

## Bioprocesses for the removal of nitrogen oxides from polluted air

Yaomin Jin, María C Veiga, Christian Kennes

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### Abstract

Nitrogen oxides (NO<sub>x</sub>) of environmental concern are nitrogen monoxide (NO) and nitrogen dioxide (NO<sub>2</sub>). They are hazardous air pollutants that lead to the formation of acid rain and tropospheric ozone. Both pollutants are usually present simultaneously and are, therefore, called NO<sub>x</sub>. Another compound is N<sub>2</sub>O which is found in the stratosphere where it plays a role in the greenhouse effect. Concern for environmental and health issues coupled with stringent NO<sub>x</sub> emission standards generates a need for the development of efficient low-cost NO<sub>x</sub> abatement technologies. Under such circumstances, it becomes mandatory for each NO<sub>x</sub>-emitting industry or facility to opt for proper NO<sub>x</sub> control measures. Several techniques are available to control NO<sub>x</sub> emissions: selective catalytic reduction (SCR), selective non-catalytic reduction (SNCR), adsorption, scrubbing, and biological methods. Each process offers specific advantages and limitations. Since bioprocesses present many advantages over conventional technologies for flue gas cleaning, a lot of interest has recently been shown for these processes. This article reviews the major characteristics of conventional non-biological technologies and recent advances in the biological removal of NO<sub>x</sub> from flue gases based on the catalytic activity of either eucaryotes or procaryotes, ie nitrification, denitrification, the use of microalgae, and a combined physicochemical and biological process (BioDeNO<sub>x</sub>). Relatively uncomplicated design and simple operation and maintenance requirements make biological removal a good option for the control of NO<sub>x</sub> emissions in stationary sources

### Keywords:

Air pollution; nitrogen oxides (NO<sub>x</sub>); BioDeNO<sub>x</sub>; nitrification; denitrification; microalgae

## INTRODUCTION

Anthropogenic emissions of nitrogen oxides (NO<sub>x</sub>) present various negative effects on ecosystems and human health, such as acidification, eutrophication, increase of ground-level ozone, contribution to the formation of particulate matter, and loss of biodiversity.<sup>1</sup> Global emissions of NO<sub>x</sub> have followed an almost exponential increase over time, although such increase has started to slow down over the past decade. In Europe the release of nitrogen oxides has even declined recently. However, the growing economies of some regions, especially in southeast Asia, are likely to overwhelm any reductions in NO<sub>x</sub> emissions that are made in Europe and North America.<sup>2</sup> The rapid economic growth and the increasing consumption of fossil fuel energy results in the emission of large amounts of NO<sub>x</sub> to the atmosphere. Approximately 24 million tons of NO were released to the atmosphere from US sources during 1998.<sup>3</sup> Titles I and IV of the 1990 Clean Air Act Amendments regulate NO<sub>x</sub> emissions from major stationary sources.<sup>4</sup> The overall goal of these programs was to achieve NO<sub>x</sub> reductions of 2 million tons per year below 1980 levels by the year 2000.

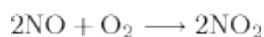
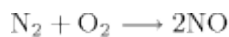
Concern for environmental and health issues coupled to stringent NO<sub>x</sub> emission standards indicate a need for the development of efficient low-cost NO<sub>x</sub> abatement technologies. Conventional post-combustion controls include selective catalytic reduction (SCR),<sup>4</sup> selective non-catalytic reduction (SNCR),<sup>5, 6</sup> adsorption,<sup>7, 8</sup> and scrubbing (absorption).<sup>9, 10</sup> The major drawback of conventional systems is the prohibitive cost for treating large volumes of air containing low to moderate concentrations of NO<sub>x</sub>. In addition, conventional systems generate secondary wastes, often requiring further treatment. The biological removal of NO<sub>x</sub> from contaminated gas streams is an emerging technology that can be applied to air pollution control in stationary sources. Other new technologies, such as pulse corona introduced plasma<sup>11</sup> and pressure swing adsorption (PSA),<sup>12</sup> are efficient and more cost-effective than conventional techniques for the removal of higher NO<sub>x</sub> concentrations, but are still expensive when treating large volumes of gas. Biological processes are alternative cost-effective treatment methods. The operation principle is relatively simple and consists generally of passing a contaminated air stream through a porous packed bed on which pollutant-degrading cultures are immobilized. Under optimal conditions, the pollutants can be converted biologically into benign end products, such as water, carbon dioxide, NO<sub>3</sub><sup>-</sup>, N<sub>2</sub> and new biomass.<sup>13–16</sup>

### Sources of NO<sub>x</sub>

Nitrogen oxides are released from both natural and anthropogenic sources, as is also often the case for many other different groups of volatile pollutants.<sup>17</sup> The production of NO<sub>x</sub> is in fact more significant in natural sources than in anthropogenic sources, mainly as the result of the activity of nitrogen-consuming microorganisms in soil. Besides, natural NO<sub>x</sub> generation has recently increased due to the larger worldwide use of fertilizers over the present and recent past decades.

The anthropogenic sources of NO<sub>x</sub> are motor vehicles (49%), electric utilities (27%), other industrial, commercial and residential sources (19%), and all other activities (5%) that burn fuels.<sup>3</sup> This shows that anthropogenic sources are generally linked to combustion processes used in either mobile sources or stationary sources. Nitrogen (N<sub>2</sub>) is the most abundant molecule in air, representing 79% by volume. Therefore, NO<sub>x</sub>

appear in exhaust gases from most motor vehicles due to their formation during the combustion of molecular nitrogen from air at high temperatures. NO<sub>x</sub> are also generated as a result of the combustion of different forms of nitrogen compounds in fossil fuels. During the oxidation of nitrogen gas, nitrogen monoxide is formed first, to be converted subsequently to nitrogen dioxide in the presence of either oxygen or ozone:



Actually, these are equilibrium reactions in which the concentrations of the products (NO<sub>x</sub>) will increase whenever the temperature, the oxygen concentration and/or the residence time in the combustion chamber are increased. Nevertheless, if reducing one or several of these three parameters slows down the formation of nitrogen oxides, it sometimes also reduces the combustion efficiency. Moreover, the incomplete combustion of fuel may result in the formation of other undesirable pollutants.

Combustion gas streams typically contain different amounts of oxygen and fluctuate in temperature depending on the post-combustion treatment. Industrial treatment technologies designed for NO<sub>x</sub> removal from flue gases must be capable of operating under these variable conditions. For example, the flue gas discharged from fuel oil-fired power plants is estimated to contain typically 13% (v/v) CO<sub>2</sub>, 2% (v/v) O<sub>2</sub>, 500 ppm (v/v) SO<sub>x</sub>, 100–300 ppm (v/v) NO<sub>x</sub>, and N<sub>2</sub>.

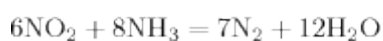
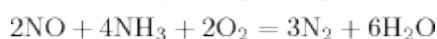
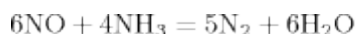
Nitrogen oxides as NO<sub>2</sub> are also found in some industrial effluents generated during production processes, as in the case of nitric acid plants, but the environmental impact is far less important than for other NO<sub>x</sub>.

## CONVENTIONAL NON-BIOLOGICAL PROCESSES

The most attractive alternative to limit pollution problems would be to reduce NO<sub>x</sub> emissions instead of applying specific treatment processes. However, this is not always feasible nor advantageous. For example, lowering the amount of excess air used in combustion processes will reduce the concentration of NO<sub>x</sub> in the waste gas. Nevertheless, it will also affect the combustion efficiency. Since lowering emission levels during combustion is not always easy, treatment technologies often need to be applied. The most important treatment technologies are described hereafter, focusing mainly on the innovative biological processes.

### Selective catalytic reduction (SCR)

In selective catalytic reduction, a reactant is added to reduce NO to molecular nitrogen. It is usually based on the following reactions between ammonia and NO<sub>x</sub> at high temperatures.<sup>4</sup>

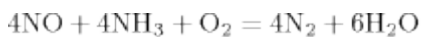


Basically the same reactions occur during selective non-catalytic reduction (SNCR) described in the next section. The difference is that the catalysts, particularly noble

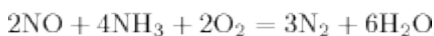
metal-exchanged zeolites and copper ion-exchanged zeolites, used exclusively in selective catalytic reduction, allow the reaction to proceed at lower temperatures. Different temperatures have been tested in the SCR process, but at lower temperatures, higher amounts of catalysts need to be used. Therefore, reducing the temperature too much may not be cost-effective. However, too high a temperature cannot be applied either since part of the oxygen would be used to oxidize  $\text{NH}_3$  to  $\text{NO}$ , resulting in reduced  $\text{NO}_x$  removal. Typically, temperatures between 500 and 750 K are applied. The major advantage of the catalytic process (SCR) compared with the non-catalytic one (SNCR) is that lower temperatures can be applied while reaching similar treatment efficiencies, thus lowering the operation's costs. However, the use of a, sometimes relatively expensive, catalyst contributes to increased investment costs. SCR is the most widely applied post-combustion  $\text{NO}_x$  control method, but it has two significant problems, which are catalyst deactivation and the presence of unreacted ammonia in the flue gas that can reach 10 ppm.

### Selective non-catalytic reduction (SNCR)

The selective non-catalytic reduction process<sup>5, 6</sup> involves the injection of a nitrogen-containing compound such as ammonia ( $\text{NH}_3$ ) or urea ( $\text{CO}(\text{NH}_2)_2$ ) in a region where the gas temperature is in the range of 1140–1420 K. The temperature window depends upon whether ammonia or urea is used. In this case,  $\text{NO}_x$  are reduced thermally and the reactions take place without the addition of any catalyst. Over this temperature range, ammonia or urea is ionized and reacts with  $\text{NO}_x$  in the presence of oxygen to form molecular nitrogen, carbon dioxide, and water. The equations for the reactions of ammonia with  $\text{NO}_x$  are:



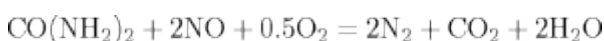
$$(\text{NH}_3/\text{NO} = 1/1)$$



$$(\text{NH}_3/\text{NO} = 2/1)$$

For temperatures between approximately 1140 and 1420 K, the first equation dominates. The higher the temperature, the larger the contribution of the second equation.

The equation for the reaction of urea is:



If the reaction temperature is too low, eg below 1000 K, part of the ammonium will not react and it will appear in the exhaust gas.

### Adsorption

Several decades ago, commercial zeolites (molecular sieves) were found to efficiently remove low concentrations of nitrogen oxides from gas streams.<sup>18</sup> The zeolite could effectively adsorb  $\text{NO}_x$  from waste gas streams in a nitric oxide plant. Adsorbed  $\text{NO}_x$  can be recovered as enriched  $\text{NO}_x$  and  $\text{HNO}_3$  by regenerating the bed at elevated temperatures with hot air and/or steam. Tests indicated that such a system could be

incorporated into an existing nitric acid plant, preventing the release of significant quantities of NO<sub>x</sub> into the atmosphere.

More recently, Wang *et al*<sup>7</sup> investigated the adsorption properties of NO on alumina-supported palladium by simultaneous thermogravimetric-analysis and differential scanning calorimetry (TGA–DSC) in a temperature range of 220–470 K.

With other common adsorbents, such as activated carbon, NO in contaminated waste gases is converted to NO<sub>2</sub>. Guo *et al*<sup>8</sup> investigated the removal of NO<sub>x</sub> by the catalytic oxidation of NO to NO<sub>2</sub> on activated carbons at 30 °C. The results showed that the NO conversion markedly decreases with the rise of gas temperature and with moisture content. To be efficient, this process requires complete de-moisturing of the flue gas prior to the reaction. The CHA process (CHA Corporation, CA, USA) is another treatment system, based on the use of carbon, that removes oxides of nitrogen from small stationary diesel engines. The diesel exhaust is adsorbed onto a moving carbon adsorbent bed. Subsequently, microwaves are utilized to regenerate the carbon adsorbent and reduce the NO<sub>x</sub>.<sup>19</sup>

## Scrubbing

A lot of research still needs to be done in order to understand NO removal in wet scrubbers. In general, additives have to be added to the scrubbing system to convert insoluble NO to soluble NO<sub>x</sub> or to form a complex, which can be removed. Possible NO oxidants are ClO<sub>2</sub> or O<sub>3</sub>.<sup>10</sup> However, these additives are quite expensive as well as very dangerous in equipment operating in the gas phase. Therefore, chemical reagents added to the liquid phase have been widely used.<sup>9</sup> Kobayashi *et al*<sup>20</sup> investigated the removal of nitrogen oxides using a number of inorganic and organic reagents. Their results showed that strong oxidizing reagents such as KMnO<sub>4</sub> and NaClO<sub>2</sub> removed NO efficiently. Chu *et al*<sup>10</sup> used NaClO<sub>2</sub>/NaOH to perform a preliminary test on a semi-continuous spraying sieve tray combined SO<sub>2</sub>/NO removal system and a continuous spraying sieve tray column. The results showed that NaClO<sub>2</sub>/NaOH worked well for the combined SO<sub>2</sub>/NO removal system.

Aqueous solutions of iron chelates have been widely employed to catalyze the absorption of NO<sub>x</sub>. Demmink and co-workers<sup>21</sup> used solutions of ferrous chelates of nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), hydroxyethylenediaminetriacetic acid (HEDTA), and diethylenetriaminepentaacetic acid (DTPA) to absorb the nitric oxide in a stirred cell reactor. They found that the absorption leads to the formation of stable ferrous NO chelates and that the mass transfer rate greatly affects the absorption rate.

Although these post-combustion controls offer sufficient treatment of nitrogen oxides, the cost is high and products requiring further disposal are often generated.

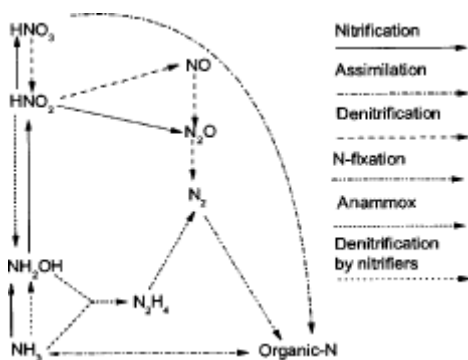
## RECENT BIOLOGICAL TECHNOLOGIES FOR NO<sub>x</sub> REMOVAL

There are many different bioprocesses, developed recently, available to control NO<sub>x</sub> emissions. The major technologies can be classified into different groups: (i) nitrification; (ii) denitrification; (iii) microalgae; (iv) BioDeNO<sub>x</sub>.

## Nitrification

Nitrification is the process by which ammonium is oxidized to nitrate, as indicated in Fig 1. This process is carried out by two distinct groups of bacteria: the ammonium oxidizers, which oxidize ammonium to nitrite, and the nitrite oxidizers, which oxidize nitrite to nitrate. Ammonium oxidizers may produce nitric oxide during oxidation of ammonium under particular growth conditions.<sup>22–25</sup> Three distinct classes of ammonium oxidizers can be distinguished, as indicated below:

- (1) The lithoautotrophic ammonium oxidizers are all Gram-negative bacteria which transduce the free energy necessary for their metabolism from the oxidation of ammonia to nitrite and obtain the majority of carbon for growth by assimilation of carbon dioxide ( $\text{CO}_2$ ).
- (2) In heterotrophic nitrification, organic and inorganic nitrogen compounds such as ammonia ( $\text{NH}_3$ ), and organic nitro-compounds are oxidized to a variety of organic and inorganic nitrogen compounds such as nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), hydroxylamine ( $\text{NH}_2\text{OH}$ ) and trihydroxamic acids. Heterotrophic nitrification only proceeds when an external organic carbon and energy source is available.
- (3) A third class of nitrifiers includes the anaerobic ammonium oxidizers. They use the so-called ‘anammox’ process which is the oxidation of ammonium coupled to the reduction of nitrite, yielding molecular  $\text{N}_2$ . It has been shown that this is a biological process although the exact biochemical mechanisms have not been elucidated yet. The dominant organism in the anammox community is a Planctomycete.



**Figure 1.**

Simplified nitrogen cycle (reproduced and modified with permission from the copyright holders, IWA).<sup>24</sup> Ammonification and nitrification are processes of ‘mineralization’. Uptake of ammonia and reductive uptake of nitrate by bacteria are known as ‘immobilization’, as N is incorporated into bacterial biomass. Denitrification takes place in low oxygen environments, where  $\text{NO}_3^-$  is used as the electron acceptor (oxidant) for the respiration of organic matter. Nitrogen fixation is the process of ‘fixing’ nitrogen from the atmosphere.

Some promise has been demonstrated in the use of autotrophic nitrifiers to degrade  $\text{NO}_x$ ,<sup>26–31</sup> although most studies have been undertaken at room temperature (Table 1) while thermophilic conditions would be more suitable for the full-scale treatment of most  $\text{NO}_x$ -containing waste gases. Chemoautotrophic organisms are capable of assimilating  $\text{CO}_2$  as carbon source and obtain energy from inorganic substrates.  $\text{NO}$  is

an intermediate metabolite in the nitrification steps that has been shown to produce 200 times more NADH (reduced-nicotinamide adenine dinucleotide) when utilized in *Nitrobacter* organisms than  $\text{NO}_2^-$ . NADH production is directly related to the production of ATP, the cellular energy storage molecule, therefore more energy is available for organisms that produce increased quantities of NADH.<sup>30</sup>

Table 1. Comparison of the different biological NO<sub>x</sub> removal methods

Method	Packing	T <sup>a</sup> (°C)	O <sub>2</sub> (%)	NO conc (ppm)	EBRT (min)	Load (g NO m <sup>-3</sup> h <sup>-1</sup> )	RE (%)	Ref
Nitrification	HFMB	20–55	5	100	1.9 s(5)a	1.48	69–75	37
Nitrification	Celite, pumice	Room	Air	100	1	7.4	10–15	29
Nitrification	Slag	Room	Air	892–1237	2	2.75–22.4	80	31
Nitrification	Bio soil	20–25	Air	1.6	1	0.12	60	35
Denitrification	Compost	30–45	Air	250	1	18	99	51
Denitrification	Compost	22, 37	—	500	1	37	90	53
Denitrification	Compost	53–55	—	500	1.3	28	95	61
Denitrification	Perlite	53–55	—	500	1.18	31	94	61
Denitrification	Biofoam	53–55	—	500	1.18	31	85.5	61
Denitrification	Sulfur, maerlb	Room	—	8978	22	4.15 mmol h <sup>-1</sup>	10	62
Denitrification	Silicate pellets	23	> 17%	60	3	0.75	75	59
Denitrification	Ceramic matter	60	Air	5	2.4	0.15	60–90	63
Fungi, denitrification	Silicate pellets	23	21	250	1	18.4	93	42
Microalgae		25	—	300	26.7	22.3 mg day <sup>-1</sup>	50–60	72
BioDeNO <sub>x</sub> , denitrification	None	50–55	Air	250			60	76

- A The gas residence time, calculated as the membrane volume divided by the gas flow rate, was 1.9 s. The overall residence time, calculated as the reactor volume divided by the gas flow rate, was 5.0 min.
- B Mixture of 60% sulfur and 40% maerl in volume.

RE: removal efficiency; —: anaerobic.

Davidova *et al*<sup>26</sup> were the first to demonstrate the potential of nitrifying bacteria for NO removal in a gas-phase biofilter packed with porous glass rings. They conducted studies using autotrophic bacteria grown on ammonium in an oxic gas stream. Degradation in that system was rate-limited, requiring long residence times of around 13.7 min to remove 90% of NO from a 100 ppm contaminant stream. Examination of the media revealed that the biofilm's appearance was rather sparse and thin. The presence of insufficient biomass for effective bio-oxidation was considered as the cause of the rather inefficient removal. The slow growth of autotrophic bacteria is attributed to the fact that approximately 80% of the energy generated by autotrophs is used to fix carbon dioxide and the amount of energy gained from the oxidation of ammonia to nitrate is small.

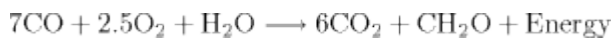
The above work was further extended using nitrite as a substrate to initially develop and enhance growth of the biofilm.<sup>27–29</sup> Trickling biofilters were used to remove NO from contaminated air with different packing materials such as natural red lava rock (1/4 inch pumice), pellets of diatomaceous earth (Celite<sup>®</sup> R-635) and a very high interfacial area artificial sintered glass (Sirian rings) medium. The measurement of NO<sub>3</sub><sup>-</sup> concentrations in the liquid phase showed that the nitrate levels slowly increased over time and that the recovery of NO as NO<sub>3</sub><sup>-</sup> exceeded 90%. This definitely confirms that the removal mechanism is nitrification. The systems operated at an EBRT (empty bed residence time) of 1 min exhibited a limited removal efficiency (10–15%), attributed to mass transfer limitation. However, reduction of the liquid film thickness temporarily yielded up to 100% improvements in removal efficiency as the aqueous medium drained. The performance did also improve whenever an ultrasonic nebulizer was used to maintain column moisture in lieu of a conventional spray nozzle. The estimated residence time for 90% removal was reduced from 13.7 min to 6 min.<sup>26</sup> Further studies demonstrated that the high EBRT of 6 min was due to mass-transfer limitation of the poorly soluble nitric oxide and oxygen. Little difference in performance was observed between the Celite and lava rock packings despite the much larger specific surface area of Celite. Clearly the internal pore structure of Celite media was unavailable for effective NO removal because even a thin biofilm covering the openings of the surface pores drastically reduces diffusion of the poorly soluble NO into the interior. Thus, the key to economical biological treatment of NO is to maximize the surface-to-volume ratio that can support the necessary biofilm without clogging of the pores. In a subsequent work, a porous, pyrolyzed carbon-foam structure was used as packing allowing to greatly reduce the mass transfer limitation.<sup>30</sup> The pore dimensions could be selected such that the biofilm grew on all surfaces of the fibrous structure, but did not bridge across the openings.

Chou and Lin<sup>31</sup> employed an aerobic trickling biofilter using a blast furnace slag as packing material with particulate sizes of 20–40 mm and a specific surface area of 120 m<sup>2</sup> m<sup>-3</sup>. Approximately 6 weeks were required to develop a biofilm for NO degradation, using activated sludge as microbial seed, while glucose, yeast powder, phosphate, and NaHCO<sub>3</sub> were added as supplementary nutrients. Removal efficiencies of 80% with a 2 min residence time were demonstrated in the case of gas streams containing 892–1237 ppm NO. Glucose was added as a carbon source supplement to encourage the growth of heterotrophic microorganisms capable of secreting polysaccharides to enhance adhesion of the biofilm to the packing surface. When glucose addition was ceased for 2 weeks, NO removal declined to 48%. This organic carbon deficiency resulted in the detachment of part of the biofilm from the packing surface. Over 90% of the eliminated NO was converted into nitrate by conversion of the NO to nitrite followed by the nitrification of nitrite to nitrate, suggesting successful removal via nitrification.

In other studies, the biofiltration of NO<sub>x</sub>, consisting of a mixture of NO and NO<sub>2</sub>, was carried out using a laboratory-scale biofilter packed with soil.<sup>32–35</sup> The removal efficiency of NO<sub>2</sub> by soil was almost 100%, and the removal efficiency of NO was 60% on average, and 86% was the maximum value. By using gamma-irradiated soil as a packing material, NO<sub>2</sub> was completely removed mainly by adsorption onto or absorption into the packing material. However, the removal efficiency of NO in the sterilized soil was only 20%, suggesting that NO in soil was removed microbiologically under aerobic conditions.

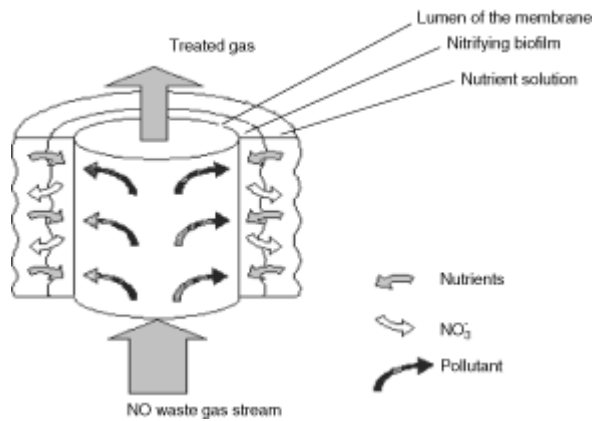


Another application of nitrifiers is in the cleaning of polluted air from road tunnels. Robra *et al*<sup>36</sup> isolated microorganisms from natural substratum and from air and dust of road tunnels. They enriched bacteria capable of eliminating low concentrations of CO, NO and NO<sub>2</sub> in a mineral medium in the absence of other nitrogen compounds in a laboratory-scale biotrickling filter packed with light expanded clay aggregates. During this process carboxidotrophic bacteria oxidize CO, and they release energy and CO<sub>2</sub> that serves as carbon source for the growth of autotrophic nitrifying bacteria according to the following reaction:



Later, they built a pilot plant to treat polluted air in a road tunnel at the Katschbergtunnel in Carinthia (Austria). The 55 dm<sup>3</sup> trickling filter exhibited good performance over 2 years of experimentation. At gas residence times ranging from 7.5 to 11 s, maximum removal efficiencies of 90% CO, 15–20% NO, 95% NO<sub>2</sub>, and 50–75% volatile organic compounds without methane were obtained.

More recently, the feasibility of using a hollow fiber membrane bioreactor (HFMB) as a means to remove NO from combustion gases was also evaluated.<sup>37</sup> A detailed description of the basic principles of operation of HFMB for air pollution control in general has been published recently.<sup>38</sup> A membrane bioreactor system may overcome many of the limitations of conventional compost biofilters. The HFMB process consists of bundles of microporous hydrophobic hollow fiber membranes, a biofilm containing nitrifying organisms and a nutrient supply. As shown in Fig 2, the synthetic combustion gas (15% CO<sub>2</sub>, 5% O<sub>2</sub>, 77% N<sub>2</sub>) containing NO passes through the lumen of the microporous hydrophobic hollow fiber membranes. The NO gas diffuses through the membrane pores and partitions into a nitrifying biofilm where it is oxidized to NO<sub>3</sub><sup>-</sup>. The hollow fiber membranes serve as a support for the microbial populations and provide a large surface area for NO and oxygen mass transfer. Activated sludge from a wastewater treatment plant was acclimated with an NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> nutrient solution over 2 months and was used to inoculate the HFMB. Over 90% of the nitrogen was converted to NO<sub>3</sub><sup>-</sup> by nitrification. The HFMB was initially fed 100 ppm NO at a gas flow rate of 100 cm<sup>3</sup> min<sup>-1</sup> for 1 month, and with a liquid recirculation rate of 300 cm<sup>3</sup> min<sup>-1</sup>. The NO removal rate was 12–14 g m<sup>-3</sup> day<sup>-1</sup>, corresponding to efficiencies between 34 and 40%. It increased to a maximum of 27 g m<sup>-3</sup> day<sup>-1</sup> with 74% removal efficiency at a recirculation rate of 600 cm<sup>3</sup> min<sup>-1</sup> at 20 °C. The NO elimination capacity of the synthetic combustion gas ranged between 24 and 26 g m<sup>-3</sup> day<sup>-1</sup> at 20 °C. When the temperature was gradually increased from 20 to 30, 40, 50, and 55 °C, the performance remained basically constant. The results showed that the gas composition and temperature have no influence on the reactor's performance (Table 1).



**Figure 2.**

Conceptual model of the HFMB for waste gas treatment.

## Denitrification

Microbial denitrification is a dissimilatory reductive process via enzyme-catalyzed reactions in which toxic nitrogen oxides are sequentially reduced to environmentally benign nitrogen gas<sup>39–42</sup> (Fig 1). A wide variety of organisms are known to denitrify, including some bacteria, fungi and simple eukaryotes.<sup>43</sup>

## Bacteria

Denitrifying bacteria are ubiquitous in nature and represent indigenous populations in composts and soils. They include a relatively wide variety of physiological and taxonomic groups. Denitrifiers have the ability to reduce oxides of nitrogen when oxygen levels become limited.<sup>44</sup> Denitrification is a dissimilatory reductive process that occurs according to the following simplified order:



Different denitrifying bacteria are thus able to use NO<sub>x</sub>. *Thiobacillus denitrificans*, a strict autotroph and facultative anaerobe, may be cultured anaerobically in aqueous phase batch reactors using NO as a terminal electron acceptor. Thiosulfate can serve as an energy source, CO<sub>2</sub> as a carbon source, and ammonium ions as a source of reduced nitrogen.<sup>45</sup> Up to 96% removal was observed with *Thiobacillus denitrificans* for a gas stream containing 5000 ppm NO. Related studies were performed to check the feasibility to simultaneously remove SO<sub>2</sub> and NO<sub>x</sub> from cooled flue gas by contact with cultures of the sulfate-reducing bacteria *Desulfovibrio desulfuricans*, converting SO<sub>2</sub> to H<sub>2</sub>S, and *Thiobacillus denitrificans*, transforming H<sub>2</sub>S to SO<sub>4</sub><sup>2-</sup> in cultures-in-series or cocultures in a single contacting stage.<sup>46</sup> However, the simultaneous combined SO<sub>2</sub>/NO<sub>x</sub> removal from flue gas was not technically feasible due to NO inhibition of SO<sub>2</sub> reduction in *D desulfuricans*, nitrate suppression, and oxygen inhibition.

In addition, the same research group found that two heterotrophic denitrifying bacteria, *Paracoccus denitrificans* and *Pseudomonas denitrificans*, utilize NO as a terminal electron acceptor, converting it into elemental nitrogen in the presence of succinate, yeast extract, and heat/alkali-pretreated municipal sewage sludge as carbon and energy

sources.<sup>40, 47</sup> Complete removal of 5000 ppm NO from a feed gas sparged into the liquid cultures was observed. All these results suggest that reduction of NO may be a common property of denitrifying bacteria. Potter and colleagues<sup>48, 49</sup> used Fourier transform infrared (FTIR) spectroscopy of vapor phase spectra in order to directly monitor the biomimetic reduction of NO to N<sub>2</sub>O by dithiothreitol in the presence of cyanocobalamin and cobalt-centered porphyrins. Reaction rates were two-fold faster for the cobalt corrin than for the cobalt-centered porphyrins. The stoichiometry showed loss of two NO molecules per molecule of N<sub>2</sub>O generated.

Denitrifiers are typically facultative organotrophs, which utilize organic carbon as an energy source.<sup>50</sup> Some authors have observed denitrification in compost-biofilters.<sup>51–55</sup> At low NO concentrations, the quantity of compost used in these studies provided adequate carbon and energy sources for removal and degradation. However, at higher NO concentrations, the amount of available carbon substrates became limited and the addition of an exogenous carbon source was required to maintain efficient NO reduction. As high as 90% removal efficiency was observed for a 500 ppm NO gas stream at an EBRT of 1.3 min when treated in the presence of a phosphate buffer containing either lactate or dextrose compared with 20% removal in a non-supplemented biofilter.<sup>53, 56–58</sup> Lactate, glucose, dextrose and molasses, as well as labile organic material inherent in compost, have all been used as carbon sources in NO denitrifying systems. Several volatile organic compounds can also be used as carbon and energy sources, and denitrification activity has been observed in a superficially aerobic toluene-treating biofilter.<sup>59</sup> In such a system, the presence of a thick biofilm creating anaerobic underlayers allowed denitrification to nitrogen gas. Removal efficiencies of 75% of 60 ppm NO streams were reported for an EBRT of 6 min. Also, the removal of NO from a simulated wet-scrubbed combustion gas was investigated with different packing materials (compost, perlite and biofoam). The results showed that all three packing materials performed well, reaching more than 85% NO removal at EBRT of 70–80 s. The compost performed better than the other packings at shorter EBRT of 13–45 s. However, inert carrier materials such as perlite and biofoam are known to offer long term stability and reduced back-pressure compared with organic filter beds as compost.<sup>60</sup> The experimental data suggest that the compost, perlite and biofoam systems, subject to further optimization, offer potential for the biological removal of NO<sub>x</sub> from gas streams.<sup>61</sup>

Another system that has been investigated is based on the pre-concentration of low concentrations of NO at a high volumic flow onto activated carbon (Pica NC 60), followed by the thermal desorption of NO<sub>x</sub> and a biological denitrification treatment of the thermally desorbed gas.<sup>62</sup> The NO<sub>x</sub> flow can be purified by *T. denitrificans* grown on a sulfur–maerl support with a superficial gas velocity of 0.5 m h<sup>-1</sup>. The NO<sub>x</sub> inlet concentration was 10 000–20 000 mg m<sup>-3</sup> and the EBRT was 22 min.

Most of the research studies published on biological NO<sub>x</sub> removal and described above were performed under mesophilic conditions, which may not be cost-effective at full-scale since NO<sub>x</sub>-polluted air is often released at rather high temperatures. Lebedeva and co-workers<sup>63</sup> isolated 82 strains of thermophilic NO-consuming bacteria in sediment samples from hot springs in the Kronot reserve (Kamchatka), corroded matter from steel pipes of the heating system in Moscow, and soils of the southern bioclimatic belt in Russia (chestnut, white alkali soils). Three of them, belonging to the genus *Bacillus* and growing at 60 °C were chosen for further investigation. Two of these organisms were

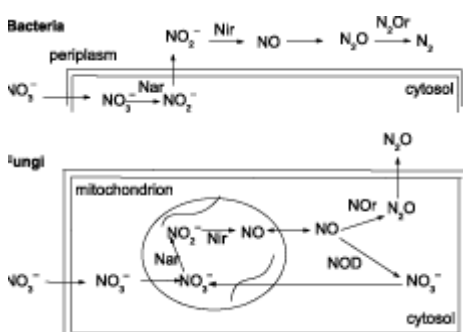
isolated from the municipal heating system, and the other one, the most active strain, came from the precipitate of a thermal spring. The latter was identified as *Bacillus stearothermophilus* strain INMI 50. Cells of this strain immobilized on a ceramic support demonstrated a high NO uptake in a 4 dm<sup>3</sup> bioreactor. Around 60–90% of NO was removed at 60 °C over 6 months of continuous bioreactor operation with inlet concentrations of 5 ppm and a gas flow rate of 100 dm<sup>3</sup> h<sup>-1</sup> (Table 1). Either glycerol or 1,2-propanediol was used as carbon and energy source.

## Fungi

Studies with filamentous fungi, investigating their capacity to degrade volatile organic and inorganic pollutants and their application to air pollution control in gas-phase bioreactors, are quite recent areas of research.<sup>64</sup> For over a century, denitrification and ammonification had been considered as processes exclusively found in prokaryotes, until denitrification was observed in the filamentous fungus *Fusarium oxysporum*<sup>65</sup> and in other fungi<sup>66</sup> which were long thought to be strictly aerobic.

Many fungi related to the genus *Fusarium* and its teleomorphs were shown to be capable of reducing nitrite anaerobically and to form NO, N<sub>2</sub>O, and/or N<sub>2</sub>. Several strains could reduce nitrate as well. N<sub>2</sub>O was the major product of the reduction of nitrate or nitrite. Several fungi could also form nitrogen. When radiolabeled nitrite was used as substrate for the N<sub>2</sub>-forming denitrification, <sup>15</sup>N<sub>2</sub>O, <sup>15</sup>NO, and <sup>14</sup>N<sup>15</sup>N were obtained as the products. These results demonstrated that many fungi have denitrifying abilities. Fungal systems maybe an attractive alternative to conventional treatment processes for the degradation of nitrogen compounds.<sup>66, 67</sup>

Denitrification is catalyzed by distinct enzyme species: NO<sub>3</sub><sup>-</sup> reductase (Nar), NO<sub>2</sub><sup>-</sup> reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (N<sub>2</sub>Or) and involves NO<sub>2</sub><sup>-</sup>, NO, and N<sub>2</sub>O as intermediate compounds (Fig 3). Denitrification is part of the bioenergetic metabolism of microbial cells in which these nitrogen oxides serve as terminal electron acceptors for the respiratory chain instead of oxygen.



**Figure 3.**

Comparison of the denitrifying systems of bacteria and fungi. Nar, Nitrate reductase; Nir, nitrite reductase; Nor, nitric oxide reductase; N<sub>2</sub>Or, nitrous oxide reductase.

The enzymes responsible for fungal denitrification have been isolated in *Fusarium oxysporum*. The unique nitrate/nitrite-inducible cytochrome P-450 (P-450<sub>dNIR</sub>) was discovered. It was expected that the fungus would be capable of metabolizing nitrate

dissimilatively. The end product of denitrification by *F oxysporum* is N<sub>2</sub>O. This indicates that the organism lacks an N<sub>2</sub>O reductase. Comparison of the cell growth during denitrification indicated that the dissimilatory reduction of nitrate to nitrite is an energetically favorable process in *F oxysporum*. However, further reduction of nitrite to N<sub>2</sub>O might be energy-exhausting and may function as a detoxification mechanism. This is unlike bacterial systems, which use a membrane-bound electron transport chain. Studies using *Fusarium solani*, *Cylindrocarpon tonkinse*, and *Chaetomium* sp have reported nitrogen as the end product of denitrification, but the enzymes responsible for N<sub>2</sub>O respiration still need to be determined.<sup>65, 68</sup>

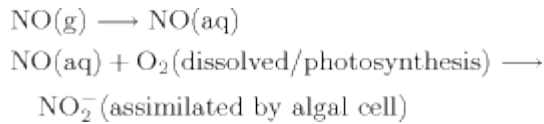
Fungi such as *Aspergillus* sp *Alternaria* sp, *F solani*, *Fusarium verticillioides*, *Fusarium dimerum*, and *Fusarium javanicum* have proved also to exhibit denitrifying abilities in aerobic environments. Denitrification in *F oxysporum* is an oxygen-inhibited process. The effects of oxygen on fungal denitrification are still under investigation.<sup>43</sup>

A toluene-degrading fungal bioreactor containing the black, dimorphic fungus *Exophiala lecanii-corni* was proven to aerobically remove NO from a contaminated gas stream, using toluene as a sole carbon and energy source.<sup>42</sup> The fungal bioreactor removed 93% NO at an inlet concentration of 250 ppm and an EBRT of 1 min, corresponding to an inlet loading rate of 17.22 g-NO m<sup>-3</sup> h<sup>-1</sup> when simultaneously supplied with 90 g-toluene m<sup>-3</sup> h<sup>-1</sup>. Also, it was observed that the removal of NO may be inhibited by the presence of high NH<sub>4</sub><sup>+</sup> concentrations in the fungal biofilm.

## Microalgae

A marine microalga, strain NOA-113, cultivated in a long tubular photobioreactor was found to simultaneously eliminate nitric oxide (NO) and carbon dioxide (CO<sub>2</sub>) from a model flue gas.<sup>69</sup> About 40 mg of NO and 3.5 g of CO<sub>2</sub> were eliminated per day in a 4 dm<sup>3</sup> reactor column with aeration of 300 ppm (v/v) NO and 15% (v/v) CO<sub>2</sub> in N<sub>2</sub> at a rate of 150 cm<sup>3</sup> min<sup>-1</sup>. The researchers used this reactor system to evaluate NO elimination by microalgae and to investigate the effects of the NO concentration, gas flow rate, and light conditions on NO elimination.

In order to thoroughly understand the process of NO removal from flue gases by an algal culture, a bioreactor system using the unicellular microalga *Dunaliella tertiolecta* was designed.<sup>70</sup> When nitric oxide, the main component of NO<sub>x</sub> in the flue gas, was supplied at concentrations ranging from 25 to 500 ppm to the algal culture in a 2 m column-bioreactor in the light, about 65% of the NO was removed. Under these conditions, cell growth was not affected by the concentration of the NO supplied, and about 1.6% O<sub>2</sub> was constantly evolved by photosynthesis. About 30% of the NO was removed in a solution without cells, at 2% O<sub>2</sub>, in which case the NO was probably photochemically oxidized by Fe<sup>3+</sup> present in the medium. However, in cell cultures without Fe<sup>3+</sup>, 65% NO removal was achieved. In the dark, on the other hand, the rate of NO removal was governed by the amount of oxygen supplied in the inlet gas, ie the obtention of an NO removal rate similar to that achieved in the light required the presence of oxygen at 2% or more, and NO removal did not occur without the supply of oxygen. It was found that the presence of both algal cells and oxygen is important in the reactor system. The following hypothesis regarding NO removal kinetics was proposed: NO in the gas is first dissolved in the aqueous phase, after which it is oxidized and assimilated by the algal cells as illustrated through the following reactions.



The results of investigations under various culture conditions indicate that the dissolution of NO in the aqueous phase is the rate-limiting step in this reactor system.

Also, the same research group used the green alga *Dunaliella tertiolecta* cultivated in a bubble column and airlift reactors to remove NO.<sup>71</sup> The gas–liquid contact area was increased by reducing the bubble size and thereby ensuring a higher NO dissolution rate. The results showed that NO removal was enhanced by increasing the dissolution of NO in water. In addition, the dissolved oxygen can also improve the NO removal. The highest level of NO removal, 96%, was achieved with a countercurrent-flow type airlift reactor aerated with small bubbles.

The uptake pathway of NO was investigated in a bubble column-type bioreactor.<sup>72</sup> It was found that little NO was oxidized in the medium before its uptake by algal cells and that NO mostly permeated directly into the cells by diffusion, based on the mass balance calculations for nitrogen and on the change in nitrate and nitrite concentrations in the medium in batch culture. NO taken up in the algal cells was then preferentially utilized as a nitrogen source for cell growth rather than nitrate, which was one of the nitrogen sources contained in the algal medium. The contents of total lipids, starch, and protein in the cells cultivated with NO were similar to those in cells cultivated without NO. Therefore, this algal system is useful for the continuous NO removal and production of algal biomass using NO as a nitrogen source. It is thought that a part of the nitrogen contained in the proteins originates from NO. Continuous NO removal by the *D tertiolecta* culture was possible under stable conditions for over 15 days in the presence of light.

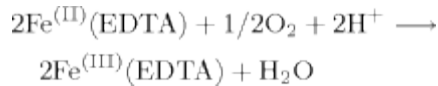
### BioDeNOx

BioDeNOx is an integrated physicochemical and biological NOx removal technique, as indicated in Fig 4. A chelating agent, Fe<sup>(II)</sup>(EDTA), is used to improve the NOx absorption into the scrubbing liquid, thereby obtaining high removal efficiencies.<sup>73, 74</sup> The NOx in the scrubbing liquid is subsequently converted into harmless nitrogen using denitrifying bacteria, with ethanol as electron donor. At the same time, the Fe<sup>(II)</sup>(EDTA) solution is regenerated.<sup>75–77</sup> Using this method, removal efficiencies of more than 80% can be achieved in such a bioscrubber. The BioDeNOx process is based on the following four reactions:<sup>76</sup>

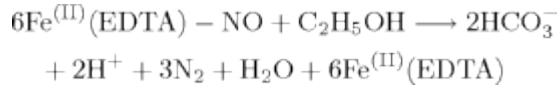
- (1) Wet absorption and complexation of NOx by Fe<sup>(II)</sup>(EDTA). The reaction rate is very fast, which enhances the mass transfer rate of NOx.



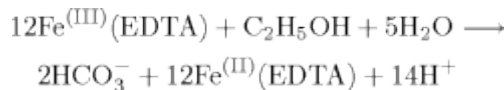
- (2) Oxidation of Fe<sup>(II)</sup>(EDTA) to Fe<sup>(III)</sup>(EDTA) by oxygen in the flue gas. This reaction should not occur as it consumes the Fe<sup>(II)</sup>(EDTA) which is needed for NO absorption.



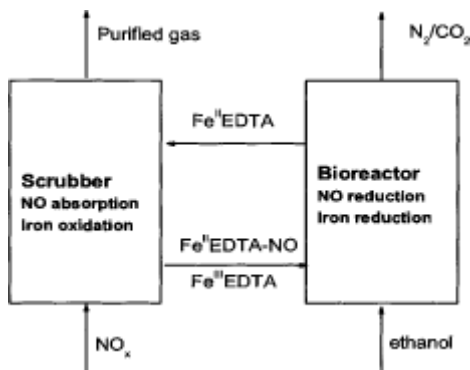
- (3) Biological reduction of NO<sub>x</sub> to N<sub>2</sub> by denitrifying bacteria, with ethanol as electron donor.



- (4) Regeneration of Fe<sup>(II)</sup>(EDTA) by reduction of Fe<sup>(III)</sup>(EDTA) with ethanol as electron donor.



The first two reactions take place in the scrubber, this enhances the mass transfer and overcomes limitation problems that other biological treatment processes usually meet. The other two microbial reactions denitrify the NO to N<sub>2</sub> and regenerate the chelating agent Fe<sup>(II)</sup>(EDTA) for NO absorption. These two microbial reactions compete for the electron flow.



**Figure 4.**

Schematic principle of BioDeNO<sub>x</sub>.

The core processes of the biological regeneration of Fe<sup>(II)</sup>(EDTA) have been investigated.<sup>78</sup> The reduction of NO to N<sub>2</sub> was found to be catalyzed biologically. The NO reduction follows first order kinetics with respect to the NO/nitrosyl concentration. Fe<sup>(II)</sup>(EDTA) serves as electron donor for NO reduction and simultaneously enhances the NO solubility in the water phase. This implies that the redox cycling of Fe<sup>(II)</sup>(EDTA) plays an important role in the biological denitrification process. However, continuous reactor experiments demonstrated that the iron reduction capacity rather than the denitrification capacity was limiting the load applicable to the bioreactor. When treating a flue gas containing 3.3% O<sub>2</sub> and 500 ppm NO, approximately 90% of the electron flow was used for Fe<sup>(III)</sup>(EDTA) reduction and only 10% for NO reduction. Batch experiments strongly suggest that the reduction of EDTA-chelated Fe<sup>(III)</sup> is not a direct enzymatic conversion, but rather an indirect nonenzymatic reaction. The redox couple S<sup>0</sup>/S<sup>2-</sup> plays an important role in the electron transfer between the bacteria and Fe<sup>(III)</sup>(EDTA). The redox couple sulfide/elemental sulfur accelerates the electron

transfer between the bacteria and  $\text{Fe}^{\text{(III)}}(\text{EDTA})$ . Thus,  $\text{Fe}^{\text{(III)}}(\text{EDTA})$  reduction is most likely a nonenzymatic conversion.

Research was recently done on the kinetics of the oxidation of  $\text{Fe}^{\text{(II)}}(\text{EDTA})$  under typical BioDeNOx conditions (temperature = 329 K,  $[\text{Fe}^{\text{(II)}}(\text{EDTA})] = 50 \text{ mol m}^{-3}$ ), on the influence of the pH on the oxidation rate in the range 5–8, and on the influence of typical BioDeNOx components, ie NaCl and biomass.<sup>79</sup> The results showed that the oxidation of  $\text{Fe}^{\text{(II)}}(\text{EDTA})$  is not influenced by the pH of the solution in the range of 5–8 under the experimental conditions of that study. Moreover, the addition of salt seems to have some positive effect on the reaction kinetics at high NaCl concentrations ( $>10 \text{ kg m}^{-3}$ ). The presence of bacteria changed the absorption profile of oxygen, influencing the physical characteristics of the solution.

Recently, van der Maas *et al*<sup>80</sup> explored the full denitrification pathway of reduction of nitrogen oxide analogs, ie the reduction of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and NO via  $\text{N}_2\text{O}$  to  $\text{N}_2$ , in aqueous  $\text{Fe}^{\text{(II)}}\text{EDTA}$  solutions. They used biomass from a wastewater treatment plant to show that in aqueous solutions of  $\text{Fe}^{\text{(II)}}\text{EDTA}$ , denitrification is accompanied by the biological reduction of  $\text{Fe}^{\text{(III)}}$  to  $\text{Fe}^{\text{(II)}}$ . Ethanol, acetate, and methanol are suitable electron donors. Moreover,  $\text{Fe}^{\text{(II)}}\text{EDTA}$  can also serve as electron donor for the chemical reduction of nitrite to NO and for the biological reduction of NO to  $\text{N}_2$  via  $\text{N}_2\text{O}$ . They suggested that the pathway of full denitrification in aqueous  $\text{Fe}^{\text{(II)}}\text{EDTA}$  solution involves both biological and chemical reduction steps with  $\text{Fe}^{\text{(II)}}\text{EDTA}$  as an electron-mediating compound. Also, they found that the free uncomplexed EDTA strongly inhibits the biological denitrification activity. This requires an excess of divalent cations to guarantee denitrification in aqueous  $\text{Fe}^{\text{(II)}}\text{EDTA}$  solutions.

## CONCLUSIONS

The different biological NOx removal methods are compared in Table 1. The studies summarized in that table demonstrate that the biological removal of NOx is a viable technology.

Denitrifying biofilters can be an effective NO control technique. However, the low oxygen requirements and the need for the addition of exogenous carbon and energy sources limits the cost-effectiveness of these systems.

In the case of autotrophic systems, chemoautotrophic organisms are capable of fixing  $\text{CO}_2$  as carbon source and obtain energy from inorganic substrates. Although high removal efficiencies can be achieved, it requires relatively long residence times of 6–12 min. This is likely caused by the NO mass transfer limitation and the slow, insufficient biomass growth. Because of the high Henry's constant of NO ( $K_{\text{H}} = 0.0019 \text{ mole fraction atm}^{-1}$ ), the BioDeNOx technology could be a good solution to this problem. This process separates the removal into a scrubbing step and a biodegradation step.

Fungal bioreactors can achieve high NO removal efficiencies with relatively short residence times of only 1 min compared with bacterial biofiltration systems. However, the mechanisms by which fungi utilize NO are not yet well understood. An alternative carbon source needs to be found since the NO waste gas stream does not necessarily contain any volatile organic compound such as toluene or other carbon sources. Moreover, the performance of fungal bioreactors under typical NO waste gas stream



operating conditions, ie high temperature and low oxygen concentrations, needs to be evaluated.

Although these biological methods show promising results for the treatment of NO-contaminated gas streams, further investigations will be required regarding, among others, the following aspects:

- Investigation of the kinetics of biodegradation of NO<sub>x</sub> in different bioreactors.
- Comparison of the performance of different kinds of bioreactors (biofilter, fungal bioreactor, hollow fiber membrane bioreactor, photobioreactor, bubble-column-type bioreactor) and optimization of the reactor design (packing material, etc).
- Optimization of operation parameters for the different systems, as, for example, the temperature, since its fluctuation beyond the optimal temperature range of the microbes can decrease their activity and even kill them. The isolation and study of thermophilic NO-consuming microorganisms would be of interest. Most of the current research considered operation at room temperature, which does not reflect the potential full-scale application. Although many flue gases are scrubbed with a water/limestone slurry to reduce sulfur dioxide emissions, gases exiting the scrubbers typically exhibit temperatures between 50 and 60 °C. It is important to find NO-consuming microorganisms active in such temperature range.
- Biological removal in concert with other control techniques.
- Since the flue gas streams can contain up to 8% oxygen and oxygen may inhibit the removal of NO<sub>x</sub> compounds by denitrifying bacteria, the influence of oxygen on the NO removal ability needs to be further investigated in such organisms.
- The presence of CO<sub>2</sub>, SO<sub>2</sub>, and heavy metals such as Hg or Cd in the flue gas may affect the growth of microorganisms. Such effect needs to be studied.
- For BioDeNO<sub>x</sub>, it is worth studying the efficiency of other alternative electron donors such as H<sub>2</sub>, besides ethanol, and other cheap complex chelating agents. In addition, for some systems which require external carbon sources, cheap alternatives are also important.
- The mechanisms by which microorganisms utilize NO<sub>x</sub> need to be understood. The application of culture-independent molecular biological techniques provides new insights into biodegradation and offers new opportunities to better understand the dynamics of microbial communities. Advanced molecular techniques as FISH (fluorescent in-situ hybridization) and PCR (polymerase chain reaction) can be used to detect and enumerate the target bacteria that are directly related to the degradation of NO<sub>x</sub>. Also, 16S rDNA, DGGE, T-REFLP enable the changes in the bacterial community to be monitored in detail.

Overall, microbial reduction of NO (and NO<sub>2</sub>) merits further study, as a potential means of removal of oxides of nitrogen (NO<sub>x</sub>). It certainly offers promise as a cost-effective novel alternative treatment method.

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