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Nitric oxide removal by wastewater bacteria in a biotrickling filter

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ABSTRACT

Nitric oxide (NO) is one of the most important air pollutants in atmosphere mainly emitted from combustion source. A biotrickling filter was designed and operated to remove NO from an air stream using bacteria extracted from the sewage sludge of a municipal sewage treatment plant. To obtain the best operation conditions for the biotrickling filter, orthogonal experiments ($L_9(3^4)$) were designed. Inlet oxygen concentration was found to be the most significant factor of the biotrickling filter and has a significant negative effect on the system. The optimal conditions of the biotrickling filter occurred at a temperature of 40°C, a pH of 8.0 and a chemical oxygen demand of 165 mg/L in the recycled water with no oxygen in the system. The bacteria sample was detected by DNA sequencing technology and showed 93%–98% similarity to *Pseudomonas mendocina*. Moreover, a full gene sequencing results indicated the bacterium was a brand new strain and named as *P. mendocina* DLHK. This strain can transfer nitrate to organic nitrogen. The result suggested the assimilation nitrogen process in this system. Through the isotope experimental analysis, two intermediate products (^{15}NO and $^{15}N_2O$) were found. The results indicated the denitrification function and capability of the biotrickling filter in removing NO.

Introduction

Nitrogen oxides (NO_x) are major constituents of exhaust gas formed during combustion process, especially combustion at high temperatures. NO_x generally refer to six compounds, namely, N_2O, NO, N_2O_3, N_2O_5, N_2O_4, and NO_2. NO is a major constituent of NO_x. More than 95% of the NO_x emitted from exhaust gas is NO, part of which is oxidized to NO_2 in ambient air. Therefore, NO_x often refer to the two compounds: NO and NO_2. Ambient NO_x causes serious environmental problems. It is responsible for tropospheric ozone formation and urban smog through photochemical reactions with hydrocarbons in the presence of sunlight. NO_x, together with SO_2 are the major contributors to acid rain.

NO_x is nasty due to its difficulty in removal. Traditional removal methods include combustion modification and exhaust gas after-treatment. Exhaust gas after-treatment methods are usefully adopted in many combustion systems. Selective catalytic reduction and selective non-catalytic reduction are proven exhaust after-treatment methods for reducing NO_x. However, both of them are costly and theirs efficiency heavily depend on the operating temperature or the use and maintenance of catalysts. Therefore, the installation and maintenance of such system are costly. The by-products (such as N_2O, NO_2) during operation also need further treatment (Brandin et al., 2012).

Biological treatment, another exhaust after-treatment method, is a cost-effective treatment technology for waste gas streams. Biological system has been considered to be

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more appropriate for the environment due to the fact that they do not give rise to further environmental problems. This type of system can be operated under ambient temperature with low energy consumption. The microbial inocula are inexpensive and can be obtained from natural environment (Yang et al., 2007). The pollutants do not enter another phase but are converted into harmless products. Also, the by-products during operation are limited or if present, are easy to disposal. This technology has been proven effective in the removal of odor and volatile organic compounds, such as benzene (Aly Hassan and Sorial, 2009), ammonia (Ryu et al., 2011), hydrogen sulfide (Ramírez et al., 2011), and phenols (Ramos et al., 2007). One such treatment technology is biotrickling filter in which a contaminated air stream is passed through a porous support material on which pollutant-degrading microorganisms are immobilized. The porous support material can provide sufficient surface area for mass transfer and biofilm growth. The integrant humidity and nutrient elements are required, which are supplied by recirculation water. Biotrickling filters have been employed to purify waste gas like valatile organic compounds (VOCs), mercury vapor and H_2S (Aroca et al., 2007; Li et al., 2008; Liu et al., 2006; Philip and Deshusses, 2008; Ramirez et al., 2007).

Researches on biological control of NO_x mission can be reflected in the nitrogen cycle. Important processes of the nitrogen cycle involved in NO_x emission control include nitrification, denitrification, assimilation and anammox. The nitrification is the aerobic oxidations of NH_4^+ to NO_2^- and NO_2^- to NO_3^-. The denitrification part of the nitrogen cycle transforms NO_3^- into N_2. It occurs in four stages, NO_3^- to NO_2^-, NO_2^- to NO, NO to N_2O and N_2O to N_2. In general, nitrogen element enters to the biosphere through the nitrogen fixation process and returns to the atmosphere as N_2 after denitrification (Niu and Leung, 2010). In a process called anammox, both NH_4^+ and NO_2^- act as respiratory electron acceptor and converted directly into N_2 (van Spanning et al., 2007). In this research, a biotrickling filter system was designed to remove NO based on the nitrogen cycle. A sample of bacteria, obtained from a multiple wastewater treatment plant, was cultivated and inoculated into the biotrickling filter. The potential of the system to treat NO was studied. In addition, the bacteria were extracted and identified by DNA sequencing technologies. The nitrogen pathway in this biotrickling filter was studied through isotope labeling technology.

1 Materials and methods

1.1 Biotrickling filter system design

Figure 1 shows a schematic diagram of the biotrickling filter system. The system was operated both at aerobic and anaerobic conditions during the experiments. The feed

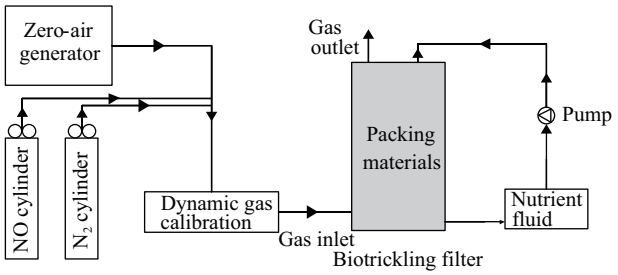

Fig. 1 Schematic diagram of the biotrickling filter system

aerobic gas was produced by a standard NO gas (1880 ppm, BOC GASES, UK) mixed with clean air generated from a zero air generator (Zero Air Module-Model 701, Teledyne API, USA). The anaerobic gas was provided by a standard NO gas mixed with N_2 (HKO, Hong Kong). The NO concentration was regulated using a dynamic gas calibration system (Model 146, Thermo, USA) both under aerobic and anaerobic conditions. The total height of the biotrickling filter was 33 cm with an inner diameter of 10 cm. The height and void of the packing was 19 cm and 55%, respectively. Ceramic beads, with a diameter between 2–4 mm, were used as packing material. The specific density and bulk density of the packing material are 1.52–2.26 g/m^3 and 0.85–1.26 g/m^3, respectively. The nutrient solution was circulated in the biotrickling filter by pumping at the top with a peristaltic pump (BT-100B, Huxi, China), and discharged at the bottom by gravity. The solution contained $MgSO_4{\cdot}7H_2O$ (0.2 g/L), K_2HPO_4 (0.5 g/L), NaH_2PO_4 (0.5 g/L) and $Na_2C_4H_4O_6$ (sodium tartrate, 5 g/L). The biotrickling filter was operated in a counter-current mode. Contaminated gas passed through the packed bed with NO-degrading biofilm. The recycled liquid trickling was maintained with a suitable pH that supports the development of microorganisms in the biotrickling filter.

1.2 Inoculums' preparation

1.2.1 Screening and enrichment of bacteria

Bacteria samples were obtained from the sewage sludge of a municipal sewage treatment plant in Hong Kong. The sludge was diluted and cultured on petri dishes at 37°C for 3 days. The ingredients of petri dish cultivation nutrient consist of the following: KNO_3 (2.0 g/L); $MgSO_4{\cdot}7H_2O$ (0.2 g/L); K_2HPO_4 (0.5 g/L); NaH_2PO_4 (0.5 g/L); $Na_2C_4H_4O_6$ (20 g/L); agar (3 g/L). The chemicals were dissolved in 1 L of pure water, and the pH of the solution was 7.2. All solutions and vessels were sterilized at 121°C for 30 min in an autoclave before cultivation. After several times' enrichment of the co-cultivation in petri dishes, some bacteria were co-cultured in a liquid nutrient solution at 37°C. The nutrient solution consists of the same composition as the petri dish cultivation nutrient except agar. The bacteria were concentrated in a centrifuge (5810R, Eppendorf, German) after 2 days' fluid

cultivation. The centrifuge was operated at 37°C, with a rotational speed of 5000 rcf, and operated for 15 min. The original volume of solution culture bacteria was 2 L, and was concentrated to less than 30 mL. Subsequently, the bacteria were inoculated in a biotrickling filter.

1.2.2 Bacterial identification

A single colony was isolated after streaking bacteria on a Luria-Betani agar plate and inoculated at 37°C overnight. It was then used as a template for polymerase chain reaction (PCR) amplification using 16S rRNA gene primers 8F (AGAGTTTGATYMTGGCTCAG) and 907R (CCGTCAATTCMTTTRAGTTT) (Nercessian et al., 2005). In a 20-μL reaction containing 1X reaction buffer, 0.25 mol/m^3 dNTP, 2 mol/m^3 $MgCl_2$, 0.2×10^{-3} mol/m^3 primers and 0.5 units of Taq DNA polymerase (Invitrogen), the PCR cycle was performed with a single step of 5 min at 95°C, followed by 30 cycles of 30 sec at 95°C, 30 sec at 53°C, and 90 sec at 72°C and then a final extension at 72°C for 8 min. The PCR product was subjected to DNA sequencing using BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) by the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). The DNA sequences were then submitted to basic local alignment search tool (BLAST) to identify their nearest relatives using the GenBank database. In this study, four samples were cloned of their PCR products after 6 months' operation of the biotrickling filter. The whole gene sequencing method is documented by Wong et al. (2012).

1.3 Analytical methods

A portable industrial combustion and emission analyzer (Greenline 800, Eurotron, UK) analyzed the concentration of NO, NO_x and O_2. The analyzer's accuracy on NO, NO_x and O_2 are ± 5 ppm, ±5 ppm and ±0.1% by volume, respectively. In this study, a confidence limit of 95% was used, and all the experimental results presented represented the mean of at least three runs. A pH meter (PHS-2F, Leici, China) measured the pH of the liquid effluents. NO_3^- and NO_2^- concentrations were monitored by ultraviolet spectrophotometric screening method and colorimetric method, respectively (Eaton et al., 1995), using a UV spectrophotometer (6105u.v/vis, Jenway, UK). COD of the liquid effluents was determined by the closed reflux method (Eaton et al., 1995). Specific surface area of the ceramic material was detected by a physisorption analyzer (Micromeritics ASAP 2020, USA).

The morphology of the bacteria was observed with a scanning electron microscope (S 4800 FEG SEM, Hitachi, Japan). The specimens were washed with normal saline and fixed in 2.5% glutaraldehyde in a cacodylate buffer (0.1 mol/L sodium cacodylate-HCl buffer pH 7.4) for 4 hr at 4–8°C, then washed well in several changes of cacodylate buffer with 100 mol/m^3 sucrose. Subsequently, the samples were dehydrated in an up-grading series of ethanol solution (30%, 50%, 70%, 90%, and 100%). The samples were dried in a critical point dryer using liquid carbon dioxide as transitional fluid and coated with a thin layer of metallic film.

The orthogonal array experiments were used with the Taguchi experimental design to determine the optimum biotrickling filter operation conditions. The Taguchi method is a complete application in design and analysis of experiments by conducting relatively less number of experiments (Taguchi, 1987; Lochnar and Matar, 1990). In this study, four relevant process parameters, i.e. temperature, pH, concentration of organics ($Na_2C_4H_4O_6$) in recycled water and system's oxygen content, were selected and their set ranges are shown in **Table 1**. The effect of oxygen to the system is not confirmed (Lee et al., 2001; Jiang et al., 2008, 2009; Wang et al., 2006; Yang et al., 2007). The effect of external carbon source depends on different packing materials (Chou and Lin, 2000; Barnes et al., 1995; Yang et al., 2007). The effects of pH and temperature on bioreactor success are analogous in several ways. Each species of microorganism is most active over a certain range of pH and temperature and will be inhibited if conditions move outside this range (Devinny et al., 2000). An orthogonal table $L_9(3^4)$ was chosen for this method, where $L_9(3^4)$ means 4 parameters and 3 levels for each parameter (Ross, 1988). Since COD is easier to determine and more universal to represent the level of organic compounds in water as compared to the concentration of $Na_2C_4H_4O_6$, therefore, COD is used in the present experiments. The orthogonal experiments consist of 9 groups of experiments, with each group lasted for 3 weeks. When the same (or nearly the same) results were obtained during two weeks operation, the system was recognized as stable condition, the data were then collected. NO removal efficiency is selected to be the response parameter of these experiments. The NO removal efficiency (R_E) is defined as:

$$R_E = 1 - c_{out}/c_{in} \quad (1)$$

where, c_{in} (μg/m^3) and c_{out} (μg/m^3) are the inlet and outlet NO concentrations, respectively. In Taguchi method, the *S/N* ratio is used to determine the deviation of the characteristics from the desired value (Tayyeb et al., 2009). There are several *S/N* ratios available depending on the type of characteristic. Here the *S/N* ratio for higher is better

Table 1 Selected process parameters and their respective levels

Parameter	Level 1	Level 2	Level 3
Temperature (°C)	24	32	40
pH	6.8	8.0	9.0
$Na_2C_4H_4O_6$ (g/L)	0	2	4
O_2 (%)	0	13	20.9

(HB) is considered. The HB statistics is given by Eq. (2):

$$S/N_{\mathrm{HB}} = -10\log\left(\frac{1}{r}\sum_{i=1}^{r}\frac{1}{y_i^2}\right) \quad (2)$$

where, S/N_{HB} is the S/N ratio of "the higher is better", r is the number of tests in a trial, y_i is the comparison variable in experiment i for a certain combination of control factor levels.

To the labeling experiments, an isotope chemical NH_4NO_3 (NO_3^--15N, 98%+, Cambridge Isotope Laboratories, USA) was used to detect the nitrogen pathway in the biofilter system. A quantitative gas analyzer (QGA, Hidden, UK) was used to test gas samples molecular fragments masses to determine the isotope gases.

2 Results and discussions

2.1 Biotrickling filter start up experiment

2.1.1 Effect of ceramic material and liquid phase on NO removal without bacteria

The NO adsorption curve of the ceramic material is shown in **Fig. 2**. The ceramic material was dried in an oven for 24 hr to sterilize the original bacteria and volatilize adsorption gases. The height of the ceramic material in the biotrickling filter was 19 cm, original NO concentration was 123 mg/m^3, and operation temperature was 37°C. The nutrient solution was circulated in the biotrickling filter, and discharged at the bottom by gravity. The solution contained $MgSO_4{\cdot}7H_2O$ (0.2 g/L), K_2HPO_4(0.5 g/L), NaH_2PO_4 (0.5 g/L) and $Na_2C_4H_4O_6$ (sodium tartrate, 5 g/L). The biotrickling filter was operated in a countercurrent mode. As shown in **Fig. 2**, the ceramic material adsorption capacity and the removal capacity of liquid phase were saturated in less than 2 min. The empty bed residence time (EBRT), a ratio of contaminated gas flow rate to the volume of biotrickling filter, was 60 sec. Thus, the effective removal capacity was saturated in less than 60 sec. This indicated the ceramic material adsorption capacity and the removal capacity of liquid were limited and saturated in a short time's operation.

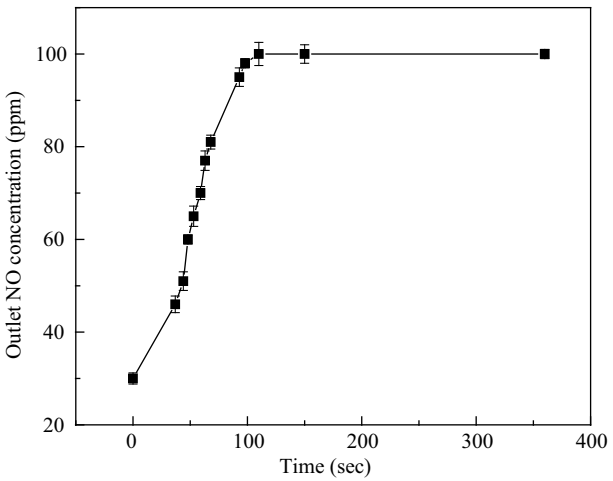

Fig. 2 Operation characteristics of the biotrickling filter without bacteria.

2.1.2 Biotrickling filter start up

The bacteria were cultured in the nutrient solution after several times' enrichment. **Figure 3** shows the NO_3^- removal efficiency during 30 hours' cultivation in a shaker incubator. Batch runs for the cultures were carried out aerobically at 37°C and stirred at 130 r/min equipped with a temperature control system. The pH of the solution was kept at 7.2 ± 0.05. The nutrient solution consists of the same composition as the petri dish cultivation nutrient without agar. The original KNO_3 concentration was 2.0 g/L. **Figure 3** indicates that NO_3^- removal efficiency reached about 85% after 24 hours' cultivation. And during this test, the NO_2^- concentration was very small and can be neglected. This indicated that there was no NO_2^- accumulation during the biological process.

The bacteria were inoculated into the biotrickling filter system for subsequent testing with influent pollutant gas. At the beginning, the nutrient fluid used consists of ingredients of KNO_3, $MgSO_4{\cdot}7H_2O$, K_2HPO_4, NaH_2PO_4 and $Na_2C_4H_4O_6$. The environmental temperature and pH of the biotrickling filter were around 24°C and 8, respectively. After developing the biofilm on the surface of the packing material for 4 days, the biotrickling filter was operated with NO as nitrogen source to substitute KNO_3. The system was operated at an aerobic environment. The operation data of the system was demonstrated in **Fig. 4**. The inlet NO concentration was 123 mg/m^3 (100 ppm) while the outlet NO concentration was between 31 and 70 mg/m^3 (25 and 57 ppm). The outlet NO concentration started to decline after inoculation for 5 hr. After 15 hr, a relatively steady

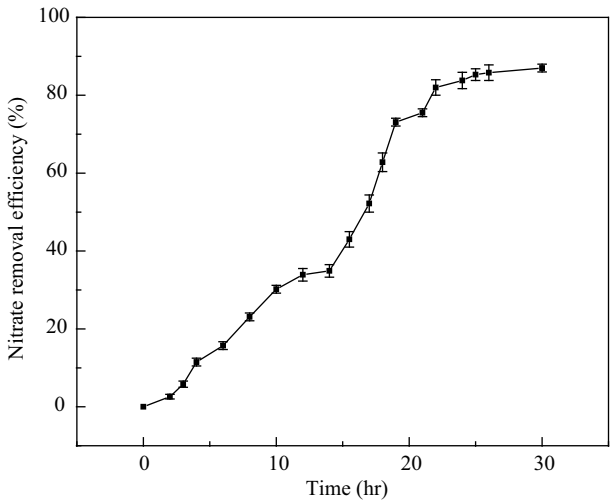

Fig. 3 Temporal variation of NO_3^- removal efficiency in the liquid culture.

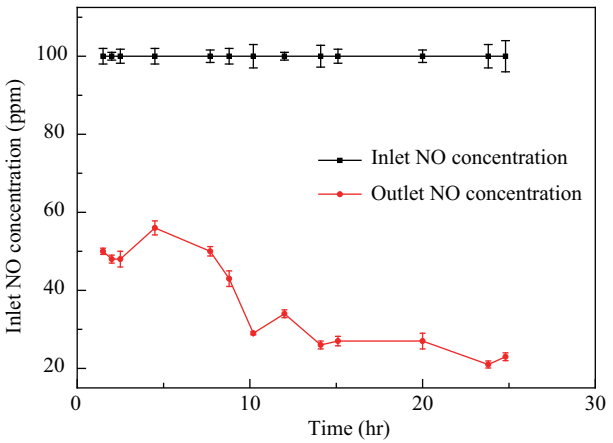

Fig. 4 Characteristics of the biotrickling filter under initial operation. Operating conditions: 24°C, pH 9, inlet gas flow rate 1.41 L/min, the trickling fluid kept the humidity of the biotrickling filter, total liquid of the system was replaced every other day.

state was achieved in the biotrickling filter system, with a removal efficiency of 73%–79%. The biotrickling filter had shown the potential capability to purify the NO in the influent gas stream.

After the bacteria were inoculated in the biotrickling filter for subsequent NO treatment, two samples were taken and examined by SEM. **Figure 5** shows the microbial communities on the samples before inoculation and after 24 hr of NO treatment. A comparison of the two micrographs on the same magnification factor shows that the microorganisms were developed on the surfaces of the packing material in the biotrickling filter, suggesting that the microorganisms can grow on the medium and promote their metabolism. As observed, the morphology of the microorganisms is of short rod shape with diameter around 1–2 μm.

2.2 Orthogonal experiment

2.2.1 *S/N* ratio

Each group of experiment lasted for 3 weeks. Data were collected when the biotrickling filter was stable. Experimental runs were based on the arrangement of the orthogonal array. Results of the orthogonal experiments are shown in **Table 2**. The inlet NO concentration and the EBRT were respectively fixed at 123 mg/m^3 and 60 sec for all the experiments.

Minitab 15 statistical software was used to find out the optimum levels of parameters. The *S/N* ratios at different levels of the parameter for the NO removal efficiency are summarized in **Table 3**. Basically, the larger the *S/N* ratio, the better the parameters for achieving higher NO removal efficiency.

Results of the orthogonal experiments (**Table 2**) indicated that the fifth group parameters (i.e. temperature = 32°C, pH = 8.0, COD = 1250 mg/L and 0% system oxygen) were the optimal conditions for the biotrickling filter operation.

Table 2 Taguchi $L_9(3^4)$ orthogonal array and results for NO gas removal efficiency

Experiment no.	Temp (°C)	pH	COD[a] (mg/L)	O_2 (%)	NO removal efficiency (%)
1	24	6.8	6.4	0	94.1
2	24	8.0	176	13.4	87.2
3	24	9.0	1280	20.9	82.5
4	32	6.8	163	20.9	86.2
5	32	8.0	1250	0	99.0
6	32	9.0	5.0	13.4	87.0
7	40	6.8	1260	13.5	88.7
8	40	8.0	156	20.9	85.5
9	40	9.0	5.8	0	98.5

[a]As the bacteria metabolism is in progress, some biofilm may desquamate, so the COD may not be zero even when the concentration of $Na_2C_4H_4O_6$ is zero in the recycled water.

Table 3 *S/N* ratios

Level	Temp	pH	COD	O_2
1	38.87	39.05	38.97	39.75
2	39.14	39.12	39.13	38.85
3	39.16	39.00	39.07	38.56
Delta	0.29	0.12	0.16	1.19
Rank	2	4	3	1

However, according to the range analysis (**Table 3**), the optimal operation conditions obtained were: temperature = 40°C, pH = 8.0, COD = 165 mg/L and 0% system oxygen. Nevertheless, **Table 3** also shows that the order of significance in decreasing order was: oxygen content > temperature > COD > pH. In the orthogonal experiments array design, all the parameters except oxygen level are suitable for the bacteria growth. Therefore, system oxygen content was the most significant factor. Other factors such as temperature, carbon source and pH value had little effects to the system. Therefore, reasonable operation conditions would be anaerobic, environmental temperature between 32 and 40°C, pH from neutral to weak alkaline and with organism (COD) in the recycled solution.

In the present biotrickling filter system, the purification of NO involves physical, chemical and biological processes. According to **Fig. 2**, the capacities of NO adsorption by the packing material and liquid phase were limited and saturated in a short time (< 2 min). The biological effect is the main process during the NO treatment.

The effect of oxygen on NO removal efficiency in the biotrickling filter has not yet been confirmed. Some researchers believe that the presence of oxygen creates negative effect on some bacteria, thus some bacteria have better ability to reduce oxides of nitrogen at low oxygen levels (Ye et al., 1994; Barnes et al., 1995). As such, some experiments are carried out under anaerobic condition

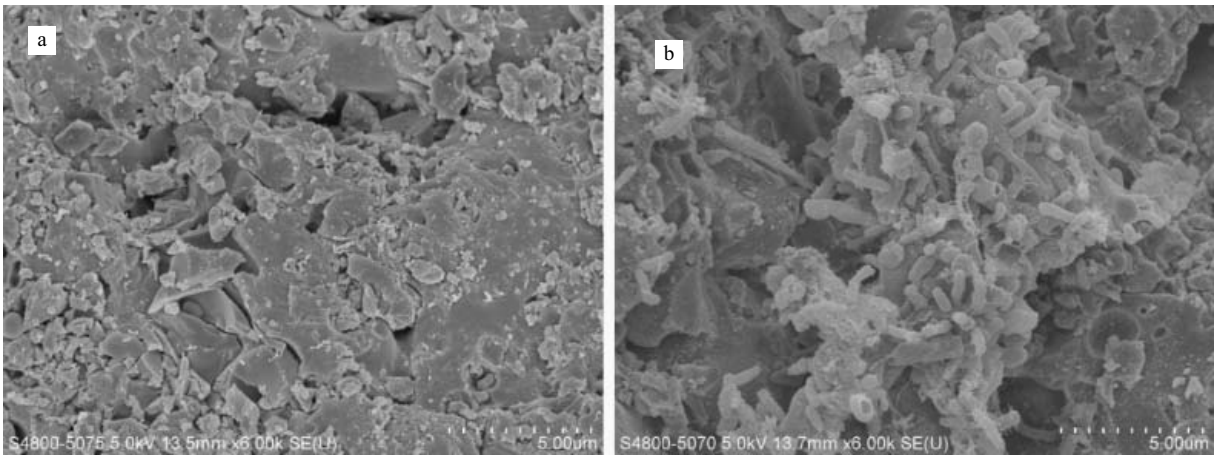

Fig. 5 SEM micrographs of the microorganisms grown on ceramic bead surfaces. (a) Before cultivation; (b) after 24 hr of NO treatment

(Barnes et al., 1995; Chagnot et al., 1998; Lee et al., 2001; Mohsen, 2005; Yang et al., 2007); while others are operated at aerobic condition (van der Maas et al., 2006; Wang et al., 2006; Jiang et al,. 2009). Wang et al. (2006) reports that the optimal inlet oxygen concentration was 5.2% when calculating the chemical oxidation (NO is oxidized to NO_2). However, Jiang et al. (2009) find that oxygen has no negative effect on the aerobic denitrifier, but rather enhances the total efficiency in part by chemical oxidation and in part by the strain activities. In this research, increasing inlet oxygen concentration has a significant negative effect on NO treatment. This may indicate that some anoxic bacteria were active in the absence of oxygen. The bacteria were extracted from the sewage sludge of a municipal sewage-treatment plant. The bacteria were cultured as mesophiles due to their original environment. Mesophiles are microorganisms with growth optima around 20 to 45°C as indicated in some researches (Fontenot et al., 2007; Kim et al., 2008). In this study, the result indicated the optimal temperature (40°C) was reasonable to former research.

The effect of carbon source to a bioreactor could be negative or positive. The outlet NO concentration decrease significantly as glucose is added into the column (Yang et al., 2007). However, a temporary decrease in NO removal is observed as dextrose is provided to a biofilter (Barnes et al., 1995). In other research, the average proportion of C:P:N=7:1:30 could be used for realistic applications (Chou and Lin, 2000). In this study, the carbon source has little effect to the system, due to the adsorption of carbon source by microporous structure in the ceramic material. However, in long term operation, an exogenous carbon source needs to add to maintain efficient NO reduction (Barnes et al., 1995).

The bacteria were cultivated at a pH of 7.2 before inoculated into the biotrickling filter. Moderate pH was considered appropriate during petri dish cultivation. However, when the biotrickling filter operation was just started, the optimal pH value for the nine experiments is 8.0 (**Table 3**). In this system, NO could be oxidized to NO_2 in air, and subsequently reacted with water to form NO_3^-, which causes a decline in pH values. Therefore, in the biotrickling filter system, the alkaline solution could protect the microorganisms from changes in pH. The pH was controlled by a buffer solution in the recycling solution. However, the solution must be changed when the buffer capacity is decrescent.

2.2.2 ANOVA

In order to determine the effective parameters and their confidence levels on the NO removal efficiency, an analysis of variance (ANOVA) was performed. An ANOVA is to investigate which selected parameter significantly affects the NO removal efficiency. Due to the degree of freedom (DOF), **Table 4** contains the ANOVA tables for removal efficiency without the parameter pH. During the whole experiments, pH was kept alkalescent in the system. The pH between 6.8 and 9.0 is reasonable to the development of microorganisms in the biotrickling filter. Therefore, in this study, the effect of pH in this range on the system was recognized limited. Matlab software was used to calculate the variances for the ANOVA. **Table 4** shows the results of the ANOVA test. In this study, the total DOF is 8. The DOF for each parameter is 2. From this, the DOF for the error is calculated as 2. The F_{cr}-critical value

Table 4 Results of ANOVA for NO removal efficiency

Source	Sum Sq.	DOF	Mean Sq.	F	Prob > F (p-value)
Temperature	16.669	2	8.334	6.83	0.1278
COD	4.882	2	2.441	2	0.3334
O_2	255.349	2	127.674	104.56	0.0095
Error	2.442	2	1.221		
Total	279.342	8			

Sum Sq. is the sum of squares; Mean Sq. is mean squares, (Sum Sq.)/DOF.

of each parameter for DOF of 2 and 2 at a confidence level of 95% is 19.0. The F values in **Table 4** show that the parameter oxygen is statistically significant. In addition, the p-value of parameter oxygen is less than 0.05, which also indicates that the parameter oxygen is significant. Similarly, temperature and COD are found to be insignificant to the results. Therefore, both the *S/N* ratio analysis and ANOVA, indicate that the parameter oxygen significantly affects the NO removal efficiency.

2.3 Characterization of bacterium

After six months' operation, four samples of bacteria were extracted to characterize the bacteria community in the system. Through DNA sequencing, the sequences were submitted to BLAST search of the GenBank database to identify their nearest relatives. These sequences of the four clones of bacteria showed 93%–98% similarity to *Pseudomonas mendocina*. The most recent observation of *Pseudomonas* sp. for treatment of NO_x is reported by Jiang et al. (2009). The researchers developed a biotrickling filter with a newly isolated strain of *Pseudomonas putida* SB1 for the effective treatment of NO (Jiang et al., 2009). Also, a strain D3 of denitrifying bacterium is isolated from an anammox reactor, and is identified as *P. mendocina* (Hu et al., 2006). Song and Ward (2003) find *P. mendocina* CH91 has nitrite reductase (*nir*K) genes. Under conditions of limited nitrogen, *P. mendocina* NK-01 can synthesize medium-chain-length polyhydroxyalkanoate anoate and alginate oligosaccharides from glucose at the same time (Guo et al., 2012). *P. mendocina* is considered as heterotrophic bacteria (Koschorreck et al., 1996; Wong et al., 2012). In this study, *P. mendocina* is heterotrophic, no chemosynthesis or photosynthesis pathway have been reported in this species.

From the result of whole genome sequencing the strain *P. mendocina* is identified as a brand new strain, and named as *P. mendocina* DLHK (Wong et al., 2012). In this research, from the Kyoto Encyclopedia of Genes and Genomes map of nitrogen metabolism (**Fig. 6**), all the pathways and intermediates related to nitrogen metabolism were shown. The gray boxes show the presence of genes related in this search. Each box has the enzyme commission number (EC number) for the related enzyme. It indicates strain *P. mendocina* DLHK has assimilatory nitrate reductases genes, nitrite reductase genes and so on. The nitrate reductases genes (EC 1.7.99.4) are molybdoenzymes that convert NO_3^- to NO_2^-. The nitrite reductases genes (EC 1.7.1.4) can convert NO_2^- to NH_3. From the information of this map, this bacterium can convert NO_3^- to NO_2^- , at the same time NO_2^- can be converted to NH_3, and then to organic nitrogen (i.e. L-glutamate, carbamoyl-P) for the growth of the bacteria. It is the assimilation

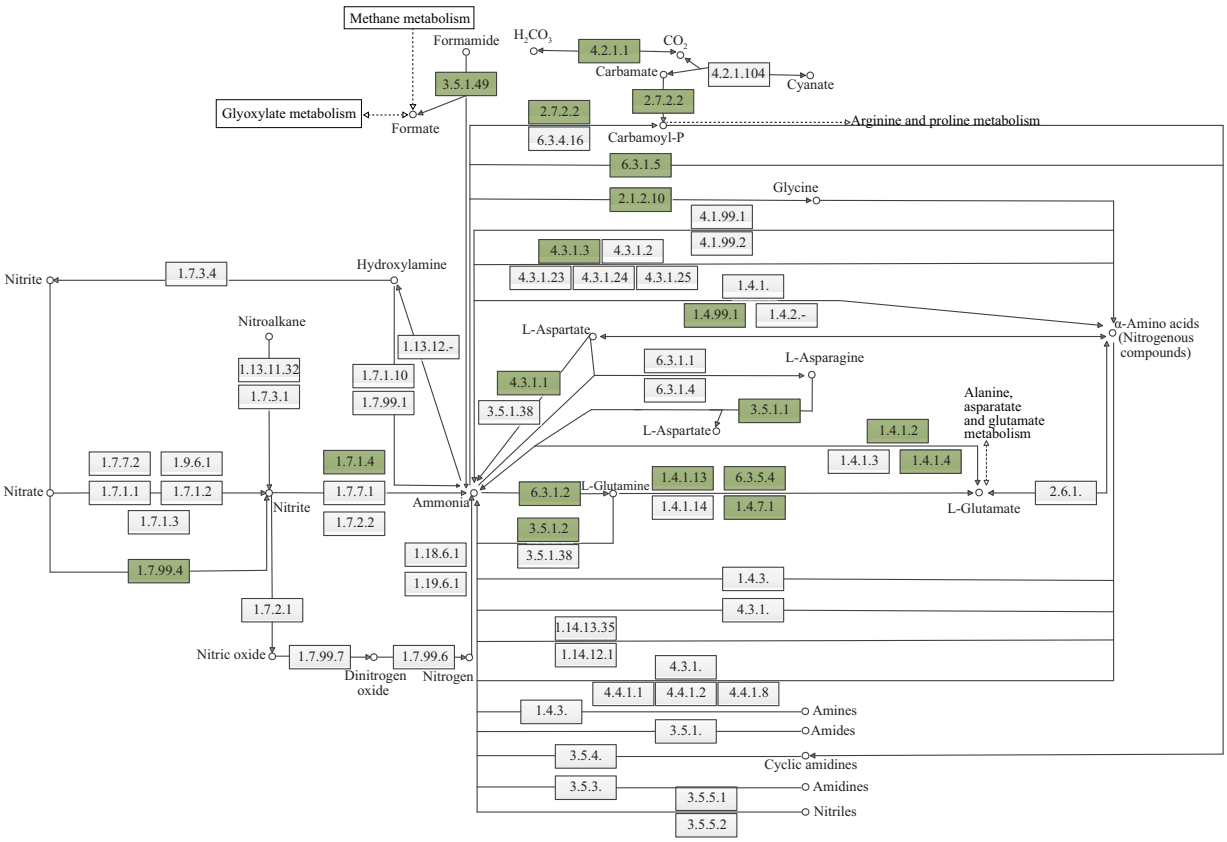

Fig. 6 Kyoto Encyclopedia of Genes and Genomes map of *Pseudomonas mendocina* DLHK's nitrogen metabolism

of nitrogen cycle. These kinds of bacteria are well documented to reduce phenolic compounds (Tian et al., 2003; Fang et al., 2004; Heinaru et al., 2005; Kao et al., 2005), monomethylamine (Pandey et al., 2006) and chromate (Rajwade et al., 1999). However, the *P. mendocina* bacteria potential for NO removal from flue gas has not yet been well explored.

2.4 Isotope labeling

In order to detect the medium products of the biotrickling filter, isotope labeling experiment was carried out. In this study, an isotope chemical NH_4NO_3 (NO_3^--15N, 98%+) was used to detect the nitrogen pathway in the biofilter system, especially the products of gas samples. The isotope chemical was added into the recycling solution to apply nitrogen source to the biotrickling filter system. The gas samples of the system were detected by a qualitative gas analyzer (QGA). The biotrickling filter carrying gas was helium to prevent the interference of complex air gases.

NO_2 and $^{15}N_2O$ have the same molecular mass weight (46). Therefore, both NO_2 and $^{15}N_2O$ were considered during the experiment. Also, N_2O and CO_2 have the same molecular mass of 44. In order to eliminate any confusion, molecular mass 44 was not detected. The relative molecular fragments masses are shown in **Table 5**. The signal response data is the strength of different fragments in single molecule, and all data are the completely gas phase ion energy data. Therefore, the detection molecular fragments masses were set as 14, 15, 30, 31, 45 and 46. The QGA ion energy was setup as 16 eV. The QGA carrying gas was helium gas, same as the sample carrying gas. Under certain conditions, such as the sudden addition of the instrument carrying gas or the difference of inlet gases, the data lines would be affected. The molecular fragments masses 14 and 15 were used as baseline data.

The composition of the recycling solution with isotope chemical were NH_4NO_3 (NO_3^--15N, 98%+) (1.0 g/L); $MgSO_4{\cdot}7H_2O$ (0.2 g/L); K_2HPO_4 (0.5 g/L); NaH_2PO_4 (0.5 g/L) and $Na_2C_4H_4O_6$ (20 g/L). The composition of the recycling solution without isotope chemical was the same as the above with the absence of NH_4NO_3 (NO_3^--15N, 98%+). The biotrickling filter system was kept at 37°C to generate sample gases. The samples were collected after 24 hr and detected by the QGA. The carrying gas was helium and the flow rate was set at 50 μL/sec. The sample temperature and furnace temperature were kept at 36°C. At this temperature, all the sample gases were considered volatilized. The results of gas sample with isotope chemical are shown in **Fig. 7**. The molecular fragments masses 14 and 15 have little effect to the whole detection data. In other words, the sudden addition of carrying gas helium in the instrument has limited effects to the results. As shown, all the molecular fragments masses 30, 31, 45 and 46 exist in the isotope gas sample. Molecular fragment mass 31 is ^{15}NO, which is the medium product of denitrification. Molecular fragment mass 46 could be both NO_2 and $^{15}N_2O$ or only $^{15}N_2O$. With the results of molecular fragment mass 45 present in **Fig. 7**, the molecular fragments masses 45 and 46 are found responsible for the existence of $^{15}N_2O$ in the isotope sample. The molecular fragment mass 45 could be in the form of ^{15}N-N-O. Molecular fragment mass 30 could come from the fragment of NO_2, $^{15}N_2O$, N_2O, NO and $^{15}N_2$, and the exact source cannot be confirmed.

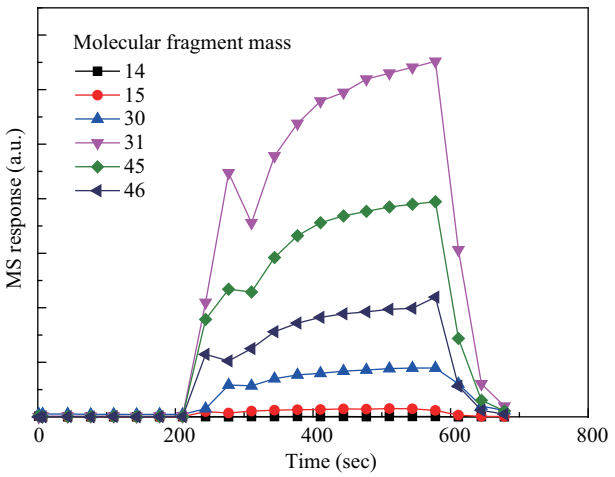

Fig. 7 Results of gas sample with isotope chemical.

Table 5 Fragments of relative gases

NO	Fragments	30	14	15	16	31	32				
	Signal response	999	75	24	15	4	2				
NO_2	Fragments	30	46	16	14	47					
	Signal response	999	370	223	96	1					
N_2	Fragments	28	14	29	15						
	Signal response	999	72	8	1						
N_2O	Fragments	44	30	14	28	16	45	46	31	29	15
	Signal response	999	311	129	108	50	7	2	1	1	1
CO_2	Fragments	44	28	16	12	45	22	46	29	13	
	Signal response	999	114	85	60	13	12	4	1	1	

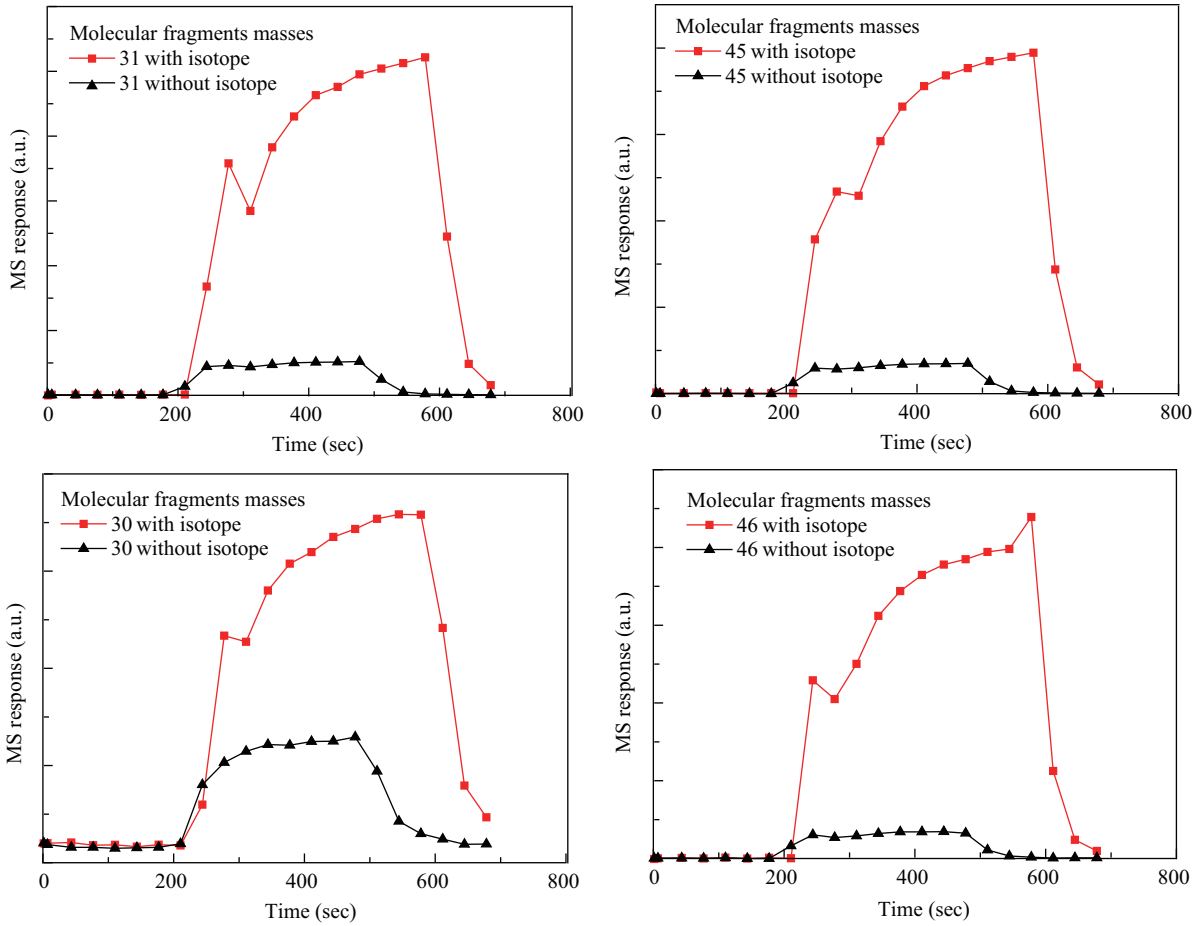

Fig. 8 Parallel results of molecular fragments masses 31, 45, 46 and 30 in the isotope sample and without isotope sample.

The results of the parallel experiments (the sample without isotope chemical) indicated that the molecular fragments masses 31, 45, 46, 30 are insignificant compared with the gas sample with the isotope chemical (**Fig. 8**).

In these parallel experiments, the labeling of 15-N was used to detect the pathway of nitrogen source metabolism in the bacteria biological reaction. Both ^{15}NO and $^{15}N_2O$ were available in the isotope gas sample. The parallel experiment without isotope chemical confirmed the existence of these intermediate products in the isotope gas sample. NO and N_2O are the intermediate products of denitrification, which is an important biological process in the nitrogen cycle. Therefore, in the biotrickling filter, the denitrification process occurred during the bacteria's metabolism using the nitrogen source. The denitrify bacteria existed in this system along with the assimilation bacteria.

However, the existence of $^{15}N_2$ cannot be confirmed due to the same molecular fragment from several gases. A feasible approach to identify $^{15}N_2$ is by the pre-treatment of gas samples to purify the gases components and changing the setup of ion energy, both of which cannot be done in the present study.

2.5 NO_3^- and NO_2^- concentrations in recycling solution

Recycling solution samples were taken during the six months' operation, NO_3^- concentration was found to be between 2.12 and 3.45 mg NO_3^--N/L, and NO_2^- concentration was not detected. The samples were diluted with two different dilution ratios (i.e. 5 and 10 times) to obtain the average values. The full gene sequencing revealed that the *P. mendocina* DLHK could use as NO_3^- and NO_2^- to assimilate the organic nitrogen in the nitrogen cycle. The denitrify bacteria could use NO_3^- and NO_2^- as electron acceptors. However, NO_3^- could be generated by the oxidation of NO_2 with O_2 in the aqueous solution. Therefore, the nitrification process in the system cannot be confirmed.

3 Conclusions

A biotrickling filter, using a mixed microbial community, was fabricated for treating NO in a simulated exhaust gas. The NO removal efficiency was found to vary from 82.5% to 99% at an inlet NO concentration of 123 mg/m^3 and an EBRT of 60 sec. In this study, the potential of biotrickling

filter to remove NO by bacteria was shown. Optimal operating conditions of the biotrickling filter were obtained through a series of $L_9(3^4)$ orthogonal experiments. The optimal conditions of the biotrickling filter were found to be at a temperature of 40°C, a pH of 8.0, a COD of 165 mg/L and 0% of system oxygen. Oxygen was found to be a significant factor influencing the efficiency of the NO removal. Increasing inlet oxygen concentration had a significant negative effect on the NO removal efficiency. The bacteria *P. mendocina* strain was detected after six months' operation. The full gene sequencing results indicated that the strain, named as *P. mendocina* DLHK, was a brand new bacterium. Nitrite reductase and nitric oxide synthase gene orthologues were found in this strain, with the reference orthologue sequence at NCBI accession number of YP_001187589 and YP_001188768, respectively. The result suggested the assimilation process in the biotrickling filter operation. The assimilation process in a biotrickling filter to remove NO is not documented before. The assimilation could supply a new way to the utilization of biomass in recycling solution. A new method was used to detect isotope gas fragments. By using this method, two intermediate products ^{15}NO and $^{15}N_2O$ of the denitrification process were observed by the isotope labeling experiment. The denitrification process was confirmed in the biotrickling filter. The final product N_2 could not be confirmed due to the technological limitation of the present experiment. However, a feasible approach was reported in this study. In this study, the biotrickling filter shows potential to remove NO by the denitrify bacteria along with the assimilation bacteria obtained from wastewater treatment sludge.

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REFERENCES

Aly Hassan, A., Sorial, G., 2009. Biological treatment of benzene in a controlled trickle bed air biofilter. Chemosphere 75(10). 1315–1321.

Aroca, G., Urrutia, H., Nunez, D., Oyarzun, P., Arancibia, A., Guerrero, K., 2007. Comparison on the removal of hydrogen sulfide in biotrickling filters inoculated with Thiobacillus thioparus and Acidithiobacillus thiooxidans. Electron. J. Biotechnol. 10(4), 514–520.

Barnes, J. M., Apel, W. A., Barrett, K. B., 1995. Removal of nitrogen oxides from gas streams using biofiltration. J. Hazard. Mater. 41(2-3), 315–326.

Brandin, J. G. M., Hulteberg, C. P., Odenbrand, C. U. I., 2012. High-temperature and high-concentration SCR of NO with NH_3: Application in a CCS process for removal of carbon dioxide. Chem. Eng. J. 191, 218–227.

Chagnot, E., Taha, S., Martin, G., Vicard, J. F., 1998. Treatment of nitrogen oxides on a percolating biofilter after pre-concentration on activated carbon. Process Biochem. 33(6), 617–624.

Chou, M. S., Lin, J. H., 2000. Biotrickling filtration of nitric oxide. J. Air Waste Manage. Assoc. 50(4), 502–508.

Devinny, J. S., Deshusses, M. A., Webster, T. S., 2000. Controlling factors and operation of biofilters. Biofiltration for air pollution control. CRC Press LLC, Boca Raton. 62.

Eaton, A. D., Clesceri, L. S., Greenberg, A. E., 1995. Standard Methods for the Examination of Water and Wastewater (19th ed.). American Public Health Associaion, Washington DC. 4-83-4-91.

Fang, J., Lovanh, N., Alvarez, P. J. J., 2004. The use of isotopic and lipid analysis techniques linking toluene degradation to specific microorganisms: Applications and limitations. Water Res. 38(10), 2529–2536.

Fontenot, Q., Bonvillain, C., Kilgen, M., 2007. Effects of temperature, salinity, and carbon:nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. Bioresour. Technol. 98(9), 1700–1703.

Guo, W., Feng, J., Geng, W., Song, C., Wang, Y., Chen, N. et al., 2012. Augmented production of alginate oligosaccharides by the Pseudomonas mendocina NK-01 mutant. Carbohydr. Res. 352, 109–116.

Heinaru, E., Merimaa, M., Viggor, S., Lehiste, M., Leito, I., Truu, J. et al., 2005. Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area. FEMS Microbiol. Ecol. 51(3), 363-373.

Hu, B. L., Zheng, P., Li, J. Y., Xu, X. Y., Jin, R. C., 2006. Identification of a denitrifying bacterium and verification of its anaerobic ammonium oxidation ability. Sci. China Ser. C-Life Sci. 49(5), 460–466.

Jiang, R., Huang, S., Yang, J., 2008. Biological removal of NO*x* from simulated flue gas in aerobic biofilter. Global Nest J. 10(2), 241–248.

Jiang, R., Huang, S., Chow, A. T., Yang, J., 2009. Nitric oxide removal from flue gas with a biotrickling filter using *Pseudomonas putida*. J. Hazard. Mater. 164(2-3), 432–441.

Kao, C. M., Liu, J. K., Chen, Y. L., Chai, C. T., Chen, S. C., 2005. Factors affecting the biodegradation of PCP by *Pseudomonas mendocina* NSYSU. J. Hazard. Mater. 124(1-3), 68–73.

Kim, J. B., Cho, K. S., Jeong, S. K., Nam, S. W., Jeong, H., Kim, J. K., 2008. Identification and characterization of a pigment-producing denitrifying bacterium. Biotechnol. Biopr. Eng.13(2), 217–223.

Koschorreck, M., Moore, E., Conrad, R., 1996. Oxidation of nitric oxide by a new heterotrophic *Pseudomonas* sp. Arch. Microbiol. 166(1), 23–31.

Lee, B. D., Apel, W. A., Smith, W. A., 2001. Oxygen effects on thermophilic microbial populations in biofilters treating nitric oxide containing off-gas streams. Environ. Progr. 20(3), 157–166.

Li, G. Y., He, Z., An, T. C., Zeng, X. Y., Sheng, G. Y., Fu, J., 2008. Comparative study of the elimination of toluene vapours in twin biotrickling filters using two microorganisms *Bacillus cereus* S1 and S2. J. Chem. Technol. Biotechnol. 83(7), 1019–1026.

Liu, Q., Babajide, A. E., Zhu, P., Zou, L. P., 2006. Removal of xylene from waste gases using biotrickling filters. Chem. Eng. Technol. 29(3), 320–325.

Lochnar, R. H., Matar, J. E., 1990. Designing for quality: An introduction to the best of taguchi and western methods of statistical experimental design. Chapman and Hall, London, pp. 163–178.

Mohsen, M., 2005. Biological treatment of waste gases containing inorganic compounds. In: Shareefdeen, Z., Singh, A., (Eds.), Biotechnology for Odor and Air Pollution Control Springer, Berlin Heidelberg. pp. 253–279.

Nercessian, O., Fouquet, Y., Pierre, C., Prieur, D., Jeanthon, C., 2005. Diversity of *Bacteria* and *Archaea* associated with a carbonate-rich metalliferous sediment sample from the Rainbow vent field on the Mid-Atlantic Ridge. Environ. Microbiol. 7(5), 698–714.

Niu, H. Y., Leung, D. Y. C., 2010. A review on the removal of nitrogen oxides from polluted flow by bioreactors. Environ. Rev. 18, 175–189.

Pandey, R. A., Gangane, R., Mudliar, S. N., Rajvaidya, A. S., 2006. Treatment of waste gas containing monomethylamine in a biofilter enriched with Pseudomonas mendocina. Waste Manage. 26(3), 233–244.

Philip, L., Deshusses, M. A., 2008. The control of mercury vapor using biotrickling filters. Chemosphere 70(3), 411–417.

Rajwade, J. M., Salunkhe, P. B., Paknikar, K. M., 1999. Biochemical basis of chromate reduction by Pseudomonas mendocina. Process Metallurgy 9, 105-114.

Ramirez, A. A., Jones, J. P., Heitz, M., 2007. Biotrickling filtration of air contaminated with ethanol. J. Chem. Technol. Biotechnol. 82(2), 149–157.

Ramírez, M., Fernández, M., Granada, C., Le Borgne, S., Gómez, J. M., Cantero, D., 2011. Biofiltration of reduced sulphur compounds and community analysis of sulphur-oxidizing bacteria. Bioresource Technol. 102(5), 4047–4053.

Ramos, A. F., Gómez, M. A., Hontoria, E., González-López, J., 2007. Biological nitrogen and phenol removal from saline industrial wastewater by submerged fixed-film reactor. J. Hazar. Mater. 142(1-2), 175–183.

Ross, P. J., 1988. Taguchi Techniques for Quality Engineering: Loss Function, Orthogonal Experiments, Parameter and Tolerance Design. McGraw-Hill, New York. 227.

Ryu, H. W., Cho, K. S., Lee, T. H., 2011. Reduction of ammonia and volatile organic compounds from food waste-composting facilities using a novel anti-clogging biofilter system. Bioresour. Technol. 102(7), 4654–4660.

Song, B., Ward, B. B., 2003. Nitrite reductase genes in halobenzoate degrading denitrifying bacteria. FEMS Microbiol. Ecol. 43(3), 349–357.

Taguchi, G., 1987. System of Experimental Design: Engineering Methods to Optimize Quality and Minimize Costs. UNIPUB/Kraus International Publications, White Plains. pp. 117–142.

Tayyeb, J.M., Nimmo, W., Mahmood, A., Irfan, N., 2009. Effect of oxygenated liquid additives on the urea based SNCR process. J. Environ. Manage. 90(11), 3429–3435.

Tian, L., Ma, P., Zhong, J. J., 2003. Impact of the presence of salicylate or glucose on enzyme activity and phenanthrene degradation by *Pseudomonas mendocina*. Process Biochem. 38(8), 1125–1132.

van der Maas, P., van den Brink, P., Utomo, S., Klapwijk, B., Lens, P., 2006. NO removal in continuous BioDeNOx reactors: Fe(II)EDTA(2-) regeneration, biomass growth, and EDTA degradation. Biotechnol. Bioeng. 94(3), 575–584.

van Spanning, R. J. M., Richardson, D. J., Ferguson, S. J., 2007. Introduction to the Biochemistry and Molecular Biology of Denitrification. In: Bothe, H., Ferguson, S. J., Newton, W. E., (Eds.), Biology of the Nitrogen Cycle. Elsevier, Amsterdam. 3.

Wang, J. D., Wu, C. Q., Chen, J. M., Zhang, H. J., 2006. Denitrification removal of nitric oxide in a rotating drum biofilter. Chem. Eng. J. 121(1), 45–49.

Wong, C. F., Niu, H., Jiang, J. W., Li, J., Chan, C. M. N., Leung, D. Y. C., Leung FCC, 2012. Genome sequence of *Pseudomonas mendocina* DLHK, isolated from a biotrickling reactor. J. Bacteriol. 194(22), 6326.

Yang, W. F., Hsing, H. J., Yang, Y. C., Shyng, J. Y., 2007. The effects of selected parameters on the nitric oxide removal by biofilter. J. Hazard. Mater. 148(3), 653–659.

Ye, R. W., Averill, B. A., Tiedie, J. M., 1994. Denitrification: production and consumption of nitric oxide. Appl. Environ. Microbiol. 60(4): 1053–1058.

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