Field Applications for NOx Removal from Flue Gas in a Biotrickling Filter by *Chelatococcus daeguensis* TAD1

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**ABSTRACT**

In this study, effects of the initial nitrate, oxygen and carbon sources on the nitrification and denitrification rate were studied in a lab-scale biotrickling filter (l-BF) inoculated with *Chelatococcus daeguensis* TAD1, and the maximum nitrification and denitrification rate achieved to 30.08 mg-N/L/h and 100.8 mg-N/L/h, respectively. Thereafter, *C. daeguensis* TAD1 was initially inoculated into a pilot-scale biotrickling filter (p-BF) to remove NOx from the real flue gas of a coal-fired power plant, and a high removal efficiency of 86.7% at about 45–50°C was obtained. Analysis by PCR-DGGE showed that TAD1 was predominant in the biofilm of l-BF, whereas TAD1 in the biofilm of p-BF coexisted with other microbes to remove NOx together. Overall, the present study demonstrated that *C. daeguensis* TAD1 was firstly found to be one of the best candidates for the efficient treatment of NOx on a large scale under high flue gas temperature.

**Keywords:** Biotrickling filter; Aerobic denitrification; *Chelatococcus daeguensis* TAD1; Flue gas; NOx.

**INTRODUCTION**

Emissions of nitrogen oxides (NOx, mainly including NO and NO2) have a detrimental effect on ecosystems and human health (Lawrence and Crutzen, 1999). NOx, together with sulfur dioxide (SO2) are hazardous air pollutants that lead to the formation of acid rain, airborne particulates, and increased ground level ozone. Recently, NOx is growing in intensity, due to the burning of a large number of fossil fuels like in power plants and traffic vehicles. Technologies used to treat NOx usually refer to two methods: (1) conventional physico-chemical control systems with the major drawbacks of high operating cost and of possibility of the secondary pollution (Flanagan et al., 2002; Jin et al., 2005); (2) biological techniques with characteristics of high efficiency and low pollution. As a result, microbial methods have attracted more and more attention, but most researches are restricted to using the simulated flue gas without consideration of the complexity of real flue gases (Okuno et al., 2000; Jin et al., 2005; Van der Maas et al., 2005; Li et al., 2007; Yang et al., 2007). My lab had utilized a small pilot-plant of biotrickling filter to make sure the feasibility of removing NOx from real flue gases, but the predominating bacteria of the biofilm in the filter showed a shift (Jiang et al., 2009), suggesting that there was possibly potential instability easily influenced by flue gas environments. Once the predominating microorganisms are replaced by those with low denitrification performance, the removal efficiency of NOx would decrease. Accordingly, it is very important to explore a stable strain with a high performance of denitrification and a strong ability to adapt to the flue gas environments.

*Chelatococcus daeguensis* TAD1 is a novel aerobic strain. When it was applied to a biotrickling filter used to remove NOx from simulated flue gas, an efficient removal of NOx was obtained in the filter (Liang et al., 2012; Yang et al., 2012), but whether TAD1 is able to adapt to the real flue gas environment remains unknown. Hence, a lab-scale biotrickling (l-BF) filter inoculated with TAD1 was set up. Considering the processing cost, the effects of nitrate concentration, oxygen and carbon sources were studied to optimize the treatment operation. Then, TAD1 was inoculated into a pilot-scale biotrickling filter (p-BF) where a mature film formed and the flue gas from a coal-fired power plant was injected to investigate the feasibility of removing NOx by TAD1 on a large scale.

**MATERIALS AND METHODS**

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Microorganisms and Media

*C. daeguensis* TAD1 was stored in 30% glycerol at \(-20^\circ\text{C}\). The culture was maintained at 4°C on agar slants.

*C. daeguensis* TAD1 was maintained on agar slants containing the following components (g/L): 1.5 KNO₃, 6 sodium succinate, 1 Na₂HPO₄, 1 KH₂PO₄, 0.2 MgSO₄, 1 peptone and 15 agar at pH 7.5. The slants were incubated at 50°C for 36 h and then stored at 4°C. The seed medium modified from Merck medium consisted of (g/L): 1.5 sodium succinate, 1.5 KNO₃, 8.6 peptone and 6.4 NaCl at pH 7.5. The slants were incubated at 50°C for 36 h and then stored at 4°C. The seed medium was cemented into the biofilter column to provide a packed bed volume of about 50 m³, and the flow rate of recycling liquid ranged from 1000 to 5000 L/h. The flue gas employed in this research was produced by a 2 × 125 MW electricity generating unit installed with low NOₓ burners, generally containing 5–10% (v/v) O₂, 67–78% (v/v) N₂, 10–14% (v/v) CO₂, 6–8% (v/v) H₂O, 298.5–893.8 mg NOₓ/m³ (including 12.3–35.3 mg NO₂/m³), 34.7–228 mg SO₂/m³ and 23–50 mg total particulate matter/m³. The temperature of the flue gas in the p-BF system was about 45–50°C.

After the biofilm of the p-BF system grew up, the main flow rate of the flue gas fluctuated around 10000 m³/h.

**Biotrickling Filter System Setup**

The l-BF system and packing materials used in this study were performed as previously described (Yang et al., 2012). Concisely, the total height was 50 cm with an inner diameter of 8 cm and a bed depth of 30 cm. It mainly consisted of a BF (A) filled with packing materials and a reservoir (B) containing the recycling liquid. The packing materials were composed of porous ceramic beads with an average diameter of 2–3 mm (void fraction is about 0.35) and polyhedral spheres made of polypropylene with a diameter of 5 cm (void fraction is about 0.9). In order to reduce the pressure drop and increase colonization, these two materials by an equal volume were mixed together.

The p-BF system is shown in Fig. 1(b), main principles and constructions of which were similar to the l-BF. Polyhedral spheres with a specific surface area of 500 m²/m³ were packed in the biofilter column to provide a packed bed volume of about 50 m³, and the flow rate of recycling liquid ranged from 1000 to 5000 L/h. The flue gas employed in this research was produced by a 2 × 125 MW electricity generating unit installed with low NOₓ burners, generally containing 5–10% (v/v) O₂, 67–78% (v/v) N₂, 10–14% (v/v) CO₂, 6–8% (v/v) H₂O, 298.5–893.8 mg NOₓ/m³ (including 12.3–35.3 mg NO₂/m³), 34.7–228 mg SO₂/m³ and 23–50 mg total particulate matter/m³. The temperature of the flue gas in the p-BF system was about 45–50°C.

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**Calculation of Nitrification Rate, Denitrification Rate and Nitrate Removal Rate**

For the calculation of denitrification rate, nitrification rate and nitrate removal rate, the following equations were defined:

\[
DR = \frac{C_{10}}{H} \quad (1)
\]

\[
NR = \frac{C_{10} - C_{10}}{H} \quad (2)
\]

Fig. 1. Schematic of the biotrickling filter (a) l-BF (b) p-BF.
where DR, NR and NRR respectively represent denitrification rate, nitrification rate and nitrate removal rate, $C_{v0}$ represents the initial NO$_3^-$-N concentration in the recycling liquid, $C_{v0}$ and $C_{v0}$ are the respective initial and final NH$_4^+$-N concentrations in the recycling liquid, $H$ represents the time when nitrate was consumed completely as well as without nitrite accumulation, and $H_1$ represents the time when nitrate was consumed completely but regardless of nitrite accumulation.

**PCR-DGGE**

For pure cultures, 1.5 mL of log phase cultures was centrifuged for 3 min at 12000 rpm. The pellet was used for DNA extractions with a bacterial genomic DNA isolation kit (Bioteke Corporation, Beijing, China). For biofilm samples, direct DNA extractions were performed by using a soil DNA isolation kit (Bioteke Corporation, Beijing, China). The kits were used according to the manufacturer’s instructions. Extracted DNA was dissolved in the elution buffer provided by the kits and quantified by measuring its absorbance at 260 nm with a spectrophotometer. The quality of the extracted DNA was analyzed by electrophoresis on a 1.0% agarose gel.

PCR was performed in a Mastercycler gradient (Eppendorf 5331, Germany) using the following primers: GC341F (5'-CGC CGC CGG CCC CCC CGG CCC CGG CCC CGG CCC CGG CCC CCT AGC GG A GGC AGC AG -3') and 907R (5'-CCG TCA ATT CCT TTG AGT TT -3'). DGGE was carried out in a Universal Mutation Detection System (BIO-RAD DCode, USA). The gel contained a gradient of denaturant ranging from 30% to 60% (100% denaturant is 7 M urea and 40% deionized formamide). DGGE was run at 200 V for 5 h at 60°C. After electrophoresis, the gel was stained with UltraPowerTM (Bioteke Corporation, Beijing, China) for 30 min and viewed with a UV transilluminator (BIO-RAD, Italy).

**Analytical Methods**

NO$_3^-$-N, NO$_2^-$-N and NH$_4^+$-N were determined based on standard procedures as described in *Standard Methods* (APHA, 1992). The NO, NO$_2$ and O$_2$ concentrations at both the inlet and outlet were analyzed with a flue gas analyzer (TESTO pro 350, Germany).

**RESULTS AND DISCUSSION**

**Nitrite Accumulation in the Recycling Liquid**

Nitrite accumulation is commonly used to remove ammonium nitrogen from wastewater by the biological nitrification-denitrification process. More and more studies suggested that nitrite accumulation arose from a combination of factors, including carbon substrates (Kelso et al., 1999), dissolved oxygen (DO) (Ruiz et al., 2006), temperature (Kim et al., 2006), ammonium levels and pH (Park et al., 2009). However, the present study implied that DO, pH and temperature had no remarkable influence on the nitrite accumulation (data not shown). The concentration of nitrate, nitrite and ammonium in the recycling liquid at 26 days as a function of time is shown in Fig. 2(a). As seen from the figure, the nitrate concentration was decreased from 274 mg-N/L to 10 mg-N/L in the first 2hs, and then was decreased to 0 at 4h without nitrite accumulation. Although the nitrate concentration was increased to 362 mg-N/L at 6h, it was still decreased to 0 without nitrite accumulation at 19h. After that, however, when the nitrate concentration was enhanced to 157 mg-N/L, it was reduced to 96.2 mg-N/L at 23h and then maintained that level. Similarly, the ammonium concentration was gradually reduced from 324 mg-N/L at 0 h to 53 mg-N/L at 23h and then it stopped reducing. These results may be attributed to the following reasons. Firstly, the aerobic microorganism grows faster than the anaerobic one, and some studies suggested that denitrification mainly occurred in the logarithmic phase (Park et al., 2009). However, the present study implied that DO, pH and temperature had no remarkable influence on the nitrite accumulation (data not shown). The concentration of nitrate, nitrite and ammonium in the recycling liquid at 26 days as a function of time is shown in Fig. 2(a). As seen from the figure, the nitrate concentration was decreased from 274 mg-N/L to 10 mg-N/L in the first 2hs, and then was decreased to 0 at 4h without nitrite accumulation. Although the nitrate concentration was increased to 362 mg-N/L at 6h, it was still decreased to 0 without nitrite accumulation at 19h. After that, however, when the nitrate concentration was enhanced to 157 mg-N/L, it was reduced to 96.2 mg-N/L at 23h and then maintained that level. Similarly, the ammonium concentration was gradually reduced from 324 mg-N/L at 0 h to 53 mg-N/L at 23h and then it stopped reducing. These results may be attributed to the following reasons. Firstly, the aerobic microorganism grows faster than the anaerobic one, and some studies suggested that denitrification mainly occurred in the logarithmic phase (Park et al., 2009). However, the present study implied that DO, pH and temperature had no remarkable influence on the nitrite accumulation (data not shown). The concentration of nitrate, nitrite and ammonium in the recycling liquid at 26 days as a function of time is shown in Fig. 2(a). As seen from the figure, the nitrate concentration was decreased from 274 mg-N/L to 10 mg-N/L in the first 2hs, and then was decreased to 0 at 4h without nitrite accumulation. Although the nitrate concentration was increased to 362 mg-N/L at 6h, it was still decreased to 0 without nitrite accumulation at 19h. After that, however, when the nitrate concentration was enhanced to 157 mg-N/L, it was reduced to 96.2 mg-N/L at 23h and then maintained that level. Similarly, the ammonium concentration was gradually reduced from 324 mg-N/L at 0 h to 53 mg-N/L at 23h and then it stopped reducing. These results may be attributed to the following reasons. Firstly, the aerobic microorganism grows faster than the anaerobic one, and some studies suggested that denitrification mainly occurred in the logarithmic phase (Park et al., 2009). However, the present study implied that DO, pH and temperature had no remarkable influence on the nitrite accumulation (data not shown). The concentration of nitrate, nitrite and ammonium in the recycling liquid at 26 days as a function of time is shown in Fig. 2(a). As seen from the figure, the nitrate concentration was decreased from 274 mg-N/L to 10 mg-N/L in the first 2hs, and then was decreased to 0 at 4h without nitrite accumulation. Although the nitrate concentration was increased to 362 mg-N/L at 6h, it was still decreased to 0 without nitrite accumulation at 19h. After that, however, when the nitrate concentration was enhanced to 157 mg-N/L, it was reduced to 96.2 mg-N/L at 23h and then maintained that level. Similarly, the ammonium concentration was gradually reduced from 324 mg-N/L at 0 h to 53 mg-N/L at 23h and then it stopped reducing. These results may be attributed to the following reasons. Firstly, the aerobic microorganism grows faster than the anaerobic one, and some studies suggested that denitrification mainly occurred in the logarithmic phase (Park et al., 2009). However, the present study implied that DO, pH and temperature had no remarkable influence on
shown in Fig. 3, it seemed that denitrification and nitrification rate elevated with increasing the initial nitrate concentration, except 24 days at which both denitrification rate and nitrification rate were decreased sharply when the initial nitrate concentration was increased from 161 to 522 mg-N/L suddenly, probably due to the fact that microorganism did not acclimate to such high nitrate concentration. Therefore, the initial nitrate concentration was gradually increased from 274 mg-N/L at 26 days up to 828 mg-N/L at 30 days, but a maximum denitrification and nitrification rate of 100.8 mg-N/L/h and 30.08 mg-N/L/h was obtained at 29 days (the corresponding nitrate concentration was 625 mg-N/L) after which they remained steady, which suggested that the initial nitrate concentration in the recycling liquid had no effect on the denitrification and nitrification rate, and the tendency that the denitrification and nitrification rate elevated with the time elapsing resulted from maturation of the biofilm not increase of the initial nitrate concentration.

Effect of the Oxygen on the Nitrification and Denitrification Rate

Many studies demonstrated that oxygen and nitrate could be utilized simultaneously by aerobic microorganisms, which suggests that the denitrifying enzyme system and the oxygen respiration system function in parallel, meaning that oxygen does not directly inhibit the expression of the denitrifying genes and the activity of the denitrifying enzymes. On the contrary, as shown in Table 1, denitrification rate rose from 35.3 to 40.3 mg-N/L/h with increasing the
air flow rate from 0 to 0.67 min⁻¹ in l-BF system, and more evidently, nitrate removal rate rose from 53 mg-N/L/h at a air flow rate of 0 to 73 mg-N/L/h at a air flow rate of 0.67 min⁻¹. Probably, these results were ascribed to the periplasmic nitrate reductase, which can reduce nitrate to nitrite under aerobic and anaerobic conditions (Reyes et al., 1996; Gavira et al., 2002), and apparently, the periplasmic nitrate reductase of C. daeguensis TAD1 preferred to aerobic environment. By the way, our previous experiments confirmed that C. daeguensis TAD1 only contained the periplasmic nitrate reductase gene, the partial sequence of which had been sequenced. As far as the nitrification rate is concerned, it is reasonable that the nitrification rate increased with increasing air flow rate in l-BF system.

**Effects of the Different Carbon Sources on the Nitrification and Denitrification Rate**

The carbon source plays an important role in denitrifying and nitrifying, but each microorganism has a different response to the different carbon source. Our previous results in batch culture showed that C. daeguensis TAD1 was able to utilize many kinds of carbon sources to denitrify. Taking glucose, sodium acetate, sodium citrate and sodium succinate for example, the denitrifying efficiency went downward in the following order: sodium succinate > sodium citrate > sodium acetate > glucose. Nevertheless, it appears that sodium succinate is unsuitable for carbon source on a large scale, and thus, effect of the four mentioned carbon sources on the nitrification and denitrification rate in l-BF system was investigated. Interestingly, as presented in Table 2, the denitrifying rate lowered in the following order: sodium citrate > sodium acetate > sodium succinate > glucose, which was different from that in batch culture except for glucose contributing to rather low denitrification rate. From the table, the denitrification rate achieved by sodium citrate and sodium acetate was 48.2 mg-N/L/h and 45 mg-N/L/h, respectively, whereas the nitrification rate achieved by sodium acetate was 37.9 mg-N/L/h that was much higher than that achieved by the other three carbon sources. The reasons responsible for these results are: (i) the microenvironment in l-BF system was quite distinct from that in batch culture, such as DO, pH, mass transfer and dynamics. (ii) although C. daeguensis TAD1 was predominant, other microorganisms also inhabited in l-BF system (Fig. 5(a)), probably influencing each other.

**Long-Term Operation in the Pilot-Scale Biotrickling Filter**

Compared to the lab-scale BF treating the simulated flue gas, the pilot-scale BF has a high capacity to remove NOx from the real flue gas, which could increase the processing cost. Therefore, in order to make TAD1 predominant, the two-third recycling liquid was replaced every three days, and all the recycling liquid was refreshed every six days with the pure cultures of TAD1 during the startup. Consequently, a thin biofilm was seen in the packing bed after 18 days, and nitrate removal efficiency achieved to 70%. From 21 days on, the flue gas was injected into p-BF accompanied with the gradually decreased nitrate concentration with an aim to adapt TAD1 to the flue gas environment, which could help microorganisms change the denitrification pathway starting at NO3⁻ to that starting at NO. Although nitrate is a good inducer favoring denitrification genes expression, too much would inhibit the NO reduction to some extent, meaning that a few nitrates probably contribute to the NO removal. Considering that the flue gas usually contained about 5% NO2 which can be converted to NO3⁻ and NO2⁻ in the water, nitrate in the recycling liquid was reduced to zero at last.

It is known that the gas empty bed retention time (EBRT) is an important factor affecting the NOx removal (Liang et al., 2012; Yang et al., 2012). In this study, EBRT was about 50–60 s because of complicate environments such as nutrients and flue gas fluctuation. Also, owing to the wide fluctuation of flue gas, the time that flue gas was injected every day added up to 10 hours and the NOx concentrations of both inlet and outlet were recorded, then the average was calculated every seven days, namely that the NOx removal efficiency (RE) was investigated using a week as a unit, and results were shown in Fig. 4. It can be seen that the NOx RE grew up from 76.4% at week 1 to 76.4% at week 7. Notwithstanding RE dropped from 78.2% at week 5 to 72.8% at week 6, RE improved on the whole, suggesting that the growth of biofilm was a process, during which the biofilm adapted itself to NOx step by step and matured gradually. Similar to week 5, RE decreased from 92.4% at week 7 to 83.4% at week 10. Probably, this phenomenon resulted from the reason that the pollu
loads elevated and therefore the biofilm needed an adaptive phase. However, compared to week 11, RE at week 12 did not increase significantly, indicating that this system had reached the maximum capacity of treating NO\textsubscript{x}. Moreover, as shown in Fig. 4, the p-BF got into the stationary phase at week 8, and RE ranged between 84.3\% and 86.7\% from week 10 on. During the whole operation, the maximum pollutional loads of 159.4 g/(m\textsuperscript{3}\cdot h) and processing capacity of 137.3 g/(m\textsuperscript{3}\cdot h) appeared at week 11, and the corresponding inlet concentration of NO\textsubscript{x} was 558 mg/m\textsuperscript{3}.

**Stability of TAD1 in the Biotrickling Filter**

In order to verify the stability of TAD1 in the biofilm, PCR and DGGE were conducted on the 16s rDNA isolated from the culture in the biofilm and the pure stain in the medium, and the results were shown in Fig. 5. It is very clear that although some unknown strains can be seen in lane of 15d and 30d, TAD1 absolutely dominated in the biofilm all the time in the lab-scale biotrickling filter (Fig. 5(a)). One reason is that the recycling liquid inoculated with 10\% pure TAD1 was regenerated every day, which always made TAD1 far more predominant than other strains. The other reason is that the high temperature inhibited the growth of many other microorganisms.

The above study had demonstrated that TAD1 was stable and predominated in the biofilm (Fig. 5(a)). In comparison, components in the real flue gas are rather complicated, and the environment in the power plant could affect significantly the selection and growth of microbes. Jiang et al studied the biofilm in the real flue gas and found that microorganisms in the film altered (Jiang et al., 2009). The predominant strains residing in the film were Klebsiella sp. and Pseudomonas sp, showing that the environment factor is crucial to the biofilm. Thus, using the biofilm of the last week before injecting the flue gas and of 12th week after injecting the flue gas as a research object, effect of a real flue gas environment on TAD1 in the biofilm was investigated. Fig. 5(b) showed the change trend of some strains. Clearly, TAD1 predominated in the biofilm before injecting the flue gas, but an unknown strain with the same luminance as TAD1 also existed. Despite the fact that the fresh pure cultures TAD1 inoculated intermittently and high
temperature inhibiting the growth of most microbes helped TAD1 predominate in the biofilm, complicate environments also naturally selected the adapted microorganisms (e.g., strain A). However, three months after the flue gas was injected, there were some changes in strains of the biofilm: (1) TAD1 and strain A were both weakened, and especially strain A was nearly eliminated. (2) Some new strains appeared, in which strain B was more predominant than others. These changes were attributed to the reason that environments after injecting the flue gas was different that before injecting the flue gas, thus leading to a completely different metabolic pathway. On the other hand, complicate components in the real flue gas like ash and heavy metals could have an important effect on the biofilm. Although TAD1 grew down quantitatively in week 12, there were not absolutely predominant strains in the biofilm. TAD1 coexisted and coordinated with other microbes to remove NOx together. Also, it can be seen from the figure that strain C and D resided in the biofilm all the time, and interestingly, environmental changes did not affect them, the reasons of which should be further investigated.

CONCLUSION

A maximum nitrification and denitrification rate of 30.08 mg-N/L/h and 100.8 mg-N/L/h was obtained, respectively, in l-BF system where TAD1 remained predominant. In p-BF system, NOx from flue gas of a coal-fired power plant was efficiently removed, the removal efficiency of which achieved to 86.7%. Although TAD1 did not predominate absolutely, it coexisted and coordinated with other bacteria to remove NOx together. In summary, TAD1 was initially found to be a good candidate for NOx removal from flue gas of a coal-fired power plant on a large scale.

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