon as an energy source.

**Substrate** - As used in the context of the proposal refers to the compounds utilized by the bacteria as a metabolic energy source. An organic substrate would be used as a source of carbon for cell growth by **heterotrophic** bacteria.

**Facultative organotrophs** - Organisms that utilize organic carbon as an energy source.

**Biofilm** - That layer of microorganism, primarily constituting bacteria, that adheres to a supporting surface. Biofilms are typically believed to have a film of water (of varying thickness) over them. More recently it has been noted that water may also run in "channels" beneath the biofilm and its supporting surface.

## Background

The subject ARB research project entitled, "Impact of Organic Substrate on NO Oxidation in Biofilters" complemented an Energy Innovations Small Grant (EISG) program project funded by the California Energy Commission. The purpose of the EISG study was to examine the feasibility of using a commercial carbon-foam packing to enhance the specific surface area on which biofilms can develop for the purpose of reducing mass transfer limitation in an aerobic biofilter removing nitric oxide (NO).

The overall goal of the complementary ARB project was to understand how to improve the effectiveness of an NO-oxidizing biofilm grown upon the carbon-foam packing, through addition of an organic substrate, glucose. A report appeared in the literature [Chou and Lin, 2000] about the time that the EISG project was initiated indicating that relatively rapid removal of high concentrations of nitric oxide (NO) was possible through addition of an organic substrate. The empty-bed contact time (EBCT) required to achieve about 80 percent removal was reported to have decreased to about 2 minutes in the 1000 ppm<sub>v</sub> range. Verification of the reported results was a primary motivation of the current research project. The following were specific objectives of this study:

- determination of the impact of organic addition on the biofilm
- determination of the role of heterotrophic microorganisms in enhancing NO removal

## Experimental Findings

### **Batch Study Comparison of NO Conversion to Nitrate with Glucose Addition**

#### Methods

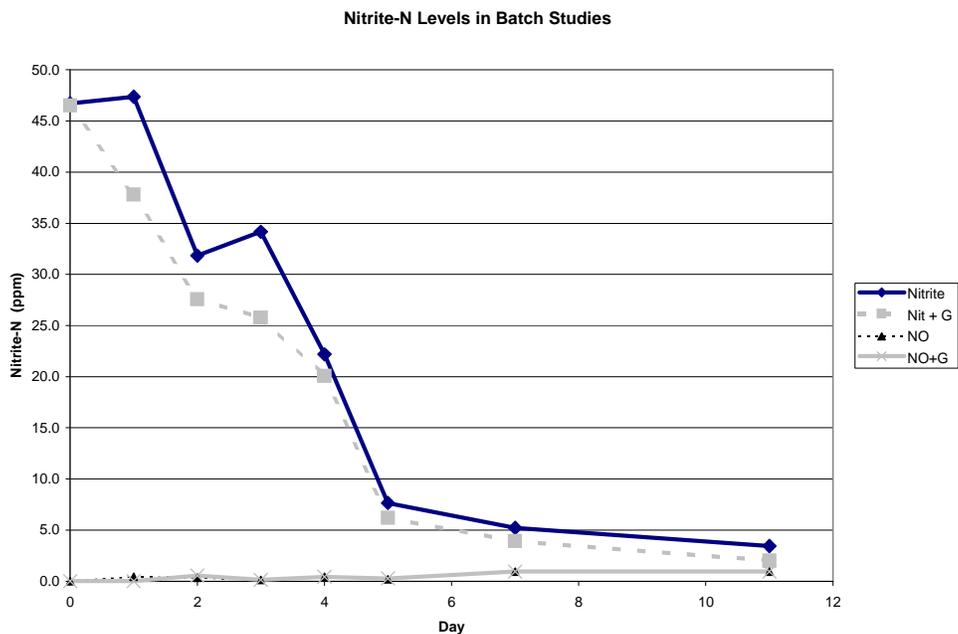
An 11-day study was conducted to compare the potential advantage of a glucose supplement on nitrification of nitrite and NO. Batch studies were initiated on four sets of 250 mL bottles seeded with 45 milliliters of mixed liquor from the nitrification ditch at the UC Davis Wastewater Treatment plant that had been centrifuged and diluted down to 130 mg SS/L and added to each bottle. Addition of nitrite only, nitrite + glucose and glucose only was then carried out to produce the following compositions: one set of bottles consisted of 50 ppm of nitrite, bacteria, and 200 ml of pure oxygen; the second set of bottles contained 50 ppm of nitrite, 40 ppm of glucose, bacteria and pure oxygen; a third set bottles consisted of bacteria, and 200 ml of a 50 ppm mixture of NO and oxygen; a fourth set of bottles contained bacteria, 40 ppm of glucose, and 200 ml of a 50 ppm mixture of NO and oxygen. Chemical oxygen demand (COD) and nitrogen analysis on one 250 ml bottle from each set were performed on the 0, 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 7<sup>th</sup>, and 11<sup>th</sup> day after seeding, respectively. The bottles were placed on a shaker table at 80 rpm and kept at a constant temperature of 23 °C. On each sampling day one of each kind of bottle was removed from the shaker table and the contents were filtered using a 0.2 µm syringe filter. Chemical oxygen demand was performed on each sample using the Hach Low Range 0-150 ppm method. Nitrite analysis was performed using Hach Nitriver-2 chemical pillows and a

spectrophotometer. The total nitrogen analysis was conducted with a Timberline total nitrogen analyzer. Ammonia and nitrate/nitrite levels were measured and then the results from the nitrite test were subtracted from the nitrate/nitrite results to find the nitrate in the sample.

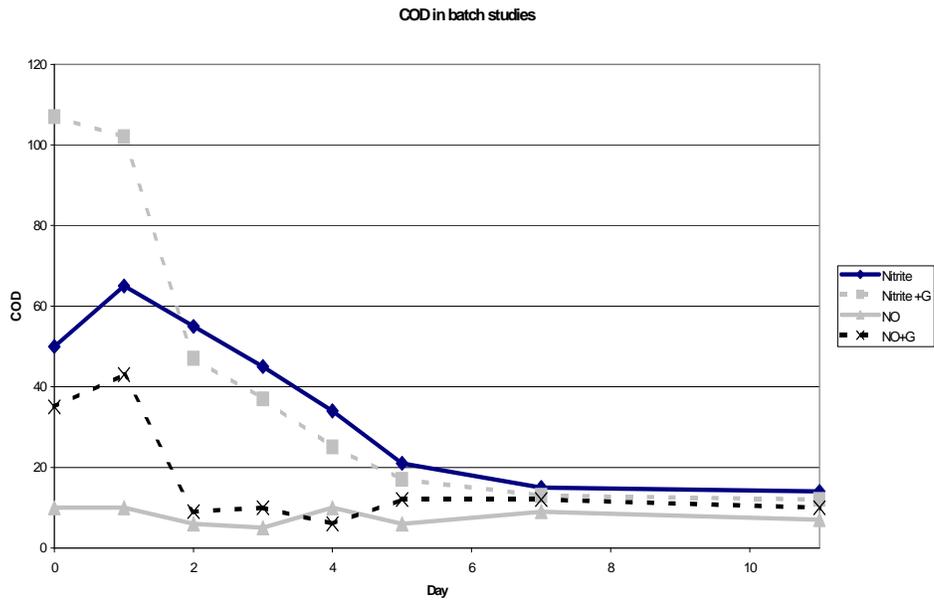
### Batch Study Results

The results of COD, nitrite analysis and total nitrogen analysis are shown in Figures 1 through 3. Each point represents the results of analysis on one bottle on one testing day. The bottle containing nitrite without glucose was represented with the nitrite line, and the nitrite with glucose addition was designated as Nit -G. The data from bottles containing only NO are designated by NO, and the data from bottles with additional glucose supplement are denoted by NO +G.

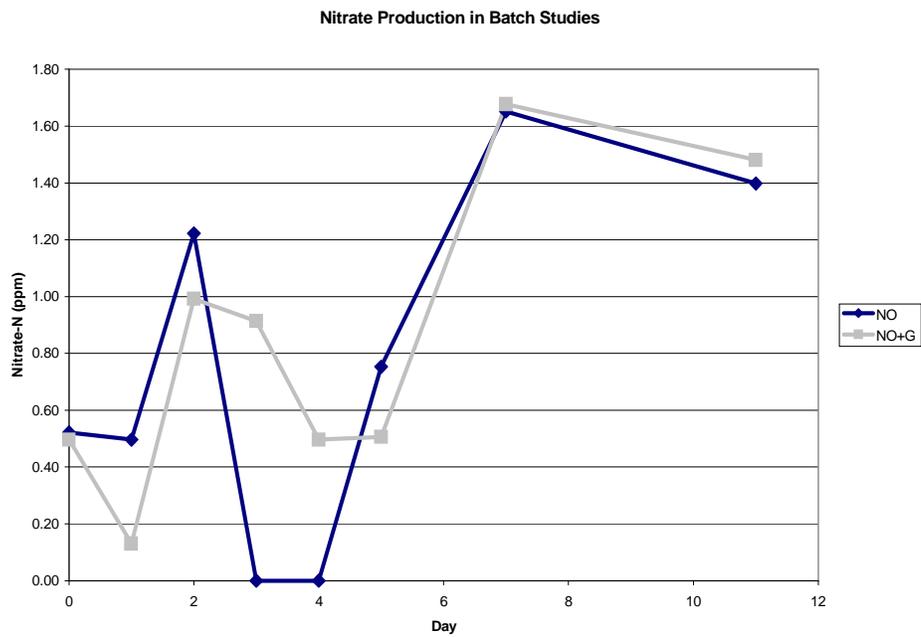
Glucose did not appear to increase the rate of oxidation of nitrite to nitrate in the bottles that utilized nitrite as a nitrogen source. A small amount of NO conversion to nitrate occurred and increased as the bottles were allowed to oxidize for a longer period. A difference between glucose additions is not evident from these data, although the uncertainty in the data is relatively large because of the low absolute amounts. However, further studies with replicated data would be needed to definitively state that there is an absence of a significant difference. Nevertheless, from these results addition of glucose as a supplemental source of carbon did not appear to result in increased nitrification by heterotrophic bacteria present in the seed.



**Figure 1. Nitrite removal profile from batch study.**



**Figure 2. Conversion of nitric oxide to nitrate from batch study.**

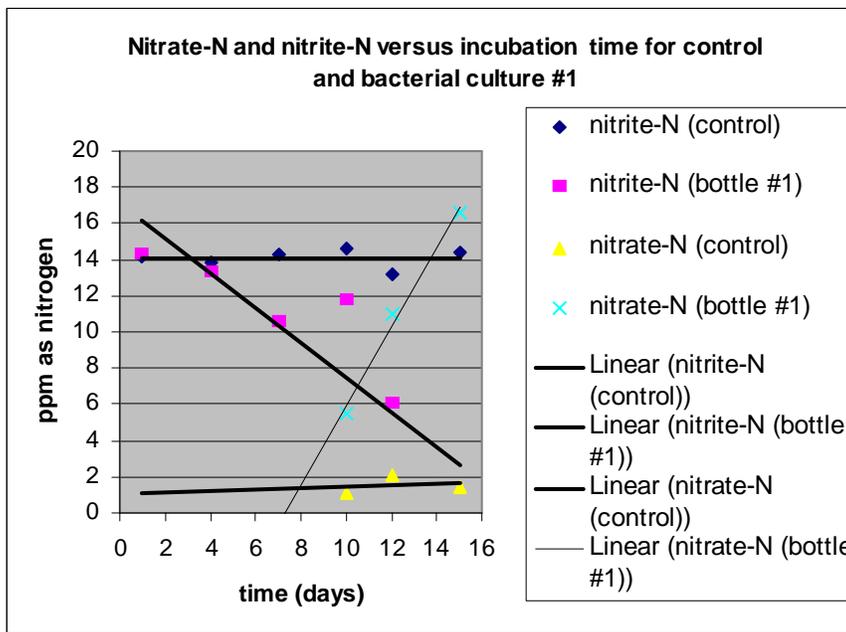


**Figure 3. Chemical oxygen demand results from batch study.**

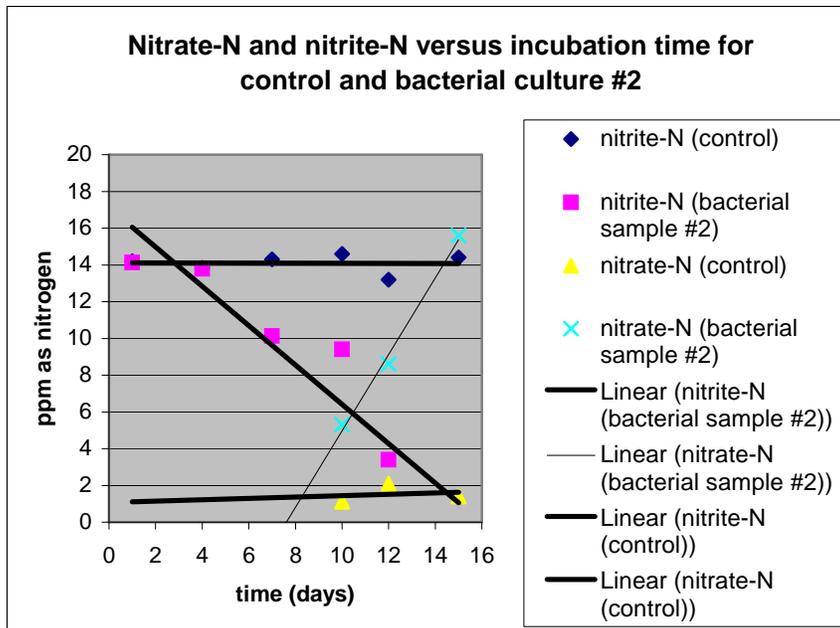
Chemical oxygen demand decreased in the bottles in three of the data sets (Figure 3). The bottles containing NO only did not exhibit an oxygen demand. The reduction of COD indicate that heterotrophic bacteria were present in the seed microorganism population, but if they are nitrifying organisms, their concentration was either too small to compete with the autotrophic bacteria or they were unable to compete with other heterotrophic organisms for the carbon source (glucose). Based upon the batch studies, it does not appear that carbon addition significantly increases NO oxidation.

### Preparation of heterotrophic mixed culture to seed carbon-foam packed columns

Mixed cultures from the UCD wastewater treatment plant were used in the packed column seeding studies supported by the EISG. A mixed culture from the same area of the UCD treatment plant was grown up in an Erlenmeyer flask to "seed" the carbon foam columns for the organic substrate addition experiments. As part of the preparation to seed the columns, activity of the cultures was tested by following nitrite to nitrate conversion in a sample as shown in the next two figures below.



(a)



(b)

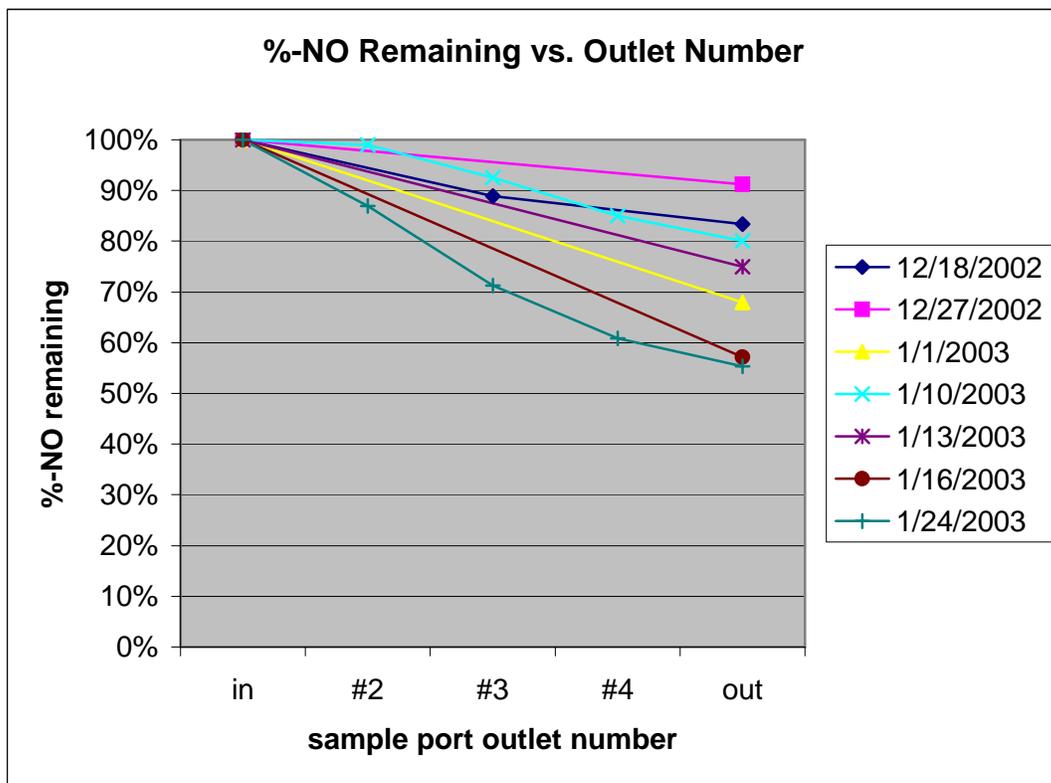
**Figure 4a,b. Nitrite to nitrate conversion in replicate seeded and control batch studies.**

### Column studies

The culture appeared to be robust and to grow rapidly in suspension. Therefore, an attempt to seed the column with the cultures in the Erlenmeyer flasks was carried out. During the first seeding procedure the column accidentally dried twice. Two weeks after seeding the column, the nitrite-conversion activity on the columns was tested, but was found to only be a few percent. The low conversion efficiency was not expected, but probably resulted from an accidental drying of the column that occurred. A second attempt to re-seed the column was undertaken and results of that seeding effort are presented in figure 5. Performance in the column as measured by nitrite conversion and NO removal was poor for the first two weeks (12/16/03) even though seeded with an active culture that was clearly growing on a nitrite/glucose/phosphate/tap water solution. The NO removal at a 2-minute EBCT was less than 10%. By way of comparison with the autotrophic study conducted for the EISG, at concentrations below 100 ppm NO and a residence time of about 2 minutes the best observed performance with only autotrophic organisms, i.e., with no carbon substrate addition, was slightly less than 30%.

As will be discussed later, in order to minimize the amount of abiotic NO conversion, the column was receiving about 50 to 70 ppm<sub>v</sub> NO during this period of time. Close examination of Figure 5 reveals that the removal of NO did not increase monotonically with time. During the first several weeks of operation, up to the period 12/18/02 to 12/27/02, very little removal was observed, and appears to have been decreasing. Prior to 12/27/03 nutrient addition to the column had been either by spray nozzle or an ultrasonic nebulizer was not operated continuously. Shortly after Christmas, a change to the method of nutrient addition was made, i.e., an ultrasonic

nebulizer was employed to continuously deliver, a glucose/phosphate/tap water aerosol to the 20 PPI carbon foam column. Biofilter performance improved within one week. By 1/1/03, the performance of the column noticeably improved to a level that matched the best observed with the autotrophic system alone, however, because the NO gas cylinder supply was nearing depletion, the NO flow rate was decreased, and was inadvertently set too low so that very little NO was being delivered to the 20 PPI column. Column performance degraded over the period of several days as the microbial culture was deprived of its primary energy source, and the NO flow was returned to its earlier setting on 1/10/03. The concentration of glucose and phosphate buffer in the nebulizer was also increased by a factor of 10 on 1/13/03. By 1/16/03 column performance had improved to levels not previously achieved with the autotrophic organisms alone (no glucose addition).



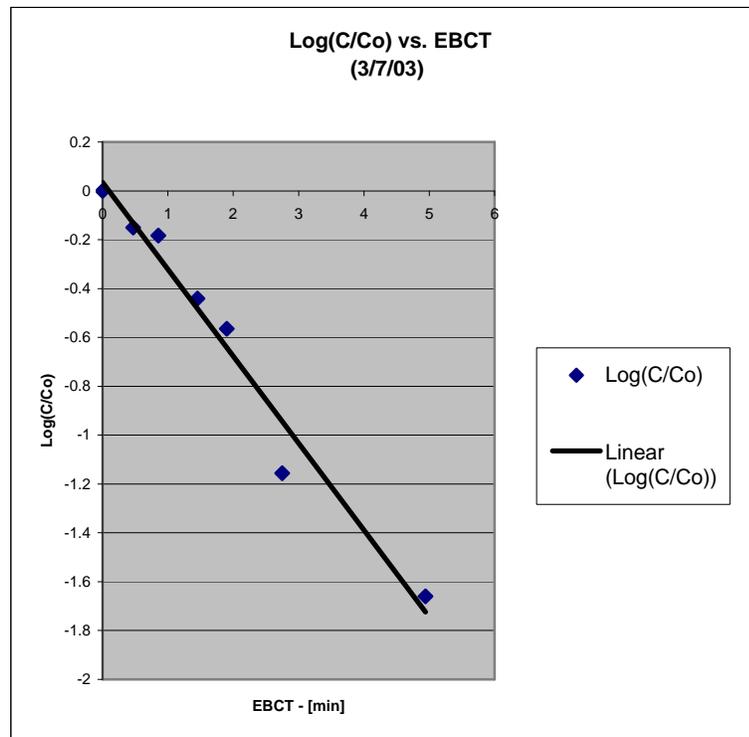
**Figure 5. NO removal in heterotrophic column study at an EBCT of 2 minutes.**

Liquid consumption in the nebulizer section could be monitored and was approximately 50 ml per day. Given the glucose concentration in the feed solution and assuming that the liquid loss was as aerosol droplets (without concentration of glucose in the nebulizer), the molar flow of glucose aerosol provided to the column could be estimated. Similarly, from the removal efficiency of the column at a given flow rate, the molar conversion of NO could also be estimated. Interestingly, at a flow rate of 0.2 Lpm and 25% NO removal, approximately  $2 \times 10^{-9}$  mol/s of NO was converted while  $6 \times 10^{-9}$  mol/s of glucose was supplied in aerosol form, and at the highest removal observed, about 60%, approximately  $4 \times 10^{-9}$  mol/s of NO was converted while  $3 \times 10^{-8}$  mol/s of glucose was supplied. Thus the molar ratio of glucose to NO appears to

be about an order of magnitude, and on a carbon basis, greater than an order of magnitude. The mass ratio of C:N is considerably larger than that reported by Chou and Lin (2000).

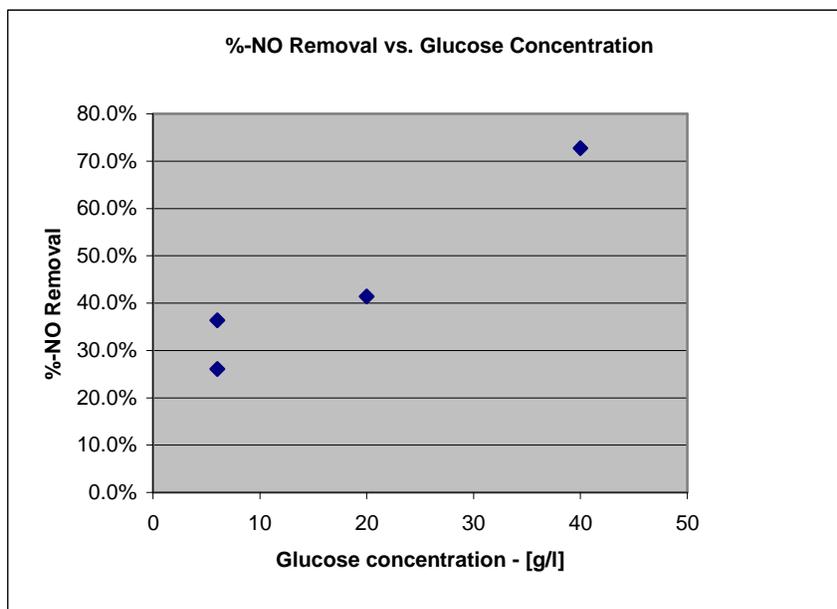
Addition of nutrients solely by ultrasonic nebulizer was continued until the third week of April when the first section of the carbon foam in the column became clogged as a result of biomass accumulation. As reported previously, by 1/24/03, at an EBCT of two minutes, NO removal efficiency had increased to about 45%, better than had been achieved at any time with the autotrophic organisms only. When the nebulizer was returned to supplying only tap water, after a week the removal efficiency dropped back down to about 20% for a two-minute EBCT. Thus we can conclude that increased activity was associated with the change to addition of increased carbon, phosphate and bicarbonate through the nebulizer.

The concentration of glucose in the nebulizer reservoir was increased by a factor of four to 20,000 ppm, 10 g/l of sodium bicarbonate and 2.2 g/l of phosphate on 2/18/03. After 6 days of operation, 2/24/03, the removal efficiency had improved to about 55% at an EBCT of 2 minutes. However, addition of the high level of bicarbonate to the nebulizer, increased the solution conductivity and rapidly corroded the piezoelectric crystal electrode leading to a nebulizer failure. The nebulizer was replaced, and the glucose concentration was again doubled to 40,000 ppm, but without sodium bicarbonate and only 1 g/l of phosphate solution. Performance was determined after 5 days of operation as a function of EBCT as shown in figure 6. A remarkable improvement in performance was noted, with removals of about 75 %, 90% and > 95% at EBCT's of 2, 3 and 5 minutes, respectively.



**Figure 6. NO penetration ( $\log_{10} C/Co$ ) through biofilter versus EBCT with 40 g/L glucose and 1 g/L potassium phosphate dibasic in nebulizer reservoir.**

It was evident from mass conservation considerations that the column would eventually clog at such a high loading rate of carbon through the aerosol. (Unlike biofilters that remove carbonaceous VOCs, nitrogen removal in this system is by incorporation into cells and thus carbon substrate would not exit the biofilter as CO<sub>2</sub> gas, but would likely remain incorporated in cells.) Thus over the next several weeks the glucose concentration in the nebulizer was decreased. For a two to three-minute EBCT, the influence of glucose concentration on nitrogen removal is noted in figure 7.



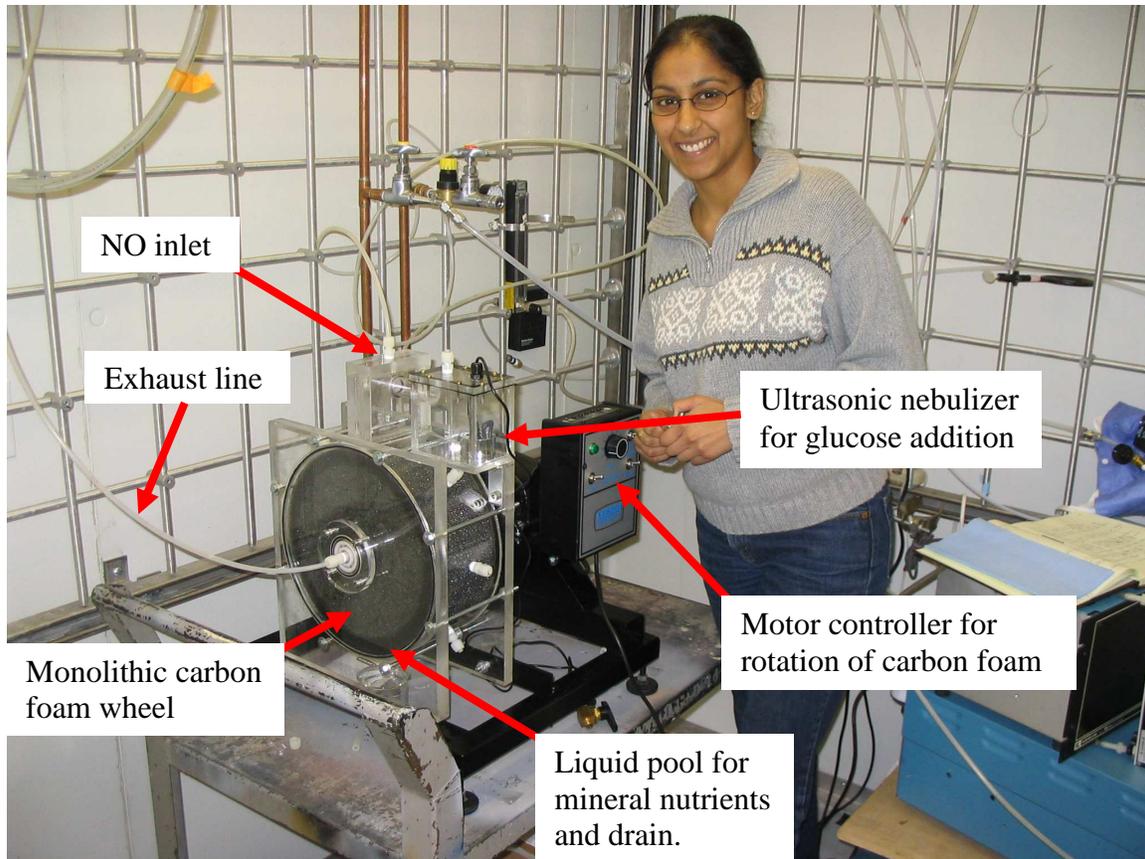
**Figure 7. Effect of glucose concentration in ultrasonic nebulizer reservoir on %-NO removed at EBCTs from 2 to 3 minutes.**

As noted above, the column became clogged with biomass. An effort to remove the biomass by washing was attempted unsuccessfully, even when water jets were directed onto the surface of the carbon foam after its removal from the column. Soaking the foam in concentrated sodium hydroxide solution, 6 N, dissolved the biofilm, but it was evident that removal of the biofilm from the carbon foam might pose a serious problem in full-scale units.

### **Rotating Biological Contactor**

It was also evident that the fixed column design would again clog at high removal efficiencies. Unlike organic compound degradation where the mass of carbon in the molecule is transformed primarily to CO<sub>2</sub>, which is carried out of the reactor in the gas phase, the nitrogen in the NO is in the form of NO<sub>3</sub><sup>-</sup> and must either be washed out of the column or is incorporated into cells. A decision was made to construct a rotating biological contactor (RBC). Ultramet Inc., the manufacturer of the rigid carbon foam was contacted and the company subsequently donated a block of carbon foam (approximately a \$5000 contribution) from which an RBC could be constructed.

A detailed design of the RBC was completed and a new unit was fabricated in the College of Engineering shops. The salient features of the design are that it permits separate introduction of the NO gas stream, a carbon source by ultrasonic nebulizer, and other mineral nutrients (such as P, S, Fe and other trace elements through the liquid pool at the bottom of the RBC). The new design solves problems of accidental drying of the biofilm, permits controlled additions of nutrients and a means for biomass, or  $\text{NO}_3^-$  to be removed from the system by draining the liquid pool. A photograph of the unit is shown in Figure 8.



**Figure 8. Rotating Biological Contactor (RBC) for future studies. Student pictured in the figure (Priya Patel) designed the unit as part of a master's project.)**

Efforts to develop a robust biofilm in the RBC have been unsuccessful to date and could not be completed within the project period. Thus the effectiveness of the new reactor design was not evaluated.

## **Determination of presence of autotrophic microorganisms in biofilm**

The biofilm from the "successful" column was also examined to determine whether the same autotrophic nitrogen-oxidizing bacteria commonly present at wastewater treatment plants are responsible for the activity observed in the column. Fluorescent *In Situ* Hybridization (FISH) probes were obtained from the American Type Culture Collection ATCC and samples from the column and its leachate were labeled. Confocal laser scanning microscopy was used to examine the samples, and positive signals for several strains of autotrophic organisms were obtained. However, there was a high total background fluorescence, even in the absence of the FISH probes, so the results obtained were considered inconclusive. Thus it cannot be stated with certainty that the autotrophic nitrifiers were the primary oxidizers of the NO, though they are the logical candidates.

## **Discussion**

### **Review of abiotic NO reactions**

The NO removal data obtained from the autotrophic column studies carried out as part of the EISG contract indicated that NO removal was non-linear, and increasing with increasing concentration. This was an unexpected result and prompted us to determine the reason for the non-linear behavior. It was suspected that because high NO concentrations (greater than 100 ppm) were being used, abiotic gas-phase oxidation or aqueous phase reactions might be occurring. The result of a literature review provided two rate expressions for estimating the potential loss of NO by abiotic means. The abiotic gas-phase thermal conversion of nitric oxide, NO, to nitrogen dioxide, NO<sub>2</sub>, is given in equation 1.



The rate expression for the oxidation of nitric oxide to nitrogen dioxide is a pseudo-second order relationship in [NO] that is given in equation 2.

$$d[\text{NO}]/dt = -2k [\text{NO}]^2[\text{O}_2] \quad (2)$$

The rate constant k is equal to  $k = 1.066 \times 10^{-5}/T^{2*} \exp(530/T)$  ppm<sup>-2</sup> min<sup>-1</sup> (Seinfeld, 1986). The overall stoichiometric aqueous phase conversion of NO to NO<sub>2</sub><sup>-</sup> is shown in equation 3.



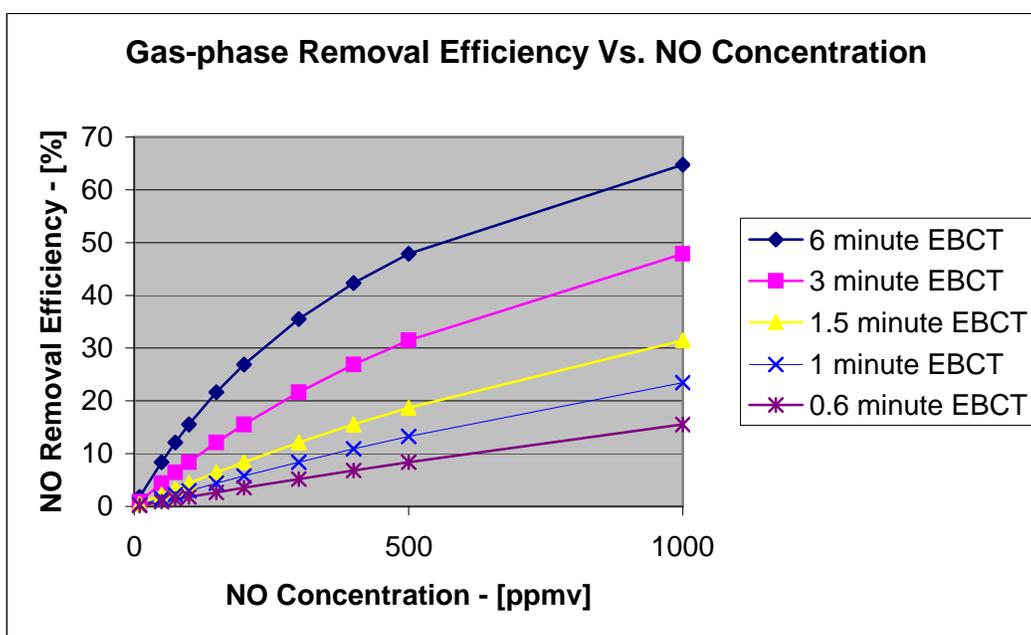
The rate expression for aqueous phase oxidation of nitric oxide has been quantified in equation 4.

$$d[\text{NO}]/dT = -4k_{\text{aq}} [\text{NO}]^2[\text{O}_2] \quad (4)$$

The value of the rate constant  $k_{\text{aq}}$  in equation 4 varies with the source as cited by several different researchers. Pogrebnaya et al. derived  $4k_{\text{aq}} = 9 \times 10^6 \text{ M}^{-2}\text{s}^{-1}$ , Wink et al. found that  $4k_{\text{aq}} = 6 \pm 1.5 \times 10^6 \text{ M}^{-2}\text{s}^{-1}$  and Awad and Stanbury obtained that  $4k_{\text{aq}} = 8 \times 10^6 \text{ M}^{-2}\text{s}^{-1}$ . Assuming that the concentration of oxygen is essentially constant at 210,000 ppm, the rate expressions can

be used to estimate the amount of NO that might be removed abiotically for a given gas-phase EBCT and liquid hold-up time in the columns. Suffice it to say that the gas and aqueous rate constants lead to about the same order of magnitude of conversion of NO for a given concentration, and that both are pseudo-second order in NO concentration.

A plot of the amount NO expected to be removed by gas-phase oxidation alone as a function of [NO] is shown in the figure 9. Clearly, at concentrations above about 100 ppm, the abiotic reactions can become significant. These new insights call into question the conclusions of the primary study by Chou and Lin (2000) upon which the current study was undertaken. Chou and Lin reported using NO concentrations as large as 1000 ppm with a 3-minute EBCT. It is evident from the kinetic expressions, that a large portion of the removal of NO observed in their system was likely the result of abiotic reaction.



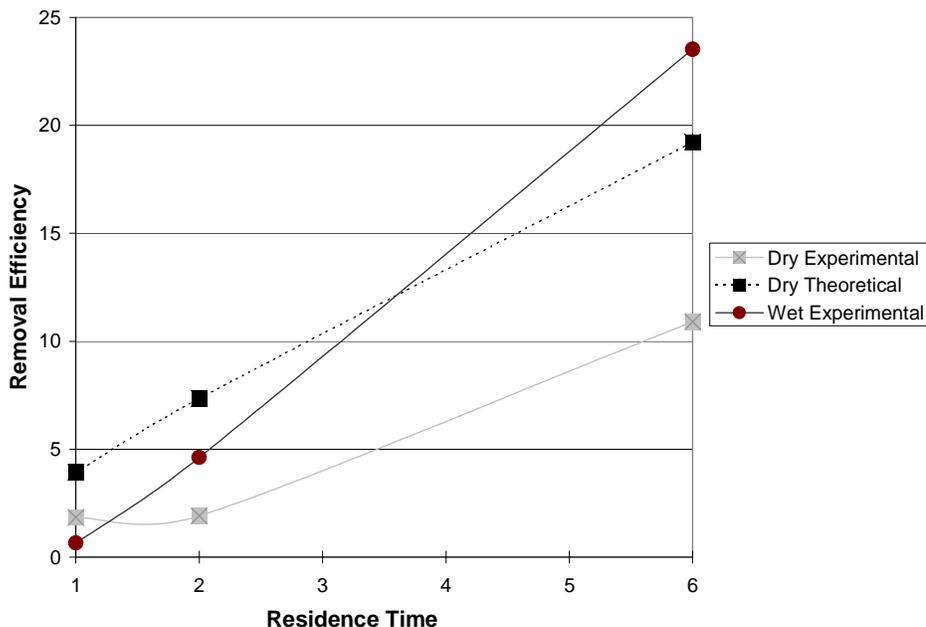
**Figure 9. Calculated abiotic, gas-phase thermal reaction removal efficiency based upon equation 2 at differing EBCT.**

In order to verify that the magnitude of the rate expressions in the literature are correct and applicable, the biofilter columns were allowed to dry out to inactivate the biofilm and any microbial degradation activity. Dry and wet abiotic removal measurements were then conducted. An example of the result for 150 ppm NO and various residence times is shown in the figure 10. Clearly, the order of magnitude of the abiotic dry oxidation rate is correct and the abiotic wet oxidation reactions roughly double the rate of removal.

### **Implications abiotic NO reactions**

The major goal of the current study was to determine whether heterotrophic organisms enhance the removal of NO in aerobic biofilters as reported in the study by Chou and Lin (2000). The lack of enhanced removal of nitrite or NO in the batch flask experiments suggests that

heterotrophic organisms do not increase the rate of NO removal. We believe that the high removal efficiencies observed in the Chou and Lin study can be explained by abiotic gas phase and aqueous phase reactions of NO coupled with the removal of NO by autotrophic bacteria. The sum of the abiotic removals and the autotrophic removal efficiency observed in our column studies roughly give the same range of removal efficiencies reported by Chou and Lin.



**Figure 9. Calculated abiotic, gas-phase thermal reaction removal efficiency compared with experimental column results under wet and dry conditions.**

The exact reason for the high removal efficiencies observed when glucose was supplied through the aerosol phase in the current study was not determined. Clearly microorganisms that can utilize the added glucose for cell production were present in the system as evidenced by biomass accumulation and the fact that autotrophic organisms strictly utilize inorganic carbon as their carbon source. It is conceivable, though seemingly unlikely, that the presence of the heterotrophic organisms provided an environment that enhanced the population of autotrophs. Chou and Lin discussed that as one possibility for the performance they observed. An alternative hypothesis is provided by the many studies that have illustrated that by depleting oxygen, rapid nitric oxide reduction is possible [Baumgartner and Conrad, 1992; Apel and Turick, 1993; Apel and Barnes, 1995; duPlessis et al., 1998; Lee and Apel, 1999]. Thus alternative explanations are 1) that the delivery of glucose in aerosol form results in locally high glucose concentrations that leads to a local depletion of oxygen, making localized nitric oxide reduction possible, or 2) that the thicker biofilm resulting from additional heterotrophic microorganisms reduces oxygen transport, again leading to nitric oxide reduction or 3) that the incorporation of nitrate produced by the autotrophs into cellular material prevents lowering of pH. As noted above, the improved performance in the column study occurred even though bicarbonate buffer was not added. The direct dependence of NO removal on glucose

concentration in the nebulizer as shown in figure 7 suggests reductive removal of NO is the most likely explanation for the improved performance.

## **Summary**

Enhanced aerobic biodegradation of NO was not conclusively observed as a result of organic substrate addition. An alternate explanation for high removal efficiencies observed at EBCTs of a few minutes was determined to be that abiotic oxidation occurs rapidly in both the gas and aqueous phases when the NO concentration exceeds about 100 ppm<sub>v</sub>. The explanations provided by the authors of the earlier study appear to be unsubstantiated. Although enhanced removal of NO was observed when glucose was added in the present study, the weight of evidence suggests that NO removal was most likely a result of reduction rather than oxidation to nitrate. As a practical matter, the ratio of carbon consumed to nitrogen removed for high removal of NO in the present study is greater than one order of magnitude. That range of ratio is consistent with other studies of NO reduction by bacteria and fungi under overall aerobic conditions.

## **Acknowledgments**

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