Journal of Hazardous Materials 186 (2011) 59–66

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# The biodegradation pathway of triethylamine and its biodegradation by immobilized *Arthrobacter protophormiae* cells

Tianming Cai, Liwei Chen*, Qian Ren, Shu Cai, Jin Zhang

*The College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, 210095, People's Republic of China*

ARTICLE INFO

*Article history:*
Received 4 July 2010
Received in revised form
27 September 2010
Accepted 3 October 2010
Available online 4 December 2010

*Keywords:*
Triethylamine
*Arthrobacter protophormiae*
Biodegradation
Immobilized cells

ABSTRACT

A bacterial strain named R4 was isolated from a wastewater treatment pool containing triethylamine (TEA) as the sole source of carbon and nitrogen. Strain R4 was identified as *Arthrobacter protophormiae* based on 16S rRNA gene sequence analysis and morphological and physiological properties. The optimal pH, temperature and concentration of NaCl for TEA degradation by strain R4 were 7.0, 30 °C and 0.5%, respectively. Strain R4 could completely degrade 100 mg $l^{-1}$ TEA to ammonia in 32 h, and could also effectively degrade diethylamine (DEA) and ethylamine (EA) to ammonia. The degradation of TEA was strongly inhibited by some metal ions ($Cu^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Ag^{+}$) (1.0 mM). Addition of either $SO_4^{2-}$ or $NH_4^{+}$ reduced the degradation efficiency of TEA by strain R4 to a certain extent. The inhibition became significant when the concentration of $SO_4^{2-}$ and $NH_4^{+}$ reached to 11 mM and 30 mM, respectively. Cell-free extracts prepared from cells grown in TEA exhibited TEA monooxygenase, DEA monooxygenase and EA monooxygenase activity. Here, we propose the metabolic pathway of TEA degradation in strain R4. The efficiency of TEA removal by immobilized cells of strain R4 was found to be equivalent to that of free cells. In addition, the immobilized cells could be reused without reduction in their ability to degrade TEA.



## 1. Introduction

Triethylamine (TEA) is widely used as a catalyst for polymerization reactions and a solvent and corrosion inhibitor in industry [1]; and it is also used as an intermediate in the production of various chemicals, including pesticides. Wastewater containing TEA causes environmental pollution problems and adverse effects on aquatic ecology. In addition, TEA is malodorous and can endanger human health [2]. Animal experiments have revealed that TEA could cause irritation to the dermal, ocular and respiratory systems, and long-term exposure to TEA could result in abnormal embryos. TEA has been considered to be a possible carcinogen [3]. Therefore, it is necessary to remove TEA from water in the environment.

Microbial degradation is considered to be the most effective means of TEA removal. However, little is known about microbial degradation of TEA. Photo-oxidation of TEA in the presence of $O_2$, $N_2$ and $H_2O$ over $TiO_2$ was previously investigated using a flat plate reactor [3]. Frings et al. [4] reported that no anaerobic degradation of TEA was observed in triethanolamine enrichment assays. Wang et al. [5] concluded that mixed bacterial cultures that were acclimated to TEA could completely remove the substance when the initial TEA concentration was below 200 mg $l^{-1}$. Rappert et al. [6] isolated two pure bacterial strains, *Pseudomonas citronellolis* RA1 and *Mycobacterium dienhoferi* RA2, that were able to use TEA and DEA (diethylamine) as the sole carbon and energy source for growth. These two isolates could only utilize up to 50 mg $l^{-1}$ in 4 days. However, the metabolic pathway of TEA degradation in these two strains was not reported.

Currently, TEA wastewater is mainly treated using a chemical and physical method; however, this method has several disadvantages [7–9]. The microbial treatment process is of great interest in TEA wastewater treatment because it could be less expensive than the chemical and physical method [8]. Immobilized cell technology can be applied to microbial treatment to enhance the efficiency and effectiveness of TEA degradation [10]. The use of immobilized cells can protect cells from toxic substances and can reduce expenses during operational periods. Alginate (AL) gel is widely employed to treat different kinds of wastewater because it has low toxicity and gels easily [11–14].

In the present study, a highly effective TEA-degrading strain, *Arthrobacter protophormiae* R4, was isolated. We found that this strain could use TEA, DEA and EA (ethylamine) as the sole source of carbon and nitrogen for growth and could completely degrade 100 mg $l^{-1}$ TEA to ammonia in 32 h. With the goal of elucidating a possible application to TEA-polluted water treatment, degradation of TEA by strain R4 was studied in liquid culture, and the pathway of TEA biodegradation by strain R4 was proposed. The efficiency of TEA degradation by both free and immobilized cells was investigated. The feasibility of reuse of AL-immobilized R4 cells for microbial degradation of TEA was also investigated. In addition,

* Corresponding author. Tel.: +86 25 84395002; fax: +86 25 84395002.
*E-mail address:* clw@njau.edu.cn (L. Chen).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.10.007

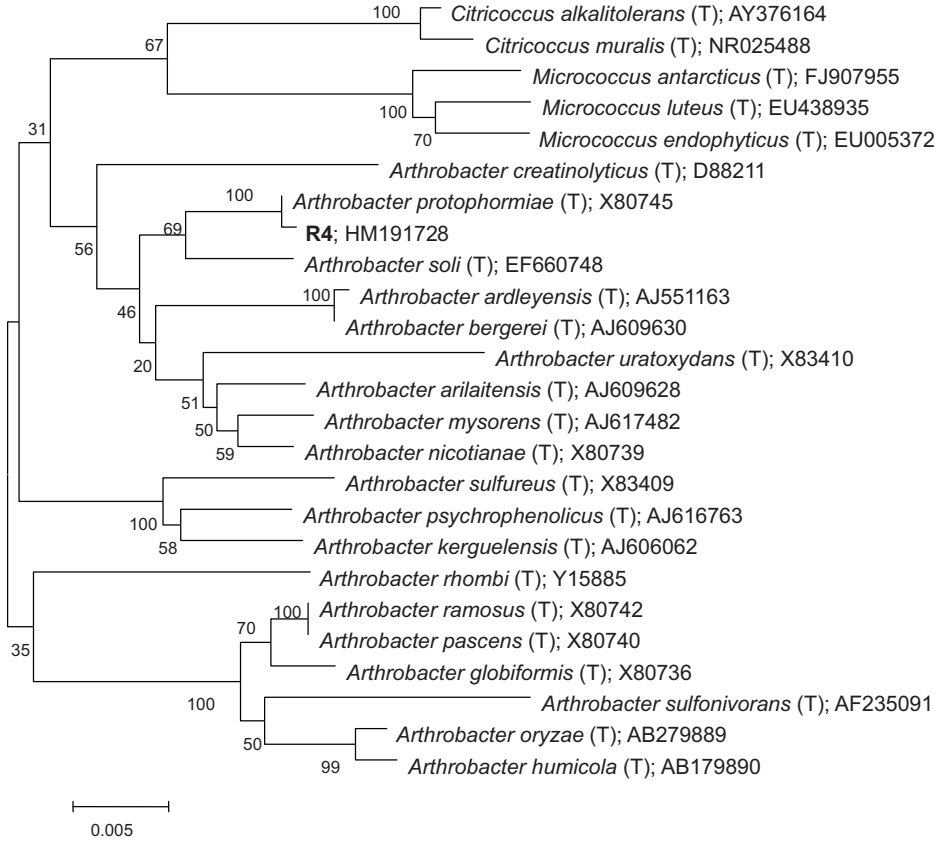


**Fig. 1.** Phylogenetic tree based on the 16S rRNA gene sequences of strain R4 and related species using the neighbor-joining approach. The GenBank accession number for each microorganism used in the analysis is shown after the species name.

some inhibitory elements were considered during TEA biodegradation. This paper highlights an important potential use of pure cultures of microbial cells for the cleanup of TEA-contaminated wastewater.

## 2. Materials and methods

### 2.1. Materials

The activated sludge sample was collected from a TEA-containing wastewater treatment pool of a pharmaceutical company in Zhejiang, China. TEA (>99% purity) and other reagents used in this study were of analytical reagent grade.

Luria-Bertani medium (LB, in $g\,l^{-1}$: 10.0 tryptone, 10.0 yeast extract, 5.0 NaCl) and mineral salts medium (MSM, in $g\,l^{-1}$: 1.0 NaCl, 1.5 $K_2HPO_4$, 0.5 $KH_2PO_4$) were used in this study. When necessary, a stock solution ($10^4$ mg $l^{-1}$, pH 7.0) of TEA, DEA, EA, or TMA (trimethylamine) was added to the medium at an appropriate concentration, and the pH of the medium was adjusted to 7.0.

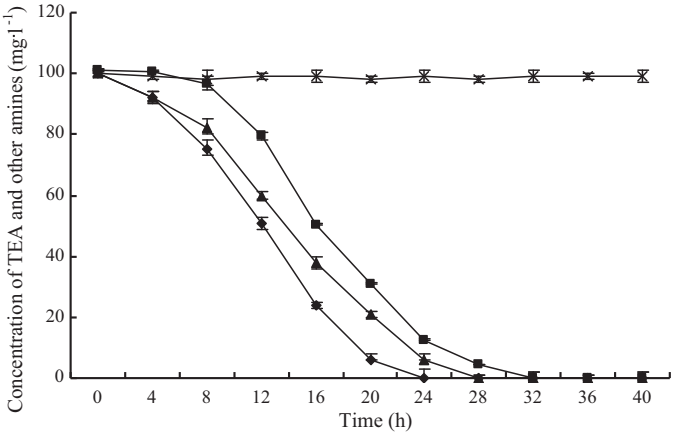


**Fig. 2.** Degradation of TEA and other amines by strain R4 in MSM containing 100 mg $l^{-1}$ of each substrate. The data are represented as the mean ± standard deviation for triplicate incubations. (■) Degradation of TEA; (▲) degradation of DEA; (♦) degradation of EA; (×) degradation of TMA.

### 2.2. Enrichment and isolation

An activated sludge sample (5 ml) was added to 100 ml MSM containing 100 mg $l^{-1}$ TEA, which was used as the sole carbon and nitrogen source, and it was incubated on a rotary shaker at 30 °C and 150 rpm for 7 days. Five milliliters of enrichment culture was then sub-cultured into fresh MSM containing TEA every 7 days. Gas chromatography (GC) was used to determine the concentration of TEA in the medium after the third passage. The ammonia content of the medium was also measured to determine whether TEA was mineralized completely. Enrichment cultures capable of degrading TEA were diluted and spread onto MSM agar containing 300 mg $l^{-1}$ TEA, and colonies grown on plates were tested for their TEA-degrading capabilities. One strain, designated as R4, which possessed the highest TEA-degrading ability and could utilize TEA as a sole carbon and nitrogen source for growth, was purified and selected for further investigation.

### 2.3. Identification of the isolate

The isolated strain was identified based on its morphological, physiological and biochemical properties with reference to Bergey's Manual of Determinative Bacteriology [15], com-

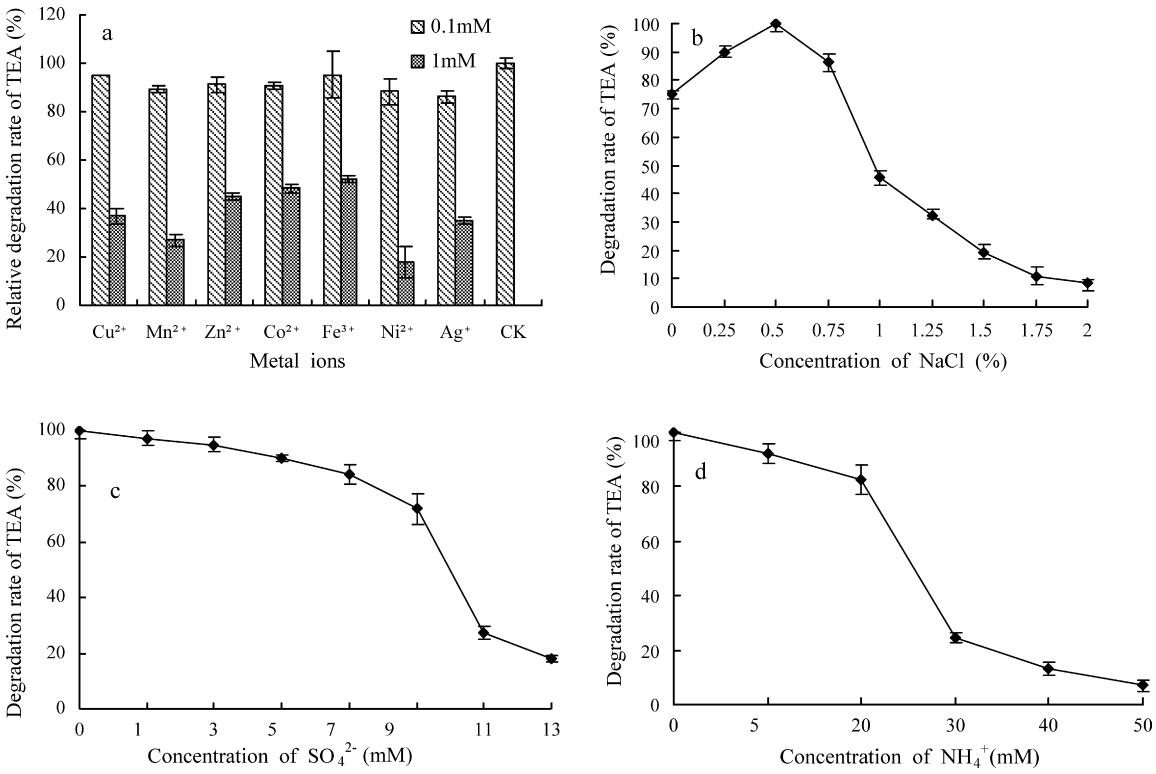


**Fig. 3.** Impact of some elements on TEA biodegradation. (a) Metal ions; (b) NaCl; (c) $SO_4^{2-}$; (d) $NH_4^+$.

bined with 16S rRNA gene sequence analysis. Genomic DNA was extracted, and the 16S rRNA gene was amplified using the polymerase chain reaction (PCR) as described previously [16]. The nucleotide sequence coding for the 16S rRNA of strain R4 was sequenced by TaKaRa Biotechnology (Dalian) Co. Ltd. Alignment with different 16S rRNA gene sequences from GenBank was performed using Clustal X 1.8.3 [17] with the default settings. Phylogenesis was analyzed with MEGA version 3.0 software, and

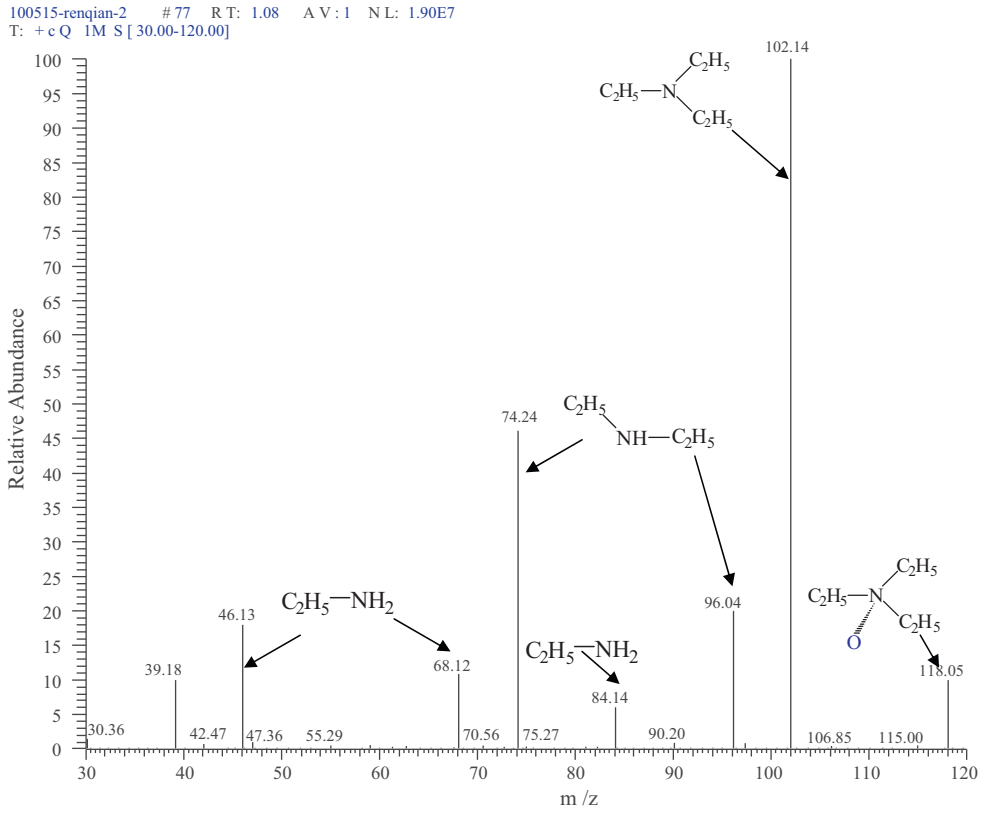


**Fig. 4.** Mass spectrum of TAE and its metabolites produced during TEA degradation by strain R4.

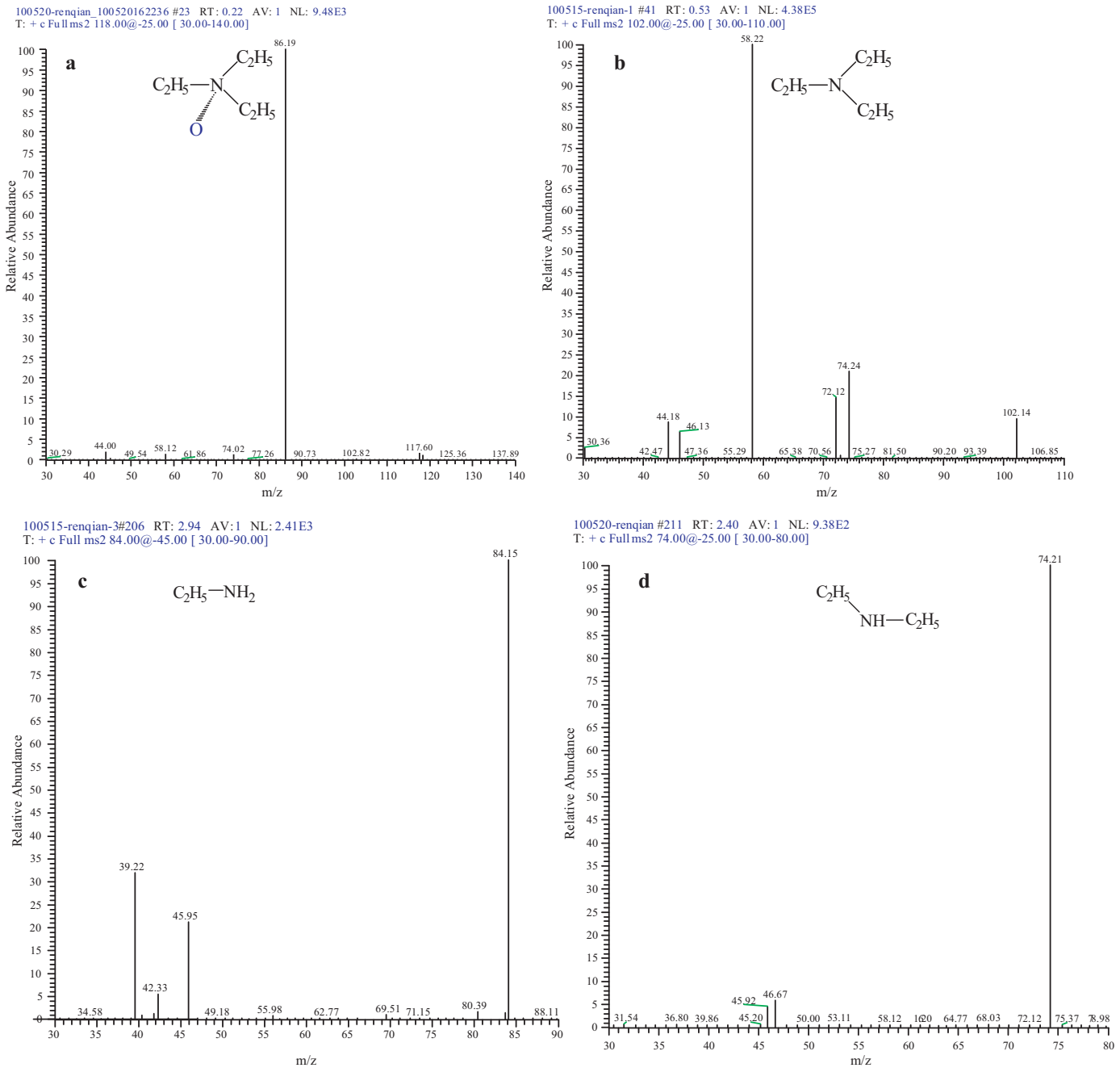


**Fig. 5.** Second-order mass spectrum of metabolites in TEA degradation. (a) TEA N-oxide; (b) TEA; (c) EA; (d) DEA.

distance was calculated using the Kimura 2-parameter distance model. A phylogenetic tree was built using the neighbor-joining method. Each dataset was bootstrapped 1000 times.

### 2.4. Degradation of TEA and other amines by strain R4

Cells of strain R4 were harvested in late-exponential phase (20 h), collected by centrifugation and washed twice and re-suspended with MSM ($OD_{600\,nm}$ = 1.0). To determine the effect of pH, NaCl concentration and temperature on the degradation of TEA by R4, the re-suspended cells were inoculated into 100 ml MSM containing 100 mg $l^{-1}$ TEA by 1% inoculums and incubated for 32 h at different temperatures (15, 20, 25, 30, 35 and 40 °C), under different pH conditions (6.0–9.0, in increments of 1.0 pH units) and at different NaCl concentrations (final concentrations of 0–2.0% (w/v) in increments of 0.25%). To determine the degradation spectrum of strain R4, TEA, DEA, EA and TMA (each 100 mg $l^{-1}$) were selected as substrates, and after incubation at 30 °C, the bacterial culture was harvested every 4 h to detect the concentration of the substrates (GC method) and ammonia. All treatments were performed in triplicate.

### 2.5. Effect of metal ions and chemical agents on TEA biodegradation

The re-suspended ($OD_{600\,nm}$ = 1.0) R4 cells were inoculated to MSM containing 100 mg $l^{-1}$ TEA by 1% inoculum. Different concentrations of metal ions ($Cu^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Ag^{+}$), NaCl, $SO_4^{2-}$ ($Na_2SO_4$) and $NH_4^{+}$ ($NH_4Cl$) were separately added to the medium. After incubation at 30 °C and 150 rpm for 32 h, the con-

centration of TEA in the medium was detected by GC method. All treatments were performed in triplicate.

### 2.6. Enzyme assays

R4 cells were cultured in 400 ml MSM medium containing 100 mg $l^{-1}$ TEA at 30 °C and 150 rpm for 32 h and were harvested by centrifugation. The cells were then washed twice with 100 mM phosphate buffer solution (PBS, pH 7.0). Washed cells were re-suspended in 1 ml fresh PBS (pH 7.0) containing 0.1 mM phenylmethanesulfonyl fluoride (PMSF) and were disrupted using an ultrasonic cell disruptor at 20 kHz for 5 min. The extract was centrifuged at 10,000 × *g* for 15 min. The supernatant was used for enzyme assays.

Monooxygenase activities were determined spectrophotometrically by measuring the oxidation of NADPH at 340 nm and 30 °C. The reaction mixture (2.5 ml) contained 100 μmol PBS (pH 7.0), 5 μmol TEA/DEA/EA as substrate, 0.2 μmol NADPH, and protein (cell-free extract). The reaction was initiated by addition of the substrate. The extinction coefficient of NADPH was $6.22 \times 10^3$ $M^{-1}$ $cm^{-1}$. One unit of enzyme was defined as the amount of enzyme required to oxidize 1 μmol NADPH per minute under these conditions. The formula of the enzyme activity was: enzyme activity (U $mg^{-1}$) = [$\Delta A$/min × 106 × Vs/($e \times L \times C \times V1$)], where Vs is the total reaction volume (μl), $e$ is the extinction coefficient of NADPH, $L$ is the cuvette diameter (cm), $C$ is the protein content (mg $ml^{-1}$), and $V1$ is the protein volume (μl). The protein content was quantified by the Bradford method using bovine serum albumin as the standard [18].

### 2.7. Cell immobilization

The R4 cells re-suspended in MSM ($OD_{600\,nm}$ = 1.0) were used for immobilization experiments. The method of immobilizing cells on AL gel beads was performed according to Chen et al. [19]. The gel beads were spheres of a 0.25 cm diameter. The hardened beads were flushed with sterilized water. Control beads were prepared without cells and were generated in the same way. The free and immobilized cells (cell masses were equal) were cultured in a 250-ml flask containing 100 ml MSM and 100 mg $l^{-1}$ TEA at 150 rpm and 30 °C for 32 h.

### 2.8. Chemical analysis

The concentration of TEA in liquid cultures was detected by GC with a flame ionization detector [20]. The column that was used for determination was a capillary column (30 m × 0.53 mm inner diameter with a film thickness of 0.01 mm). The column was conditioned and operated at 30 °C. The detector temperature was 250 °C, and the injector temperature was 200 °C. The flow-rate of carrier gas, hydrogen and air were 15, 45 and 450 ml $min^{-1}$, respectively. Toluene was used as the internal standard compound. Dichloromethane (1 ml) [21] and NaOH (1 mol $l^{-1}$, 1 ml) were added to the liquid cultures (10 ml) which were filtered through organic microporous membrane (0.22 μm) to release free TEA. All samples were severely oscillated, and then left undisturbed for 10 min. The lower liquid was used for injection. Standard methods were used to analyze ammonia produced in the culture [22].

The metabolites produced during TEA degradation were identified by MS/MS (Mass Spectrometry), Finnigan TSQ Quantum Ultra AM (Thermal, USA). Liquid culture samples, which were filtrated with a microporous membrane (0.22 μm), were collected when the degrading rates of TEA were 30, 50 and 70%. The metabolites in the sample were then separated and confirmed by standard MS, ionized by electrospray with a positive polarity and scanned in the normal mass range, from 30 $m/z$ (mass to charge ratio) to 300 $m/z$.

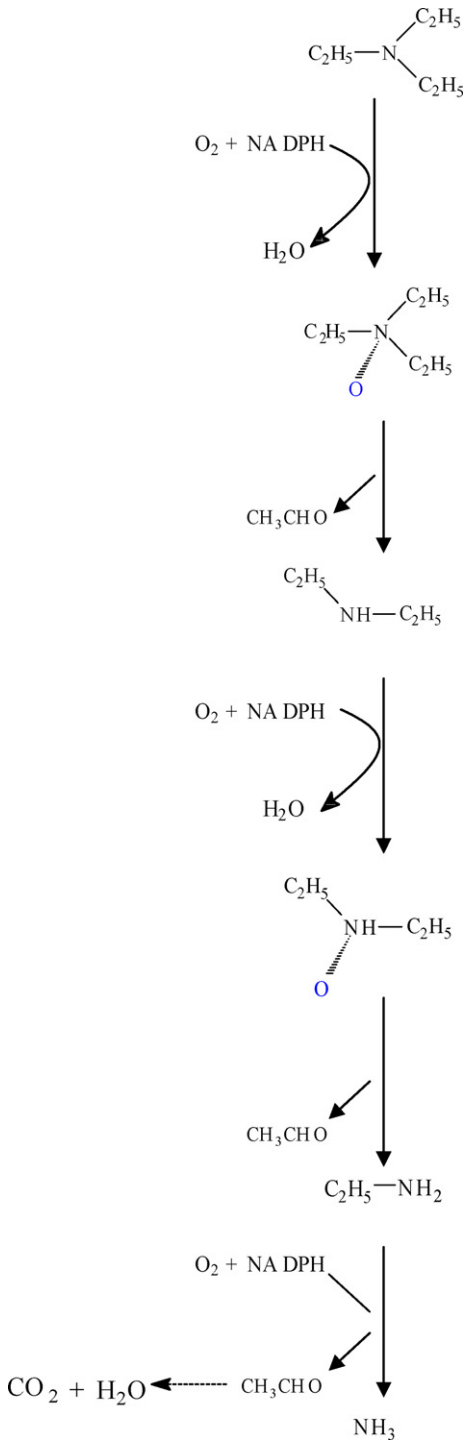


**Fig. 6.** Proposed pathway of TEA metabolism in strain R4.

Characteristic fragment ions were detected with second-order MS.

## 3. Results

### 3.1. Strain isolation and identification

A number of bacterial strains were isolated from the activated sludge using the enrichment culture technique. All of these strains were tested for their ability to degrade TEA. A bacterial strain designated as R4 was selected for further study. Strain R4 showed the

highest degrading ability and could completely degrade 100 mg $l^{-1}$ TEA to an undetectable level in 32 h.

When cultured on LB agar, strain R4 formed white, circular, convex colonies, and was characterized as a Gram-positive, non-spore-forming, rod-shaped bacterium with a flagellum. Strain R4 tested negative for oxidase activity, but tested positive for lactose fermentation and nitrate reduction. The 16S rRNA gene sequence analysis revealed that strain R4 was homologous with *Arthrobacter protophormiae* DSM20168 (99.93% similarity, X80745) (Fig. 1). Therefore, based on its morphological, physiological and biochemical properties, and the 16S rRNA gene sequence analysis, strain R4 was identified as *Arthrobacter protophormiae*. The nucleotide sequence coding for the 16S rRNA of strain R4 (1484 bp) was deposited in the GenBank database with accession number HM191728.

### 3.2. Degradation of TEA and other amines by strain R4

Strain R4 could degrade TEA (100 mg $l^{-1}$) at 15, 20, 25, 30, 35, and 40 °C (pH 7.0) in 32 h with an efficiency of 41.1, 67.4, 81.6, 100, 80.2 and 53.2%, respectively. In addition, the efficiency of TEA degradation by strain R4 at pH 6.0, 7.0, 7.5, 8.0, and 9.0 (30 °C) in 32 h was 73.4, 100, 84.1, 76.2 and 59.7%, respectively. Thus, the optimal pH and temperature for TEA degradation by strain R4 were 7.0 and 30 °C, respectively. Concentrations of NaCl higher than 1% were inhibitory to TEA biodegradation, and the optimum concentration of NaCl for TEA degradation by strain R4 was 0.5% (Fig. 3b). High concentrations of TEA (up to 1000 mg $l^{-1}$) could also be degraded by strain R4.

Degradation spectrum results (Fig. 2) showed that strain R4 could utilize TEA, DEA and EA as sole carbon and nitrogen sources for growth and could effectively degrade TEA (R4 could completely degrade TEA in 32 h). In addition, DEA and EA could be degraded more rapidly than TEA. However, TMA could not be degraded by strain R4 under these conditions. The concentrations of ammonia produced by TEA, DEA and EA were 8.3, 14.1 and 25.2 mg $l^{-1}$, respectively, these values are similar to the theoretical values of ammonia when TEA, DEA and EA (100 mg $l^{-1}$) are completely mineralized. Substrates in control samples (without inoculated cells) were not degraded at all (data not shown).

### 3.3. Effect of metal ions and chemical agents on TEA biodegradation

Low concentrations of $Cu^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Ag^{+}$ (0.1 mM) slightly inhibited the degradation of TEA (less than 10% inhibition), while high concentrations of $Cu^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Ag^{+}$ (1.0 mM) strongly inhibited the degradation efficiency (50–80% inhibition, Fig. 3). Addition of either $SO_4^{2-}$ or $NH_4^{+}$ reduced the degradation efficiency of TEA to a certain extent. The inhibition became significant (more than 80% inhibition) when the concentration of $SO_4^{2-}$ and $NH_4^{+}$ reached 11 mM and 30 mM, respectively.

### 3.4. The TEA biodegradation pathway in strain R4

The metabolites produced were identified first by MS. The mass spectrum of TEA and its metabolites is shown in Fig. 4. The prominent protonated molecular ions were found at $m/z$ = 118 $[M+H]^+$, 102 $[M+H]^+$, 96 $[M+Na]^+$, 90 $[M+H]^+$, 84 $[M+K]^+$, 74 $[M+H]^+$, 68 $[M+Na]^+$ and 46 $[M+H]^+$, and the compounds corresponding to these protonated molecular ions were designated as products A, B, C, D, E, F, G and H.

The $m/z$ of product A was 118 $[M+H]^+$, enabling the assignment of the molecular ion ($M^+$) at $m/z$ = 117. Product A, with the characteristic second-order MS fragment ion peaks at $m/z$ = 102.22, 86.19, 74.02, 58.12, 44 and 30.29 (Fig. 5a), was formed by TEA N-oxide.

The positive-ion chemical ionization of product B showed a prominent protonated molecular ion at $m/z$ = 102 $[M+H]^+$ and characteristic second-order MS fragment ion peaks at $m/z$ = 74.24, 72.12, 58.22, 46.13, 44.18 and 30.36 (Fig. 5b), so the molecular weight of product B is 101. On the basis of the molecular weight and the characteristic fragment ion peaks, product B was identified as TEA.

Product C showed a prominent protonated molecular ion at $m/z$ = 96 $[M+Na]^+$, enabling the assignment of the molecular ion ($M^+$) at $m/z$ = 73. Product F showed a base peak at $m/z$ = 74 $[M+H]^+$, also enabling the assignment of the molecular ion ($M^+$) at $m/z$ = 73 (Fig. 5d). Products C and F were identified as DEA.

The positive-ion chemical ionization of product E showed a prominent protonated molecular ion at $m/z$ = 84 $[M+K]^+$ and characteristic second-order MS fragment ion peaks at $m/z$ = 45.95, 42.33 and 39.22 (Fig. 5c), so the molecular weight of product D is 45. Product G showed a prominent protonated molecular ion at $m/z$ = 68 $[M+Na]^+$, enabling the assignment of the molecular ion ($M^+$) at $m/z$ = 45, and product H showed a prominent protonated molecular ion at $m/z$ = 46 $[M+H]^+$. Therefore, products E, G and H were all identified as EA.

Product D, which was present at trace concentrations in the degradation pathway, was presumed to be DEA N-oxide because it showed a prominent protonated molecular ion at $m/z$ = 90 $[M+H]^+$.

The monooxygenase activities are shown in Table 1. The cell-free extract exhibited TEA monooxygenase, DEA monooxygenase and EA monooxygenase activities in the TEA degradation pathway.

The proposed pathway of TEA metabolism by strain R4 is depicted in Fig. 6. TEA was first oxidized to TEA N-oxide and then transformed to DEA. The metabolism of DEA was similar to TEA, and EA was produced; EA was then degraded to ammonia and acetaldehyde, and acetaldehyde was then completely mineralized.

**Table 1**
Monooxygenase activities of strain R4.

| Substance | Enzyme activity<br>Enzyme activity (U $mg^{-1}$) in cell-free extract from strain R4 |
|---|---|
| TEA | 244.25 ± 1.2 |
| DEA | 105.27 ± 1.0 |
| EA | 45.61 ± 1.0 |

### 3.5. The efficiency of TEA removal by immobilized versus free cells

The efficiency of TEA removal by free and immobilized cells of *Arthrobacter protophormiae* R4 was determined over a temperature range from 20 °C to 40 °C and a pH range from 6.0 to 9.0. The optimal temperature and pH for the degradation of TEA by both free and immobilized cells were 30 °C and 7.0, respectively (Table 2), and the efficiency of TEA removal by free and immobilized cells was almost equal. These results indicated that use of immobilized *Arthrobacter protophormiae* R4 cells was feasible.

**Table 2**
Efficiency of TEA removal by immobilized versus free cells.

| | TEA removal efficiency (%) | |
|---|---|---|
| | Free cells | Immobilized cells |
| Temperature (°C) | | |
| 20 | 32.4 | 30.1 |
| 30 | 100 | 100 |
| 40 | 43.5 | 44.7 |
| pH | | |
| 6.0 | 54.7 | 52.3 |
| 7.0 | 100 | 100 |
| 8.0 | 49.5 | 43.7 |
| 9.0 | 17.6 | 18.9 |

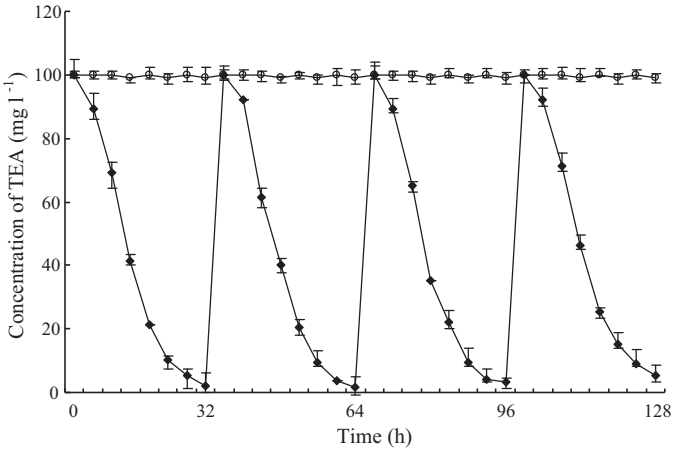


**Fig. 7.** Evaluation of the reuse potential of immobilized cells. (○) Control (immobilized beads without cells); (♦) immobilized cells.

### 3.6. Reuse of immobilized cells for TEA degradation

The feasibility of reuse of immobilized *Arthrobacter protophormiae* R4 cells for TEA degradation was evaluated. The immobilized cell beads were reused in four consecutive degradation experiments, and TEA was almost completely degraded in each cycle (Fig. 7). Results from this experiment revealed that the immobilized cells could be reused without reduction in their TEA degradation capability. This phenomenon could reduce expenses during operational periods.

## 4. Conclusions

TEA is malodorous, and it can severely endanger human health when present in the water environment. Microbial degradation has been considered to be the most effective method for TEA removal. However, little is known about the microbial degradation of TEA. In this paper, a highly effective, broad-spectrum amine-degrading and strictly aerobic strain, *Arthrobacter protophormiae* R4, was isolated using the enrichment culture technique. It was found that *Arthrobacter protophormiae* R4 can completely degrade 100 mg $l^{-1}$ TEA to ammonia in 32 h. This strain can also degrade DEA and EA completely but fails to degrade TMA.

The monooxygenase activity was determined spectrophotometrically by measuring the oxidation of NADPH at 340 nm and 30 °C. This method has been referred to as the TMA monooxygenase detecting method [23].

Some metal ions and $SO_4^{2-}$ are often found in industrial wastewater at high concentrations and can inhibit the growth and degradation capacity of the microorganisms in wastewater treatment facilities [24]. Here, we report that high concentrations of $Cu^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Ag^{+}$ (1.0 mM), and $SO_4^{2-}$ (11 mM) significantly inhibited the biodegradation of TEA. Moreover, ammonia was one of the products of TEA biodegradation, and when the concentration of ammonia dissolved in wastewater was 30 mM, TEA biodegradation was greatly inhibited. Therefore, it may be necessary to reduce these inhibitory elements when strain R4 is used to treat industrial wastewater.

In this paper, the pathway of TEA metabolism in strain R4 was investigated by metabolite identification and enzymatic studies, and the proposed pathway is shown in Fig. 6. TEA is first oxidized to TEA N-oxide and then transformed to DEA. The metabolism of DEA is similar to TEA and results in production of EA; EA is then degraded to ammonia and acetaldehyde, and acetaldehyde is then completely mineralized.

The degradation of TEA by both free and immobilized cells was investigated. Results from these experiments revealed that immobilized *Arthrobacter protophormiae* R4 cells (in AL gels) are feasible for TEA degradation. Results also indicated that immobilized cells could be reused without reduction in their capacity for TEA degradation, and this phenomenon could reduce expenses during operational periods.

Thus, in light of its broad-spectrum substrate specificity, relatively high degradation activities, and feasibility in AL gel immobilization, the application of strain R4 has the potential to remove TEA and other amines residues from industrial wastewater.

## Acknowledgments

We gratefully acknowledge Dr. Zhichao Xu of Nanjing Science and Technology University for excellent assistance in MS analysis. This work was supported by Provincial Environmental Protection Scientific Research Projects of Jiangsu Provience (2009001), National Technology Support Project (2008BAD96B05).

## References

[1] B. Åkesson, T. Florén, S. Skerfving, Visual disturbances after experimental human exposure to triethylamine, Br. J. Ind. Med. 45 (1988) 262–268.
[2] L. Belin, U. Wass, G. Audunsson, I. Mathiasson, Amines possible causative agents in the development of bronchial hyperreactivity in workers manufacturing polyurethanes from isocyanates, Br. J. Ind. Med. 40 (1983) 251–257.
[3] H. Aimin, L.X. Cao, C. Jie, F.J. Spiess, L.S. Steven, N.O. Timothy, O.H. Steve, D.F. James, Photocatalytic degradation of triethylamine on titanium oxide thin films, J. Catal. 188 (1999) 40–47.
[4] J. Frings, C. Wondrak, B. Schink, Fermentative degradation of triethanolamine by a homoacetogenic bacterium, Arch. Microbiol. 162 (1994) 103–107.
[5] C.C. Wang, K.L. Lu, X.Y. Chen, Removal of triethylamine from synthetic wastewater by acclimated mixed bacteria cultures, Int. Bioteter. Biodegr. 59 (2007) 202–205.
[6] S. Rappert, R. Ayoub, R. Li, K. Botsch, I. Obi, R. Müller, Biologischer Abbau von geruchsaktiven Einzelstoffen und von realen Vielstoffgemischen. Erfassung und Minimierung von Gerüchen, in: B. Niemeyer, A. Robers, P. Thiesen (Eds.), Messungund Minimierung von Gerüchen, Hamberburger Berichte, 2004, pp. 203–220.
[7] T. Li, J. Liu, R. Bai, D.G. Ohandja, F.S. Wong, Biodegradation of organonitriles by adapted activated sludge consortium with acetonitrile-degrading microorganisms, Water Res. 41 (2007) 3465–3473.
[8] R.R. Dash, A. Gaur, C. Balomajumder, Cyanide in industrial wastewaters and its removal: a review on biotreatment, J. Hazard. Mater. 163 (2009) 1–11.
[9] M.D. Adjei, Y. Ohta, Isolation and characterization of a cyanide-utilizing *Burkholderia cepacia* strain, World J. Microbiol. Biotechnol. 15 (1999) 699–704.
[10] A.Y. Dursun, O. Tepe, Internal mass transfer effect on biodegradation of phenol by Ca-alginate immobilized *Ralstonia eutropha*, J. Hazard. Mater. B 126 (2005) 105–111.
[11] G. Gonzalez, M.G. Herrera, M.T. Garcia, M.M. Pena, Biodegradation of phenol in a continuous process: comparative study of stirred tank and fluidized-bed bioreactor, Bioresour. Technol. 76 (2001) 245–251.
[12] M.N. Takako, K. Kohtaro, K. Kuniki, Degradation of dimethyl sulfoxide by the immobilized cells of *Hyphomicrobium denitrificans* WU-K217, Biochem. Eng. J. 15 (2003) 199–204.
[13] R. Muñoz, M. Jacinto, B. Guieysse, B. Mattiasson, Combined carbon and nitrogen removal from acetonitrile using algal-bacterial bioreactors, Appl. Microbiol. Biotechnol. 67 (2005) 699–707.
[14] J.P. Chen, Y.S. Lin, Decolorization of azo dye by immobilized Pseudomonas luteola entrapped in alginate-silicate sol–gel beads, Process Biochem. 42 (2007) 934–942.
[15] J.G. Holt, N.R. Krieg, P.H. Sneath, J.T. Staley, S.T. Williams, Bergey's Manual of Determinative Bacteriology, 9th ed., 2004.
[16] R. Li, X. Guo, K. Chen, J. Zhu, S.P. Li, J.D. Jiang, Isolation of an isocarbophosdegrading strain of *Arthrobacter* sp. scl-2 and identification of the degradation pathway, J. Microbiol. Biotechnol. 19 (2009) 1439–1446.
[17] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeamougin, D.G. Higgins, The Clustal-X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucleic Acids Res. 25 (1997) 4876–4882.
[18] M.M. Bradford, Arapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.
[19] C.Y. Chen, C.M. Kao, S.C. Chen, Application of *Klebsiella oxytoca* immobilized cells on the treatment of cyanide wastewater, Chemosphere 71 (2008) 133–139.

[20] S.G. Kim, H.S. Bae, S.T. Lee, A novel denitrifying bacterial isolate that degrades trimethylamine both aerobically and anaerobically via two different pathways, Arch. Microbiol. 176 (2001) 271–277.
[21] K. Tamura, J. Dudley, M. Nei, MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0, Mol. Biol. Evol. 24 (2007) 1596–1599.
[22] T. Mehrdad, et al., Ammonia disinfection of animal feeds—laboratory study, Int. J. Food Microbiol. 122 (2008) 23–28.
[23] C.A. Boulton, M.J.C. Crabble, P.J. Large, Partial purification of a trimethylamine monooxygenase from pseudomonas aminovorans and its role in growth on trimethylamine, Biochem. J. 140 (1974) 253–263.
[24] W.K. Chun, B.R.S. Genthner, Effect of added heavy metal ions on biotransformation and biodegradation of 2-chlorophenol and 3-chlorobenzoate in anaerobic bacterial consortia, App. Environ. Microb. 62 (1996) 2317–2323.