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Degradation of 2,4,6-trinitrotoluene (TNT) by immobilized microorganism-biological filter

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ABSTRACT

The combined process of immobilized microorganism-biological filter was used to degrade TNT in an aqueous solution. The results showed that the process could effectively degrade TNT, which was not detected in the effluent of the system. GC/MS analysis identified 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT) and 2,4-diamino-6-nitrotoluene (2,6-DA-4-NT) as the main anaerobic degradation products. In addition, the Haldane model successfully described the anaerobic degradation of TNT with high correlation coefficients (R^2 = 0.9803). As the electron donor, ethanol played a major role in the TNT biodegradation. More than twice the theoretical requirement of ethanol was necessary to achieve a high TNT degradation rate (above 97.5%). Moreover, Environment Scan Electron Microscope (ESEM) analysis revealed that a large number of globular microorganisms were successfully immobilized on the surface of the carrier. Further analysis by Polymerase Chain Reaction (PCR)-Denaturing Gradient Gel Electrophoresis (DGGE) demonstrated that the special bacterial for TNT degradation may have generated during the domestication with TNT for 150 days. The dominant species for TNT degradation were identified by comparing gene sequences with Genebank.

1. Introduction

Due to its low melting point, chemical and thermal stability, low sensitivity to impact, friction, and high temperature, 2,4,6-trinitrotoluene (TNT) is a widely used explosive [1]. Since it is toxic and mutagenic to humans and animals at low concentrations [2–5], decontamination is necessary. Physical and chemical methods have widely used to treat TNT wastewater or soils contaminated by TNT, including advanced oxidation [6–8], adsorption [9], and incineration. Each of these methods has its limitations. Incineration is the most effective and widely used process, but the method is expensive due to the cost of fuel [10]. Moreover, it usually generates secondary pollution during the treatment [11,12]. Advanced oxidation demands high level of reaction conditions and is difficult for TNT plants to carry out. Problems with adsorption include the retention of untreated compounds on granular activated carbon, incomplete degradation of TNT and the requirement of additional nutrients [13]. Therefore, recent studies have focus on the biotreatment, which is both highly efficient and cost-effective.

Most previous studies on TNT biodegradation addressed the treatment of TNT contaminated soils [14–16]. There have been only a few studies [17,18] on the degradation of TNT in an aqueous solution. Furthermore, the literature contains few detailed reports on the process of TNT degradation using immobilized microorganisms. The immobilized microorganisms process has been widely used to treat refractory wastewater, including landfill leachate [19], coking effluent [20,21], oil field wastewater [22], textile wastewater [23], phenols and dye wastewater [24–28]. Both Biological Aerated Filter (BAF) and Anaerobic Filter (AF) are types of immobilization reactor that can maintain high hydraulic loading rates while retaining a high biomass concentration [22,29–31]. This reduces the environmental shock, resulting in less sludge formation, and promoting the growth of microorganisms [20].

This paper reports a laboratory study evaluating the capability of immobilized microorganisms to degrade TNT. Early studies [5,17] on the biodegradation of TNT suggested that TNT was resistant to biological treatment in aerobic process such as the activated sludge system. As shown in previous studies [32], anaerobic biological processes could transform toxic organic compounds such as polychlorinated phenols and nitroaromatics, which were considered to be recalcitrant to aerobic treatment processes. The metabolites of anaerobic reaction could be degraded effectively by aerobic biological processes. Therefore, the process of AF combined with BAF was

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Table 1
Composition of trace element.

Composition	Concentration (mg l^{-1})
$FeCl_2{\cdot}4H_2O$	35
$CaCl_2{\cdot}2H_2O$	30
$CoCl_2{\cdot}6H_2O$	2
KI	2.5
$MnCl_2{\cdot}6H_2O$	0.5
$ZnCl_2$	1
Na_2MoO_4	0.5
H_3BO_4	1.5

developed in the present study. Microorganisms were immobilized on the special carrier [33] and filled in the two filters. To study the metabolites of TNT, GC/MS was employed to detect the degradation products. PCR-DGGE, previously applied to research on wastewater [34] and contaminated soils [35] treatment, was used to analyze the bacterial community structure of the two reactors. ESEM analysis was conducted to determine whether the microorganisms could be immobilized on the special carrier and to obtain the morphology of the immobilized microorganisms. Furthermore, the degradation kinetics model and effect of ethanol on TNT degradation were also studied.

2. Materials and methods

2.1. Materials

2.1.1. Wastewater

TNT was supplied by the Number 375 ammunition plant. The wastewater used in the experiment was simulant water. The TNT concentration of the wastewater was about 2.77–94.62 mg l^{-1}. Ethanol was added into the wastewater as the electron donor, and the concentration was about 100 mg l^{-1}. In addition, KH_2PO_4 and trace element solution were also put in the wastewater. Table 1 shows the composition of trace elements.

2.1.2. Reactor system

Fig. 1 shows the two upflow and submerged biological filters used in this study. Each reactor is made of polymethyl methacrylate and has an effective volume of 3.9 l, a diameter of 100 mm, and a height of 500 mm. Air diffusers are located 50 mm from the downside inlet. The self-made patented FPUFS [33] carrier was used to immobilize microorganisms. The synthesized polymer carrier is a 20 mm × 20 mm × 20 mm cube employed as exclusively fluidized media. The pores in the carrier guarantee better three-phase mixing of air, wastewater and carrier, and add three-phase mass transfer propulsion. At the same time, the pores can be used to immobilize microorganisms.

2.1.3. Immobilized microorganisms

The microorganisms called B925 used in the study were purchased from BIONETIX Co. (Canada). The marked performance of microorganisms in degrading aromatic compounds has been investigated experimentally [36,37]. In the study, 5 g of B925 microorganisms were placed in the each reactor.

2.2. Experimental procedure

The test can be divided into two stages, the domestication and immobilization of the microorganisms, and the stable reaction operation stage. For the first 3 days, the reactors were filled with the simulant wastewater with a low concentration of TNT and the air blowers were turned on, but with the influent and effluent stream valves closed. Then wastewater was continuously pumped into the reactors and the hydraulic retention time (HRT) of the system was 36 h. TNT concentration of the influent was gradually increased and TNT in the effluent was measured daily. When the biodegradation rate of TNT was higher than 80%, the first stage was basically complete and the system began to enter the stable reaction operation stage.

2.3. Methods

2.3.1. Wastewater quality detection

TNT was quantified by HPLC (Agilent, USA) according to EPA method 8330. COD was measured by the potassium dichromate oxidation method (Hach heating system, Hach Corporation, USA). pH was detected by pH meter (pH-201, Hanna Corporation, Italy). Measurements of the parameters above were conducted by repeated sampling, and the results were obtained as mean values. The relative errors of these measurements were less than 5%.

2.3.2. GC/MS analysis

GC–MS was used to detect the metabolites in the effluent of the reactors. A 200 ml sample of wastewater was extracted by 10 ml of dichloromethane (100%, Fisher Corporation, USA) three times for pH 2, 7 and 12. The three extract layers were combined and dried using nitrogen, and the residue was dissolved in a 1 ml solution of dichloromethane. Then, 1 μl of pretreated sample was analyzed by the 6890N/5973 GC/MS system (Agilent Corporation, USA). Pure He gas (99.999%) was employed as the carrier gas at flow rate of 1 ml min^{-1}. A DB-35MS capillary column with inner diameter of 0.25 mm and length of 30 m was adopted in the separation system. The oven temperature was 280 °C. The temperature program was as follows: 40 °C for 3 min, from 40 to 280 °C at a rate of 3 °C min^{-1} and kept at 280 °C for 3 min. The electron energy and the electron double voltage were set at 70 eV and 1200 V, respectively.

The substance analysis was conducted with reference to the NIST98 mass spectral library database.

2.3.3. Biology observation

The carrier was moved from the middle part of the reactor to the refrigerator (−20 °C). When the carrier was frozen, we removed it to the freezer dryer and dried it. Finally, the carrier was photographed with a FEI QUANTA 200F Environment Scan Electron Microscope.

2.3.4. DNA extraction and purification

20 g (wet weight) of biofilms was added into 20 ml of auto-claved extraction buffer (100 mM Tris–HCl, 100 mM EDTA–Na_2, 200 mM NaCl, 1% PVP, 2% CTAB, pH 8.0). Then 50 μl of 10 mg ml^{-1} of Proteinase K was added and the mixture was incubated at 37 °C for 45 min while being shaken at 150 rpm. 1.5 ml of 2% SDS was added to the mixture, which was then incubated at 65 °C in a water bath for 1 h and shaken every 10 min. The mixture was then centrifuged at 12 000 rpm for 10 min. The supernatant was collected and extracted three times by mixed organic reagent (phenol:chloroform:isoamyl alcohol = 25:24:1). The DNA was precipitated in isopropanol for 12 h at room temperature and then was centrifuged (13 000 rpm, 20 min, 4 °C). The precipitate was collected and washed twice with 5 ml of cold 70% ethanol. After drying, the precipitate was dissolved in 50 μl of Tris–EDTA buffer solution (pH 8.0).

2.3.5. Amplification of DNA

The variable region V3 of the 16S rDNA was amplified using primers GC-357f [38] (5′-CGC CCG CCG CGC GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and PRUN518r [39] (5′-ATT ACC GCG GCT GCT GG-3′).

PCR included 41.25 μl of UV-sterile water, 5 μl of $MgCl_2$ (20 mmol l^{-1}), 1.0 μl of 2,4-DNTP (10 mmol l^{-1}), 1.0 μl of primers GC-357f and PRUN518r (10 μmol l^{-1}), 0.25 μl of Taq polymerase (5 U), and 10 ng of DNA template. The PCR amplification procedure was as follows: one preliminary at 94 °C for 4 min, 30 cycles each involved denaturation at 94 °C for 30 s, anneal at 52 °C for 1 min, extended at 72 °C for 7 min. The results were analyzed by 1.2% agarose gel eletrophoresis (120 V, 30 min).

2.3.6. DGGE analysis

Samples of PCR product (50 μl) were loaded onto 8% (w/v) polyacrylamide gels in 1× TAE buffer (40 mmol l^{-1} of Tris–HCl, 40 mmol l^{-1} of ethanoic acid, 1 mmol l^{-1} of EDTA, pH 8.0). The gel contained a gradient of denaturant ranging from 35 to 65% (100% denaturant is 7 M urea and 40% deionised formaide). The electrophoresis was run at 60 V for 20 min and then at 180 V for 5 h. After electrophoresis, the gels were stained with goldview (Beijing SBS Genetech Co., Ltd.), a new nucleic acid stain. The gel was photographed with the gel photo system and the photographs were analyzed with Bio-Rad Quantity One software.

3. Results and discussion

3.1. TNT degradation by biological filter

Fig. 2 shows TNT degradation time history for the combined process and Fig. 3 is the detailed view. The system operated for 150 days. Because the microorganisms were perhaps unaccommodated to TNT at the early stage, the concentration of TNT in the influent was held below 5.0 mg l^{-1} over the first 8 days. Even so, about 1.2 mg l^{-1} of TNT was detected in the effluent of the anaerobic reactor, which can be seen in Figs. 2 and 3. In addition, the degradation rate of TNT varied greatly at the early stage, indicating that microorganisms in the reactor were not well accommodated to TNT and the system was still in the stage of microorganisms domestication. After the system had operated 20 days, the concentration of TNT in the influent had increased gradually, but the TNT in the effluent of the anaerobic and aerobic reactors remained stable. The mean concentration of TNT in the effluent of the anaerobic

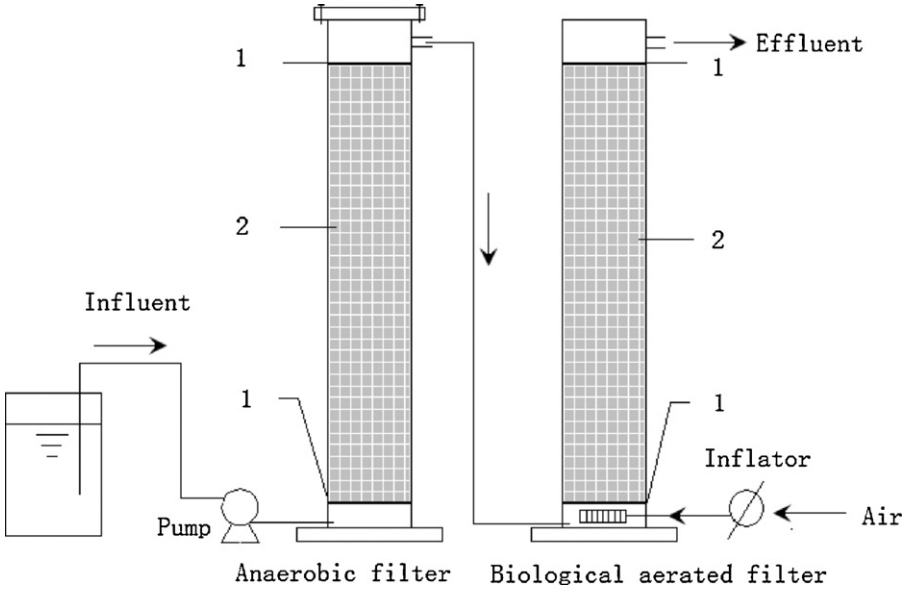

Fig. 1. Reactor system: (1) clapboard, (2) carrier and microorganisms.

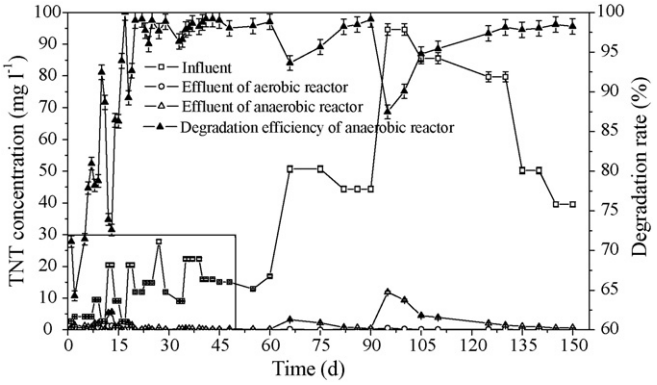

Fig. 2. Efficiency of TNT degradation biological filter.

reactor was below 0.3 mg l^{-1} and no TNT was detected in the effluent of aerobic reactor. This demonstrated that the system entered the stable reaction operation stage after 20 days. Throughout the stable reaction operation stage, TNT was not detected in the effluent of the aerobic reactor. In other words, the combined process could degrade TNT completely. The result was superior to the previous studies [1–3,40], which used physical and chemical methods

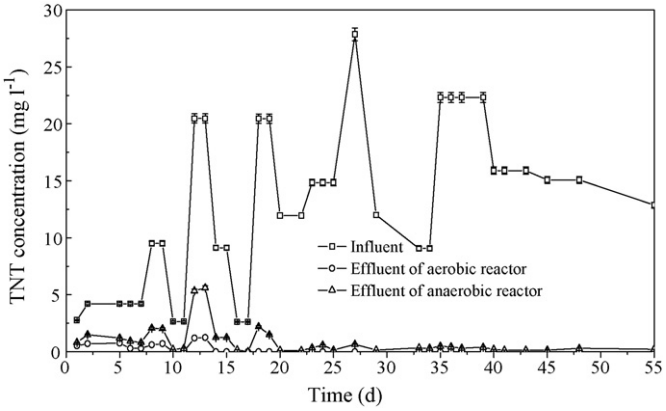

Fig. 3. Detailed view of Fig. 2.

or traditional biotreatment. Besides, the system has the advantage in reducing environmental shock.

3.2. TNT degradation metabolites analysis

TNT degradation metabolites were detected by GC/MS. Fig. 4 shows the organic composition of influent and effluent from the two reactors. It can be seen from Fig. 4(a) that TNT was the main organic compound. However, it was reduced to 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT) and 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT) during the anaerobic process, as shown in Fig. 4(b). The result was in agreement with previous studies [13,40,41]. As mentioned above, ethanol was added to the influent, and can be fermented to acetate and H_2 by anaerobes [5,42]. It is well known that H_2 has strong ability in reduction and $-NO_2$ can be reduced to $-NH_2$. Therefore, aminodinitrotoluenes and diaminonitrotoluenes were found in the anaerobic degradation products. In addition, the relative content of 2,6-DA-4-NT (51%) was much greater than that of 2,4-DA-6-NT (5%), deviating from some other reports [41]. The methyl group is the electron-donating group, while the nitro group is the electron-withdrawing one. In the molecular structure of TNT, the electron density of the nitro group beside the methyl group was greater than that of other nitro groups. So, the nitro group beside the methyl group is more easily reduced by H_2 and there was more 2,6-DA-4-NT than 2,4-DA-4-NT in the metabolites. According to the above analysis, we can deduce the proposed degradation pathway of TNT, shown in Fig. 5. Besides, as shown in Fig. 4(c), no organic compounds were detected in the effluent of the aerobic reactor, which indicates that the combined process could effectively biodegrade not only TNT but also its metabolites.

3.3. Effect of ethanol on TNT degradation

Ethanol has widely been used as electron donor to reduce sulfate [43], carbonyl compounds [44], uranium [45], selenium oxyanions [46], and so on. However, to date, few reports have been published on the application of ethanol in TNT degradation, using immobilized organisms process. In the present study, ethanol was employed to reduce TNT. It was concluded that ethanol could be fermented to acetate and H_2 under anaerobic conditions [5,42],

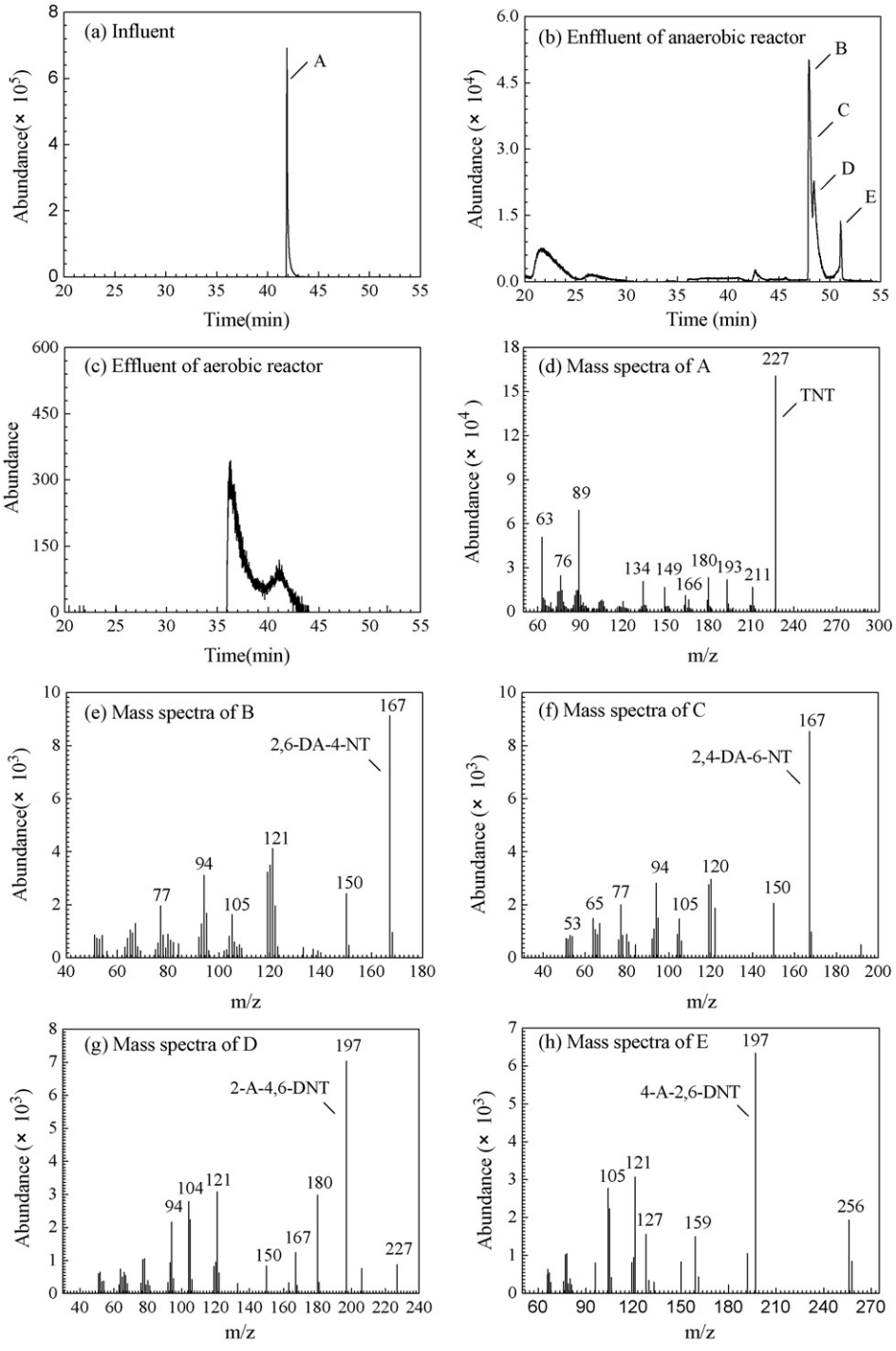

Fig. 4. Spectrum of influent and effluent organic components for anaerobic and aerobic reactors.

as shown in Eq. (1). Davel [47] investigated the biodegradation of TNT in a fluidized-bed reactor and put forth the equation of TNT biodegradation as shown in Eq. (2):

$$C_2H_6O + H_2O \overset{\text{microorganisms}}{\longleftrightarrow} CH_3COO^- + H^+ + 2H_2,$$
$$\Delta G^{0'} = 9.6\,\text{kJ/reaction} \quad (1)$$

$$\underset{\text{(ethanol)}}{1.5C_2H_6O} + \underset{\text{(TNT)}}{C_6H_2CH_3(NO_2)_3} \overset{\text{microorganisms}}{\longrightarrow} \underset{\text{(TAT)}}{C_6H_2CH_3(NH_2)_3} + 3CO_2 + 1.5H_2O \quad (2)$$

The concentration of TNT in the influent was 80 mg l^{-1}. According to Eq. (2), the influent should contain at least 24.3 mg l^{-1} of ethanol so as to completely transform TNT to TAT. Fig. 6 illustrates the effect of ethanol on TNT degradation at different concentrations. It can be seen that ethanol played a major role in the TNT biodegradation. When the concentration of ethanol was in the range of 100–200 mg l^{-1}, TNT was effectively degraded and the degradation rate was above 99%. As the concentration decreased from 100 to 50 mg l^{-1}, the degradation rate of TNT slowly decreased. It was about 97.5% when ethanol was 50 mg l^{-1}. However, while the concentration of ethanol continued to decrease, TNT in the effluent began to increase sharply. For growth, microbial used

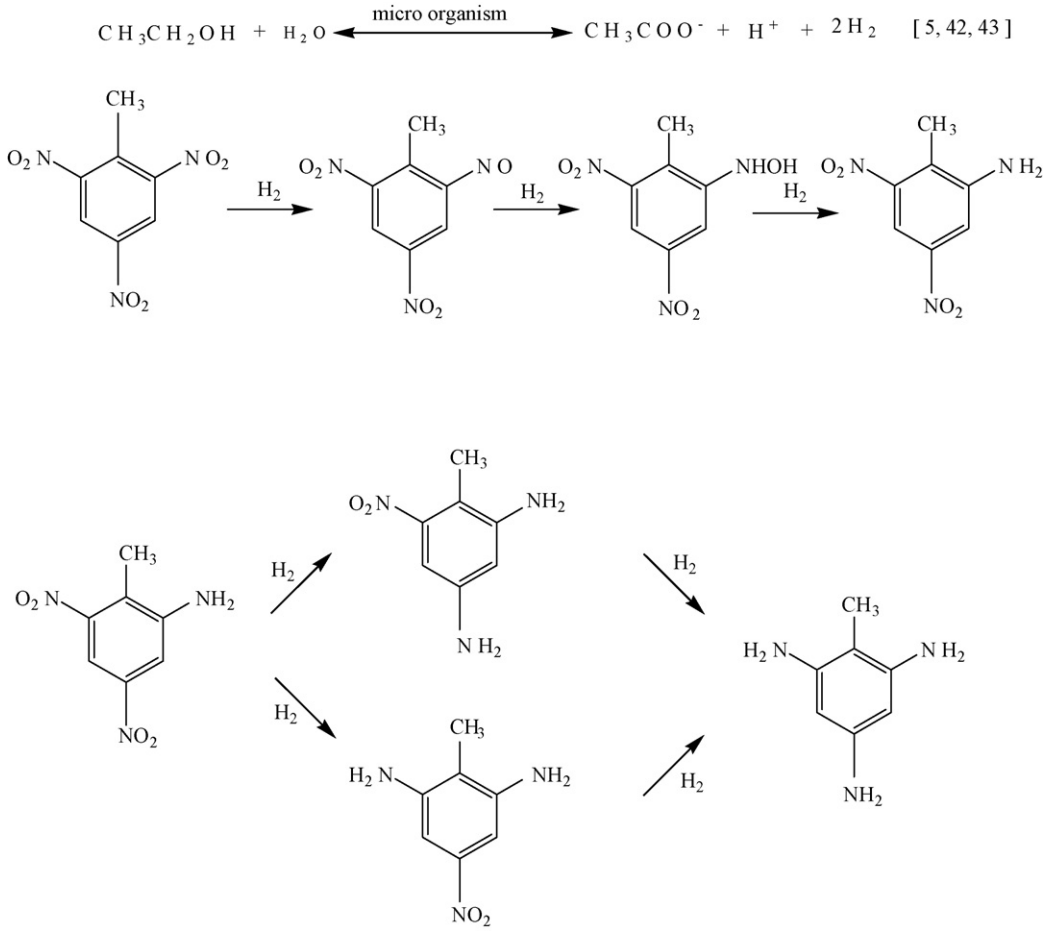

Fig. 5. Proposed degradation pathway of TNT.

part of ethanol as an auxiliary carbon source [47]. Therefore, the remaining ethanol may have been much less than required for the complete reduction of TNT to TAT with a low initial concentration of ethanol. Furthermore, only 30% of the TNT was degraded, as the concentration of ethanol was 25 mg l^{-1}, a little more than the theoretical requirement (24.3 mg l^{-1}). This confirmed the explanation discussed above. In addition, it was concluded from Fig. 6 that approximately more than twice the theoretical requirement of ethanol was needed to get a high TNT degradation rate (above 97.5%), similar to previous reports [47].

3.4. TNT anaerobic degradation kinetics

TNT has been generally known to inhibit the biodegradation reaction [48]. To describe the anaerobic biodegradation of TNT, it is necessary to use an inhibition model of substrate. In previous studies, several inhibition models of substrate have been tested, and the Haldane model fits to the experimental data best [49,50]. In addition, the Haldane model has been used successfully to describe the biodegradation of phenol, but little is known about its application

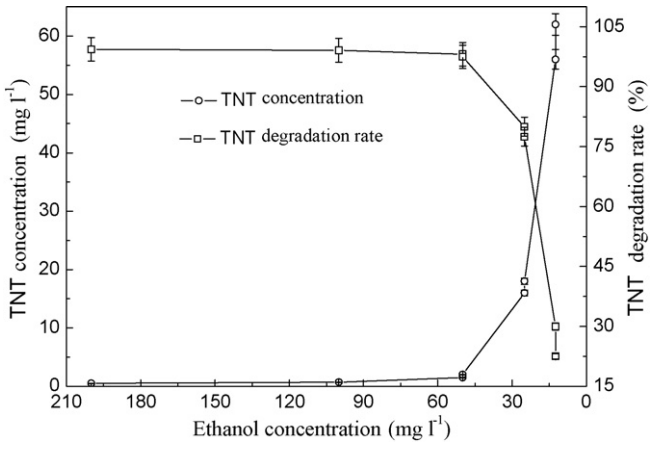

Fig. 6. Effect of ethanol on TNT degradation.

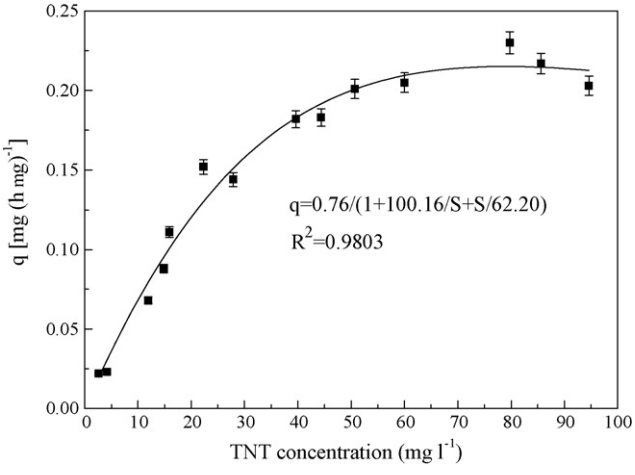

Fig. 7. Experimental data simulation of the TNT degradation kinetics equation.

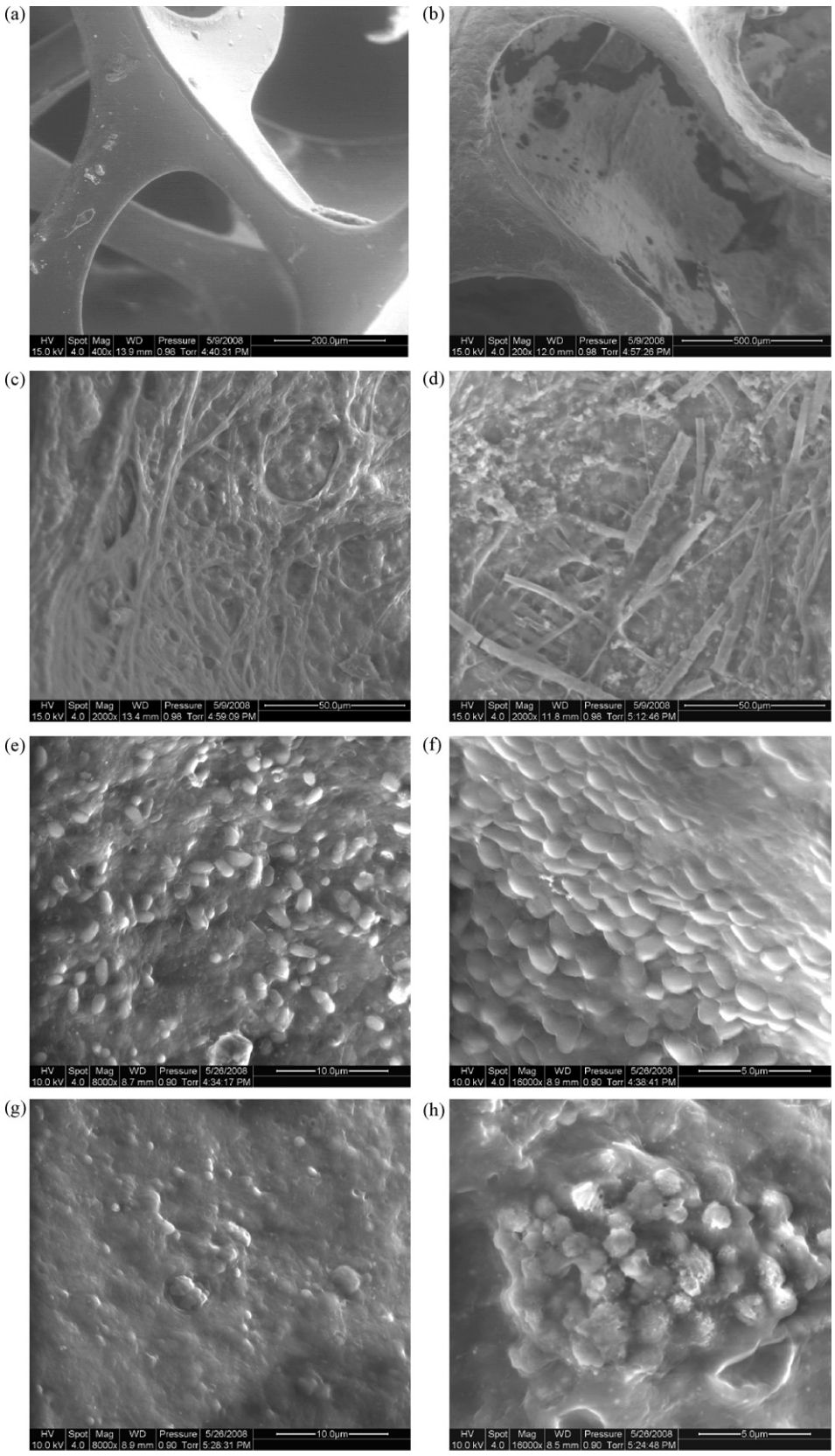

Fig. 8. Carrier and microorganisms observed in reactor by ESEM.
(a) 400× carrier without microorganisms, (b) 200× carrier with biofilm, (c) 2000× carrier with microorganisms in anaerobic reactor at the 20th day, (d) 2000× carrier with microorganisms in aerobic reactor at the 20th day, (e) 8000× carrier with microorganisms in anaerobic reactor at the 40th day, (f) 16 000× carrier with microorganisms in anaerobic reactor at the 40th day, (g) 8000× carrier with microorganisms in aerobic reactor at the 40th day, (h) 16 000× carrier with microorganisms in aerobic reactor at the 40th day.

Table 2
TNT degradation kinetics date.

Initial concentration ($mg\,l^{-1}$)	Experimental value of q [$mg\,(h\,g)^{-1}$]	Theoretical value of q [$mg\,(h\,g)^{-1}$]
2.65 ± 0.16	0.022 ± 0.001	0.020
4.20 ± 0.31	0.023 ± 0.001	0.031
11.94 ± 0.53	0.068 ± 0.003	0.079
14.84 ± 0.69	0.088 ± 0.004	0.095
15.89 ± 0.76	0.111 ± 0.006	0.100
22.31 ± 1.32	0.152 ± 0.007	0.130
27.85 ± 1.51	0.144 ± 0.007	0.151
39.60 ± 1.85	0.182 ± 0.009	0.182
44.37 ± 2.30	0.183 ± 0.009	0.191
50.70 ± 2.51	0.201 ± 0.011	0.200
60.00 ± 2.95	0.205 ± 0.012	0.209
79.75 ± 3.62	0.230 ± 0.012	0.215
85.60 ± 3.97	0.217 ± 0.011	0.214
94.62 ± 4.66	0.203 ± 0.009	0.212

in TNT biodegradation [49,51]. The Haldane equation is shown in Eq. (3).

$$q = \frac{q_{max}}{1 + K_s/S + S/K_i} \quad (3)$$

where q is the specific degradation rate [$mg\,(h\,g)^{-1}$], S is the initial concentration of TNT ($mg\,l^{-1}$), q_{max} is the maximum specific degradation rate [$mg\,(h\,g)^{-1}$], K_s is the substrate saturation constant ($mg\,l^{-1}$), and K_i is the substrate inhibition constant ($mg\,l^{-1}$).

Origin software numerical simulation better fits the experimental data, shown in Table 2. According to the simulation results, q_{max}, K_s and K_i were 0.76 $mg\,(h\,g)^{-1}$, 100.16 $mg\,l^{-1}$ and 62.20 $mg\,l^{-1}$, respectively. All the degrees of confidence of the parameters were 0.99. Moreover, the kinetic constants obtained were comparable to those in previous studies [49,52] and the difference maybe explained by the various treatment processes and reaction conditions. Fig. 7 illustrates the experimental data simulation to the Haldane equation. The simulation curve described the trend of the experimental data satisfactorily and the correlation coefficient was 0.9803.

3.5. Microbiological morphology

The carrier is micro-porous, with each pore's diameter >200 μm [20,21]. The pores provide space for gas and liquid to pass though the microorganisms [22]. The carriers in the two reactors were detected using ESEM when the system had operated 20 and 40 days, respectively.

Fig. 8(a) shows the carrier without microorganisms immobilization; Fig. 8(b) shows the carrier with microorganisms. The microorganisms were successfully immobilized onto the inside and outside surface of the carriers. The pores were not blocked by the biofilm and they permitted the gas and liquid to transfer efficiently to the microorganisms as before. Fig. 8(c) and (d) shows the microorganisms immobilized on the carriers after the reactors had been operating for 20 days. The carriers both in the anaerobic reactor and the aerobic reactor were immobilized with many globular microorganisms. Moreover, some bacilli were found in the anaerobic reactor. Fig. 8(e)–(h) shows the carriers and the microorganisms after 40 days of reactor reaction. The figure shows that the microorganisms flourished in the reactors with longer operation time. A large number of globular microorganisms were immobilized on the surface of the carriers from the anaerobic reactor. These globular microorganisms may play an important role in the reduction of TNT. As Fig. 8(g) shows, most of the globular microorganisms were packaged in biofilm. In addition, after the system had operated 20 days, some protozoa were found in the aerobic reactor, representing the healthy operating state of the aerobic reactor in concurrence with earlier research [22]. Fig. 2 shows that no TNT was detected in the effluent of the aerobic reactor after the system had operated 20 days, confirming the hypothesis.

3.6. Bacterial community analysis

The method of PCR-DGGE based on 16S rDNA and denaturing gradient gel electrophoreses fingerprinting technology has been increasingly used to assess changes in microbial communities [53,54]. However, most researchers have ignored the application of PCR-DGGE to the treatment of TNT wastewater. The strength of DGGE as a screening method for diversity is its ability to monitor spatial and temporal changes in community structure in response to changes in environmental parameters [55]. In this study, PCR-DGGE was employed to investigate the changes in microbial community structure in both reactors after 150 days of operation. The dominant bacteria for TNT degradation was identified. Fig. 9 shows DGGE profiles of amplified 16S rDNA fragments from the samples. Each of the distinguishable bands in the separation pattern represents an individual bacterial species [56].

Fig. 9 shows that lane A, with 19 as its Shannon diversity index (H'), had more bands than lanes B and C. Compared to lane A, the H' values of lanes B and C were only 15 and 14, respectively. Moreover, some bands in lane A were not found in the same position of lanes B and C. This indicates that some bacterial groups unaccommodated to TNT died out after the system operated for 150 days. In addition, some bands (b, f and g) were found at the same position in lane A, demonstrating that B925 contained some dominant bacterial groups for TNT degradation. Besides, some special crisp bands (a, c, d and e) were found in lanes B and C, indicating the special bacterial for TNT degradation may have generated during the domestication with TNT for 150 days.

To identify the specific dominant species in the bacterial community, specific bands were excised from the gel, and sequenced and compared with Genebank. Fig. 10 shows the phylogenetic trees for the partial bacterial 16S rDNA sequences. Sequence c showed 99.9% similarity to *Pseudomonas* sp. SRU_14 (FJ482111). In the previous reports, strains of *Pseudomonas* sp. demonstrated a strong ability in TNT degradation [49,57,58]. In addition, bands a, b, d, f and g were most closely related to *Flavobacteriales* sp., *Chryseobac-*

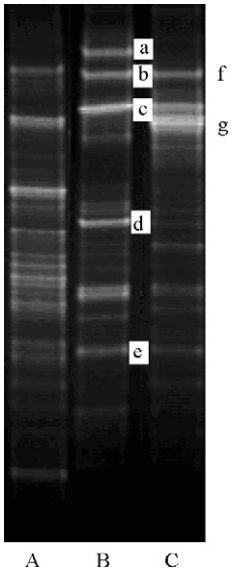

Fig. 9. DGGE profiles of amplified 16S rDNA fragments from the samples (lanes A–C: microorganisms B925, microorganisms in anaerobic reactor, microorganisms in aerobic reactor, respectively).

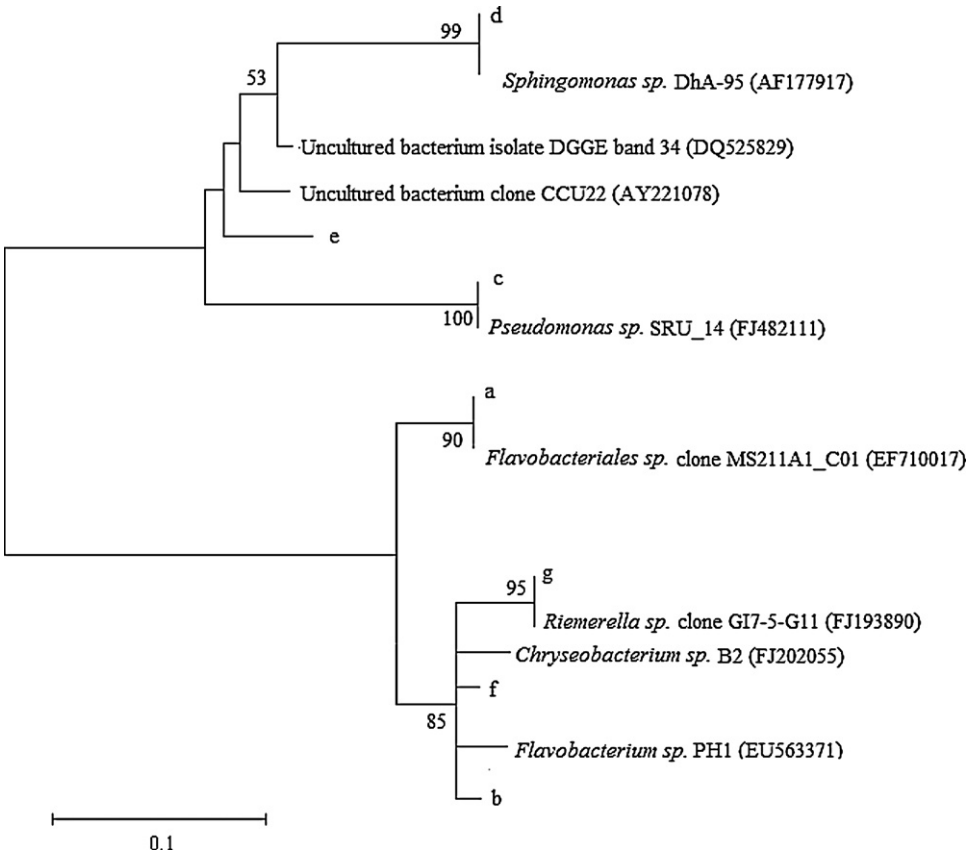

Fig. 10. Phylogenetic trees for the partial bacterial 16S rDNA sequences. The tree was obtained using a neighbor-joining algorithm. Bootstrap values are based on 1000 runs and are shown where >50.

terium sp., *Sphingomonas* sp., *Chryseobacterium* sp. and *Riemerella* sp., respectively.

4. Conclusions

This study investigated the feasibility of the combined process (AF-BAF) as a novel method for TNT degradation. We drew the following conclusions:

(1) The combined process could effectively degrade TNT. At the stable reaction operation stage, TNT was not detected in the effluent of the system. Moreover, the system was effective in reducing environmental shock.
(2) TNT was reduced to 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT), 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT) and 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT) during the anaerobic process. The anaerobic degradation products contained more 2,6-DA-4-NT than any other metabolite. Besides, no organic compounds were found in the effluent of the system.
(3) As the electron donor, ethanol played a major role in the TNT biodegradation. More than twice the theoretical requirement of ethanol was necessary to achieve a high TNT degradation rate (above 97.5%).
(4) The Haldane model effectively described the anaerobic biodegradation of TNT could be described by q_{max}, K_s and K_i were 0.76 mg (h g)$^{-1}$, 100.16 mg l^{-1} and 62.20 mg l^{-1}, respectively.
(5) The microorganisms were successfully immobilized on the surface of the carrier. After the anaerobic reactor had operated 40 days, a large number of globular microorganisms were found on the surface carrier using ESEM.
(6) The special bacterial for TNT degradation may have generated during the 150-day domestication with TNT. The dominant species for TNT degradation were identified using PCR-DGGE.

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