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Microbial removal of NO_X at high temperature by a novel aerobic strain *Chelatococcus daeguensis* TAD1 in a biotrickling filter

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ABSTRACT

The removal of NO_X at high temperature by *Chelatococcus daeguensis* TAD1 in a biotrickling filter was studied. Media components of the recycling liquid were screened using Plackett–Burman design and then were optimized using response surface methodology, which enhanced the efficiency of nitrate removal by TAD1. The optimal medium was used to perform long-term experiments of NO_X removal in a biotrickling filter under high concentrations of O_2 and NO in simulated flue gas. Results showed that the biotrickling filter was able to consistently remove 80.2–92.3% NO_X when the inlet NO concentration was 600 ppm under the conditions of oxygen concentration ranging between 2% and 20% and empty bed residence time (EBRT) being 112.5 s. Analyses by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR–DGGE) indicated that TAD1 was always predominant in the biofilm under a flue gas environment. Overall, the present study demonstrated that utilizing a biotrickling filter inoculated with the aerobic denitrifier TAD1 to remove NO_X at high temperature was practically feasible.

1. Introduction

Nitrogen oxides (NO_X) are hazardous pollutants leading to the formation of the acid rain and the elevation of the ground level ozone [1]. Recently, emissions of NO_X are undergoing severe increase, thus needing the effective technology to decrease its concentration in the air. However, traditional methods used to control NO_X emissions from power plant, such as selective catalytic and non-catalytic reduction, usually require high temperature and the use of catalysts, therefore resulting in high costs. In comparison, the biological method by which nitrogen oxides are converted to nitrogen gas is a promising alternative. Among these methods, a biotrickling filter (BF), usually comprising inert packing material which can provide sufficient surface area for gas/liquid mass transfer and biofilm growth, is a good example and has been applied to the waste gas treatment [2,3], particularly to flue gas. Flue gas from a power plant consists of CO_2, SO_X, NO_X and O_2, the primary pollutants of which are SO_X and NO_X, with insoluble NO being about 90% [4,5]. Moreover, the temperature of flue gas after wet desulfuration is about 50 °C, but most studies suggested that the optimum temperature at which denitrification occurred ranged between 20 and 40 °C [6,7], and reports on NO_X removal at high temperature are scarce. Herein, it is valuable to explore a novel strain suitable for high temperature to remove NO_X in flue gas.

Traditionally, denitrification has commonly been known to occur only under limiting-oxygen conditions [8–10], which was well evidenced by the denitrifying bacteria *Paracoccus denitrificans* [11] and *Pseudomonas denitrificans* [12] in an anaerobic environment. As far as NO_X treatment is concerned, many studies indicate that the oxygen in flue gas inhibits NO_X removal and reduces removal efficiency [13,14]. However, with reports on denitrifying bacteria increasing, aerobic denitrification attracted a lot of attention for easier operation and higher denitrification rate than anaerobic denitrification. Accordingly, both pure strain [7,15] and biofilm [16] were investigated in detail, especially in the BF system inoculated with aerobic denitrifier. Jiang et al. [17] isolated an aerobic denitrifying strain SB1, and then it was inoculated to a BF system. Results showed that 82.9–94.2% NO was removed under the conditions of the inlet NO concentration being 400 ppm and oxygen concentration ranging between 2% and 20%. In order to remove nitrogen oxides (NO_X) from the flue gas of a coal-fired power plant, a biotrickling filter packed with polyhedral spheres was used and consistently removed 64–95% of the NO_X after start-up and acclimation under dynamic conditions [18].

It is known that there are a lot of factors affecting the denitrification rate such as temperature, pH, dissolved oxygen (DO),

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C/N ratio and so on, and most studies focused on these factors [7,19,20]. However, it is worth noticing that the effects of media constituents on the denitrification rate were ignored. The recycling liquid which is an important part of BF plays a critical role in removing NO_X, because it not only supplies the energy to the biofilm but also influences the activity of the biofilm. Meanwhile, as far as we know, the potential of *Chelatococcus daeguensis* TAD1 for NO_X removal from flue gas has not yet been explored. Therefore, in the present study, medium components of the recycling liquid in BF system were first optimized using Plackett–Burman design [21] plus response surface methodology (RSM) [22]. Then, the optimized medium inoculated with *C. daeguensis* TAD1 was applied to the BF system to evaluate the denitrifying capability, stability and NO_X removal efficiency of TAD1 in BF system.

2. Materials and methods

2.1. Microorganism

C. daeguensis TAD1 used in this study was newly isolated [23], the nucleotide sequence of the 16S rRNA gene of which was 1385 bp (Accession No. HM000004). *C. daeguensis* TAD1 was stored in 30% glycerol at −20 °C. The culture was maintained at 4 °C on agar slants.

2.2. Medium

C. daeguensis TAD1 was maintained on agar slants containing the following components (g L^{-1}): KNO_3 1.5, sodium succinate 6, Na_2HPO_4 1, KH_2PO_4 1, $MgSO_4$ 0.2, peptone 1 and agar 15 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}): sodium succinate 1.5, KNO_3 1.5, peptone 8.6, NaCl 6.4 at pH 7.5. The seeds cultivation was conducted in 150-ml shake flasks for 12 h with 200 rpm at 50 °C.

2.3. Experimental design and data analysis

In this section, nitrate is selected as the response value due to the fact that nitrate can be measured accurately and is an essential factor of inducing the denitrification enzyme genes [24].

2.3.1. Plackett–Burman design

The Plackett–Burman design is an efficient way for screening the important factors among a great number of variables. Therefore, it was used to evaluate the relative importance of various nutrients for denitrification by *C. daeguensis* TAD1 in this part. Table 1 illustrates the experimental designs with the name, symbol code, and actual levels of the variables in the study, whereas the detail of the design is presented in Table 2. 12 experimental runs, where the denitrification was carried out for 9 h, were used to screen the significant factors of six variables. In all the experimental runs that were performed in duplicates using the initial nitrate concentration of 1 g L^{-1}, KH_2PO_4 concentration of 1.5 g L^{-1} and Na_2HPO_4 concentration of 0.8 g L^{-1}, denitrification rate was recorded as the response that was simply calculated from the following equation:

$$D = \frac{C_0 - C}{H} \quad (1)$$

where D is the denitrification rate (mg L^{-1} h^{-1}), C_0 and C are, respectively, the initial and final NO_3^-–N concentrations (mg L^{-1}), and H represents the time (h). Then the variables with P less than 0.05 were used for further optimization.

2.3.2. Central composite designs and response surface methodology

On the basis of the results obtained from Plackett–Burman design for screening the media constituents, response surface methodology (RSM) based on central composite design (CCD) with five levels was employed to determine the optimal conditions of the screened factors. The level of each factor and the design matrix are shown in Table 3.

All the optimization experiments were conducted in 50-ml shake flasks with 30 ml medium for 12 h with 120 rpm at 50 °C. Seed culture (5%, v/v) was inoculated to the denitrifying media. MINITAB 15.0 (Minitab Inc., Pennsylvania, USA) was used for the experimental designs and subsequent regression analysis of the experimental data. To evaluate the analysis of variance, statistical analysis of the model was performed. The quality of the polynomial model equation was judged statistically according to the coefficient of determination R^2, and the significance of the regression coefficients was tested by a t-test. All experiments were repeated at least three times.

2.4. Biotrickling filter system (BF)

The BF system used in this study is displayed in Fig. 1, the total height of which 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 material, a reservoir (B) containing the recycling liquid and a humidifier (C) used to mix N_2, NO and O_2. The outside of the BF was wrapped with heat tape controlled by a digital temperature controller, and the reservoir was loaded with 2 L recycling liquid, the temperature of which was controlled by a water jacket. Another water jacket controlled the temperature of the humidifier. BF, reservoir and humidifier were all operated at 50 ± 1 °C. The recycling liquid supplied to the top by a peristaltic pump was set at a flow rate of 90 ml min^{-1}, and the flux of NO, N_2 and O_2 controlled by a mass flow controller (MFC, D07-7B, Sanxing, China) flowed from the bottom to the top.

Packing materials used in this BF system were porous ceramic beads with an average diameter of 2–3 mm (void fraction is about 0.35) and polyhedral spheres made of polypropylene. Although porous ceramic beads gave surface and retention properties suitable for microbe growth, their dense packing led to a remarkable pressure drop. By contrast, polyhedral spheres with a diameter of 5 cm (void fraction is about 0.9) caused a small pressure drop, though not favorable for microbe growth. Herein, the two packing materials of the same volume were mixed together to reduce the pressure drop and increase colonization.

2.5. DNA extraction

For pure cultures, 1.5 ml of log phase cultures was centrifuged for 3 min at 12,000 rpm. The pallet 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.

2.6. Polymerase chain reaction and denaturing gradient gel electrophoresis

For investigating the stability of TAD1 during the whole experiment, the polymerase chain reaction and denaturing gradient gel electrophoresis (PCR–DGGE) were conducted on the cultures in the

Table 1
Plackett–Burman design for screening variables in denitrification.

Factors (g L^{-1})	Variables	Low level (−1)	High level (+1)	Effect	Coef	*t*-Value	*P*-value
Sodium succinate	*X*1	4	16	−0.4026	−0.2013	−2.00	0.102
Ammonium citrate	*X*2	0.2	1	1.5518	0.7759	7.72	0.001
Magnesium sulfate	*X*3	0.1	0.5	0.2843	0.1422	1.41	0.217
Ammonium molybdate	*X*4	0	0.02	−0.1099	−0.0549	−0.55	0.608
Copper sulfate	*X*5	0	0.02	−0.4699	−0.2349	−2.34	0.067
Ferrous sulfate	*X*6	0	0.02	1.1926	0.5963	5.93	0.002

R^2 = 95.52%, R^2(adj) = 90.13%, Coef. = coefficient.

Table 2
Plackett–Burman design variables in coded levels with denitrification rate as response.

Run	Variable levels						Denitrification rate (mg L^{-1} h^{-1})
	*X*1	*X*2	*X*3	*X*4	*X*5	*X*6	
1	1	−1	1	−1	−1	−1	0.3317
2	1	1	−1	1	−1	−1	1.3358
3	−1	1	1	−1	1	−1	1.2083
4	1	−1	1	1	−1	1	0.7925
5	1	1	−1	1	1	−1	0.7650
6	1	1	1	−1	1	1	2.4983
7	−1	1	1	1	−1	1	3.3425
8	−1	−1	1	1	1	−1	0.2950
9	−1	−1	−1	1	1	1	0.7550
10	1	−1	−1	−1	1	1	0.6842
11	−1	1	−1	−1	−1	1	3.1208
12	−1	−1	−1	−1	−1	−1	0.1017

Table 3
Design and results based on CCD.

Run	Ammonium citrate		Ferrous sulfate		Denitrification rate (mg L^{-1} h^{-1})
	Code *X*1	*X*1 (g L^{-1})	Code *X*2	*X*2 (g L^{-1})	
1	−1	0.2	−1	0.005	0.4710
2	1	3.8	−1	0.005	1.5876
3	−1	0.2	1	0.075	3.4624
4	1	3.8	1	0.075	6.0095
5	−1.41421	0	0	0.04	2.0861
6	1.41421	4.545	0	0.04	5.0786
7	0	2	−1.41421	0	0.2955
8	0	2	1.41421	0.089	6.5655
9	0	2	0	0.04	7.2766
10	0	2	0	0.04	7.1534
11	0	2	0	0.04	7.3473
12	0	2	0	0.04	7.4059
13	0	2	0	0.04	7.3066

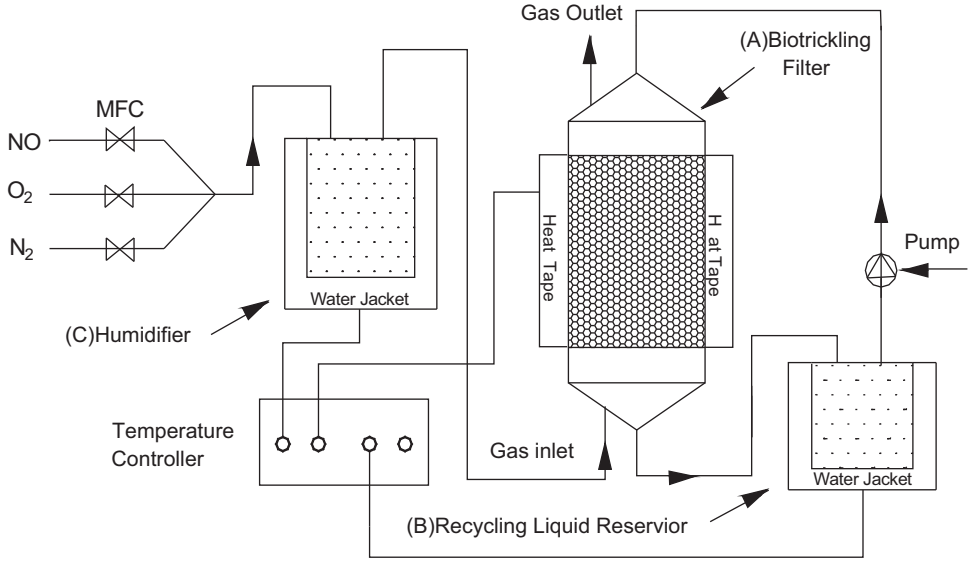

Fig. 1. Schematic of the biotrickling filter.

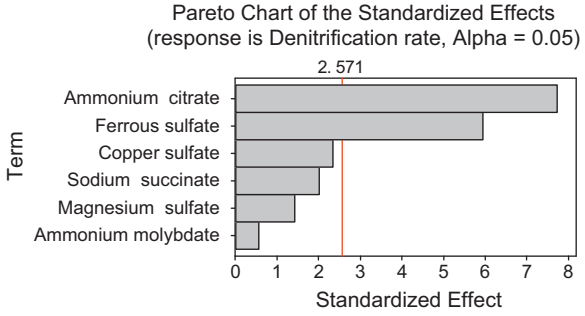

Fig. 2. Pareto chart of six factors standard effects on denitrification rate.

biofilm and the pure strain TAD1 in the medium. Chromosomal DNA from the pure culture TAD1 or the biofilm in the BF system was isolated, and then was used as a PCR template to amplify the 16S rRNA genes. PCR was performed in a Mastercycler gradient (Eppendorf 5331, Germany) using the following primers: GC341F (5′-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC G CCT ACG 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^{TM}, USA). The gel contained a gradient of denaturant raging 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 UltraPower^{TM} (Bioteke Corporation, Beijing, China) for 30 min and viewed with a UV transilluminator (BIO-RAD, Italy).

2.7. Analytical methods

NO_3–N and NO_2–N were determined based on standard procedures as described in *Standard Methods*. The biomass concentration was measured by the optical density (OD) at 480 nm in a spectrophotometer (TU-1810, CHINA). The NO, NO_2 and O_2 concentrations at both the inlet and outlet were measured with a flue gas analyzer (KANE 940, UK), which is also capable of determining the concentration of NO_X (NO plus NO_2).

3. Results and discussion

3.1. Plackett–Burman design

For the denitrification rate, 12 experiments were used to screen the significant factors from six variables in this section. The media constituents selected were sodium succinate, ammonium citrate, magnesium sulfate, ammonium molybdate, copper sulfate and ferrous sulfate, the lower and upper levels of which were chosen on the basis of the preliminary investigation.

The experimental data analysis showed that there was a wide variation of denitrification rate from 0.1017 to 3.3425 mg L^{-1} h^{-1} in the 12 experiments, manifesting that medium optimization was extremely important for enhancing dentrification rate. As shown in Table 1, ammonium citrate and ferrous sulfate had the greatest positive impacts on denitrification rate according to the analysis of the regression coefficients and the *P*-values of six factors. In addition, the *t*-value exhibited that magnesium sulfate had a positive effect on denitrification rate, whereas sodium succinate, ammonium molybdate and copper sulfate had a negative effect on denitrification rate. From the results, R^2 and R^2(adj) were found to be 95.52% and 90.13%, respectively, which indicated that the model could explain the total variation in the system well.

The Pareto chart (Fig. 2), in which the maximal effect was in the upper portion and then went down to the minimal effect, showed clearly that the most important factors affecting the denitrification rate were ammonium citrate and ferrous sulfate. Although it is uncertain whether ammonium citrate and ferrous sulfate could improve the activation of denitrifying enzymes or not, they are important for the strain growth. Hence, out of the seven media constituents, the two media constituents of ammonium citrate and ferrous sulfate were further chosen for optimizing their levels.

Table 4
Regression coefficients and their significance for response surface model.

Term	Coef.	Standard error	*t*-Value	*P*-value
Constant	−2.150	0.870	−2.472	0.043
*X*1	3.130	0.566	5.524	0.001
*X*2	204.060	29.188	6.991	0.000
$X1 \times X1$	−0.660	0.110	−5.985	0.001
$X2 \times X2$	−1844.420	289.171	−6.378	0.000
$X1 \times X2$	5.680	6.480	0.876	0.410

R^2 = 95.06, R^2(adj) = 91.52%.

3.2. Response surface methodology

Based on the above results, the significant independent variables (ammonium citrate and ferrous sulfate) were further optimized using central composite design (CCD). Their central points chosen for experimental designs were: ammonium citrate 2 g L^{-1} and ferrous sulfate 0.04 g L^{-1}, respectively. Table 3 lists the CCD and the corresponding response. With the experimental data subjected to multiple regression analysis, the following second-order polynomial equation was obtained, depicting the relationship between the denitrification rate (*Y*) and the test variables (the concentrations of ammonium citrate (*X*1) and ferrous sulfate (*X*2)).

$$Y = -2.15 + 3.13X1 + 204X2 - 0.657X1^2 - 1844X2^2 + 5.68X1X2 \quad (2)$$

Table 4 displays regression coefficients and their significance for response surface model. Generally, the *t*-test and *P*-values are used to identify the effect of each factor on the denitrification rate. A *P*-value below 0.05 indicates that the model terms were significant. As such, ammonium citrate and ferrous sulfate and their quadratic terms had significant effects on the denitrification rate ($P < 0.05$). Contrarily, the *P*-value above 0.05 of *X*1*X*2 suggested no obvious interaction of ammonium citrate and ferrous sulfate. From this model, the determination coefficient R^2 was found to be 95.06%, further showing that 95.06% of the variability in the response can be explained by the model. Meanwhile, the adjusted determination coefficient (adj R^2) was calculated to be 91.52% that was also high to support a high significance of the model. R^2 value of this model was higher than 0.9, which could be regarded as having a high correlation. Thus it was reasonable to use the regression model to analyze the trends in the response.

In this section, the 3D response surface and 2D contour plots were also employed to check the effects of ammonium citrate and ferrous sulfate on the denitrification rate. Specially, the 2D contour plots are graphic representation of relationship between response and experimental variables that can be used for determining the optimum conditions [25,26]. Fig. 3 exhibits main relationship between ammonium citrate and ferrous sulfate, the 3D response surface of which with conspicuous convexity suggested well-defined optimum conditions. Clearly, the 2D contour plots were almost circular, suggesting no obvious interaction of ammonium citrate and ferrous sulfate. According to the equation model and the plots, the optimal concentrations of ammonium citrate and ferrous sulfate were 2.62 g L^{-1} and 0.059 g L^{-1}, respectively. The corresponding predicted maximum denitrification rate was 8.0318 mg L^{-1} h^{-1}.

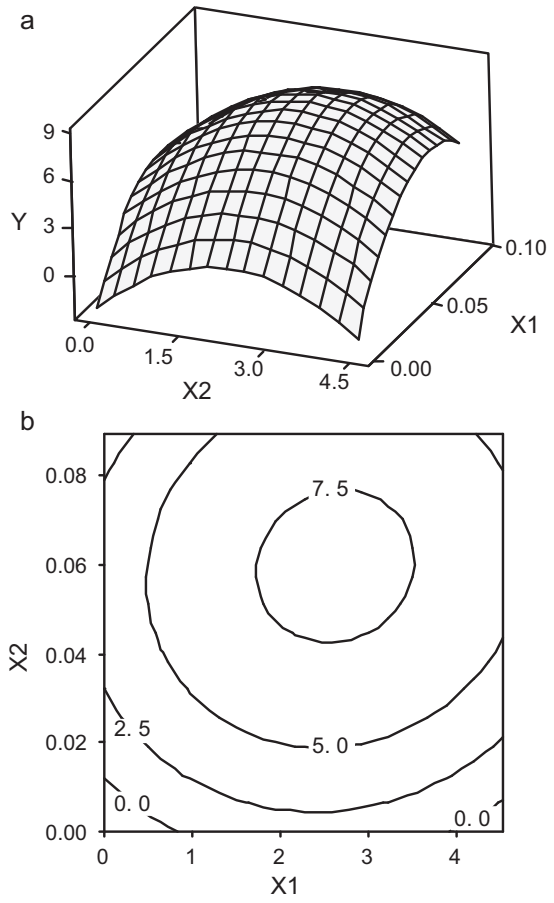

Fig. 3. Response surface figure (a) and corresponding contour (b) of the mutual effects of ammonium citrate ($X1$) and ferrous sulfate ($X2$) on denitrification rate.

3.3. Verification of the second-order polynomial equation

Based on the results of medium optimization, the optimal media constituents for denitrification rate by *C. daeguensis* TAD1 are as follows (g L^{-1}): sodium succinate 5, ammonium citrate 2.62, KNO_3 1, KH_2PO_4 1.5, Na_2HPO_4 0.8, $MgSO_4$ 0.2, $FeSO_4$ 0.059 and trace elements solution (consisting of ammonium molybdate and copper sulfate) 2 ml/1000 ml. With these components, the verification experiment was carried out in triplicates to confirm the availability of the model equation for predicting the maximal denitrification rate. Fig. 4 displays time course of nitrate, nitrite, OD_{480} and pH under the optimized condition. It was determined that NO_3–N decreased to 40.7 mg L^{-1} at 12 h. The corresponding denitrification rate was 8.11 mg L^{-1} h^{-1} (the repeated denitrification rates obtained were 7.98, 8.08 and 8.25 mg L^{-1} h^{-1}, respectively, so the average value was 8.11 ± 0.14 mg L^{-1} h^{-1}), which was obviously in good conformity with the model predicted maximal value of 8.0318 mg L^{-1} h^{-1}. Although NO_2–N accumulated up to 43.4 mg L^{-1} at 12 h, it gradually reduced to 0.7 mg L^{-1} at 24 h, indicating no final nitrite accumulation. Additionally, it was found that OD_{480} and pH achieved to 4.5 at 15 h and 9.3 at 15 h, respectively, meaning that the denitrification by *C. daeguensis* TAD1 mainly occurred in the logarithmic phase, which is in good agreement with the preliminary results [23]. Most importantly, compared with the initial OD_{480} of 0.55 [23], OD_{480} of 4.5 obtained in this study showed that the optimized medium condition could provide a more suitable environment for the cell growth and the denitrification. Thus, the optimized medium favored nitrate removal by *C. daeguensis* TAD1.

3.4. Long-term operation performance in BF

The optimized medium for the recycling liquid was used to carry out long-term operation in the BF system. In order to keep TAD1 predominant in BF all the time, the recycling liquid inoculated with fresh seed cultures (10%) of *C. daeguensis* TAD1 was regenerated every day. Consequently, the yellow biofilm was macroscopic after 13 days, and nitrate removal efficiency increased from 40% at 14 days to 100% at 29 and 30 days (data not shown), indicating that a mature biofilm had formed. Therefore, from 31 days on, the variable simulated flue gas of 0.8 L min^{-1}, corresponding to an empty bed residence time (EBRT) of 112.5 s in a 1.5 L packed volume, was injected to the BF, and nitrate concentration in the recycling liquid was reduced gradually to zero at 38 days in order to promote an alternative denitrification metabolism pathway changing from $NO_3^- \rightarrow N_2$ to $NO \rightarrow N_2$. After injecting the flue gas, the recycling liquid, components of which were the same as that in the startup except for nitrate, was regenerated every two days to maintain the activity of the biofilm.

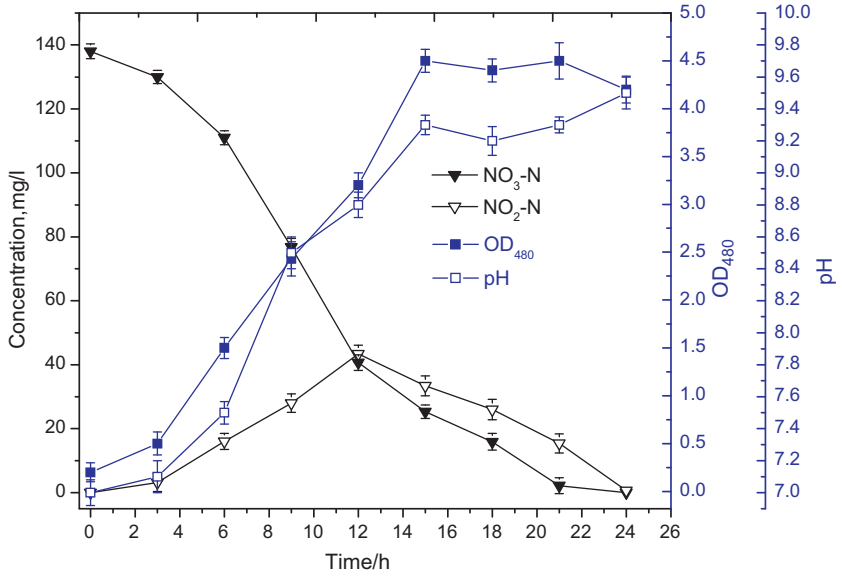

Fig. 4. Time course of nitrate, nitrite, OD_{480} and pH in the optimized medium.

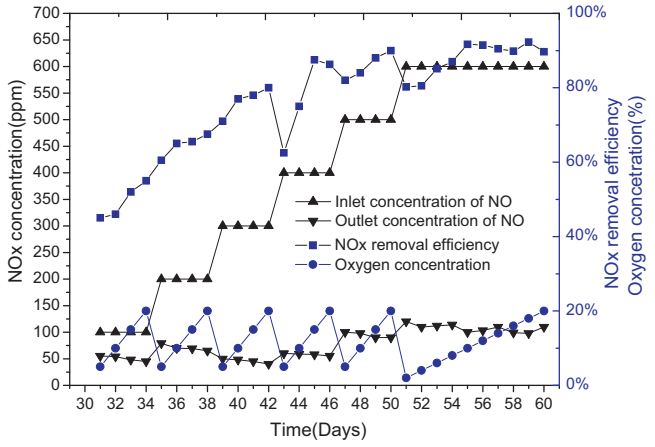

Fig. 5. Performance of the biotrickling filter for removing NO_X using different oxygen and NO_X loading.

Jiang et al. studied in detail the effect of oxygen on the NO removal in a biotrickling filter, showing that the oxygen did not inhibit NO removal but rather enhanced NO removal in part by chemical oxidation of NO. However, this abiotic removal of NO accounted for a minor part. Hence, the NO_X removal was investigated in the present study. The NO_X removal efficiency (RE) and eliminate capacity (EC) was, respectively, defined as:

$$\text{RE}\ \ (\%) = \left(\frac{[NO_X]_g^{in} - [NO_X]_g^{out}}{[NO_X]_g^{in}} \right) \times 100\% \quad (3)$$

$$\text{EC} = \left(\frac{[NO_X]_g^{in} - [NO_X]_g^{out}}{\text{filter bed volume}} \right) \times \text{flow} \quad (4)$$

where $[NO_X]_g^{in}$ and $[NO_X]_g^{out}$ are the measured NO_X concentrations in the inlet and outlet of the system.

Fig. 5 exhibits the performance for a month in BF packed with inert material with a variable load and oxygen content. In the first two days, NO_X RE was about 45% with inlet NO of 100 ppm, but in the following two days NO_X RE grew up to 55%. On the whole, NO_X RE increased gradually with increasing inlet NO from 100 to 500 ppm, except for 43 days at which NO_X RE abruptly decreased to 62.5%. This phenomenon might be ascribed to the sensitivity of TAD1 to the different denitrifying substrate, since the substrate was switched from nitrate to NO, meaning the two different denitrifying pathways. And another reason responsible for this phenomenon was its very low solubility limiting the transfer of NO from gas to biofilm. However, once the biofilm adapted to the changed pathway, NO_X RE ranged between 82% and 90%. Then, the NO load was enhanced to 48.21 g m^{-3} h^{-1}, which corresponded to 600 ppm. 89.6–91.7% RE was obtained and maintained for 6 days with EBRT 112.5 s. Also, it should be noted that NO_X RE quickly decreased from 90% at 50 days to 80.2% at 51 days, which might be caused by abruptly increasing the NO load. In summary, results evidenced that the BF system has a high denitrification performance, probably due to both the mature biofilm and the high cellular activity.

Fig. 5 also presents the effect of oxygen concentration from 2% to 20% on NO_X removal. Theoretically, more NO is oxidized to NO_2 as more O_2 is injected, which would result in phenomenons that the recycling liquid should contain more nitrate and NO_X RE should increase in part. However, NO_3–N concentration in the recycling liquid was less than 2.5 mg L^{-1}, and clearly, the oxygen had no significant effect on RE (Fig. 5). This might be attributed to the following two points: (i) as an electron acceptor, nitrate was utilized much more easily than NO, probably resulting from both the insolubility of NO and the capability of nitrate to induce denitrifying genes and (ii) the existence of nitrate maybe inhibited the reduction of NO to N_2 to some extent, which possibly counteracted the chemical oxidation of NO to NO_2, thus leading to no obvious change of NO_X RE on the whole.

Table 5
Effect of EBRT on the NO_X removal.

EBRT (s)	NO_X concentration (ppm)[a]		Average efficiency of NO_X removal (%)[b]
	Inlet	Outlet	
75	720	208	72.2 ± 2
90	720	159	78.8 ± 1
112.5	720	73	90.3 ± 3
150	720	65	91.1 ± 3

[a] The corresponding concentrations of inlet NO and oxygen were 600 ppm and 10%, respectively.
[b] Values represent mean ± S.D. of two replicates.

3.5. Effect of the empty bed residence time (EBRT) on the NO_X removal

Many studies demonstrated that EBRT was a very important factor influencing the total efficiency of the BF system [27,28]. Accordingly, the effect of EBRT on the NO_X removal was investigated under the condition of the inlet NO of 600 ppm, and the results are listed in Table 5. As expected, the NO_X RE was in proportion to EBRT. From the table, the average NO_X RE was 72.2%, 78.8%, 90.3% and 91.1% when EBRT was 75 s, 90 s, 112.5 s and 150 s, respectively. This was mainly because that the NO insolubility limited the mass transfer process. The shorter resistance time caused NO to discharge out of the BF owing to the incomplete reaction, whereas the longer resistance time made the microbe and NO have sufficient time to react upon each other, therefore resulting in good NO_X removal effect. However, NO_X RE did not increase significantly when EBRT increased from 112.5 s to 150 s, suggesting that the optimal EBRT for this BF system was 112.5 s.

3.6. Stability of TAD1 in the biofilm

Stability of TAD1 in the biofilm during the long-term operation of the BF system was checked by PCR–DGGE. 16S rDNA fragments amplified from genome of the pure TAD1 and the bacteria in the biofilm were analyzed by DGGE to identify the predominant microbes before and after 30 days of NO_X exposure. DGGE has been used to monitor and identify microbial populations for a long time, and has proven to be a powerful tool [29]. The microbial population analyzed by DGGE is shown in Fig. 6. Clearly, TAD1 was always predominant in the biofilm before and after exposure to NO_X, especially in lanes of 15 d and 30 d where some relatively faint strains could be found. However, an unknown strain with the same luminance as TAD1 appeared in lane of 60 d, which was distinct from the

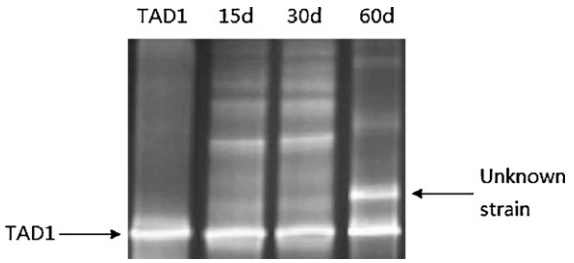

Fig. 6. DGGE profile of the 16S rRNA gene fragments amplified from DNA extracted from TAD1 and the biofilm at 30 d, 45 d and 60 d.

lanes of 15 d and 30 d. The possible reason for these phenomenons was that the recycling liquid inoculated with the fresh pure culture of TAD1 was regenerated every day during the startup, which made TAD1 far more predominant than other microbes. Another important explanation could be that the high temperature inhibited the growth of a great number of microbes. Furthermore, the flue gas environment naturally selected the dominant cultures. Thus, as for the unknown strain, it is reasonable that once some microorganisms adapted themselves to such environment, they would function together with TAD1.

4. Conclusions

A suitable medium component of the recycling liquid for NO_X removal by TAD1 using a sequential statistical experimental design was obtained. Based on the optimized medium, a biotrickling filter was set up and carried out to remove NO_X in simulated flue gas at high temperature. Ultimately, the BF system was able to consistently remove 80.2–92.3% NO_X under conditions of the inlet NO of 600 ppm and the oxygen concentration raging between 2% and 20%. Bacterium TAD1 was stable in the biofilm and favored the flue gas environment.

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