BIOMEDICAL AND ENVIRONMENTAL SCIENCES 16, 398-404 (2003)

Microbial Degradation of Aniline by Bacterial Consortium[1]

JIAN-LONG WANG[*,2], ZE-YU MAO[†], AND WEI-ZHONG WU[△]

[*]Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, China; [†] Department of Hydraulic Engineering, Tsinghua University, Beijing 100084, China; [△]School of Environmental Science, Peking University, Beijing 100871, China

Objective To investigate the characteristics of microbial degradation of aniline by a stable bacterial consortium. **Methods** The bacterial consortium was isolated from activated sludge treating chemical wastewater using aniline as the sole source of carbon and nitrogen by enrichment and isolation technique. The biomass was measured as optical density (OD) at 510 nm using a spectrophotometer. Aniline concentrations were determined by spectrophotometer. The intermediates of aniline degradation were identified by GC/MS method. **Results** The bacterial consortium could grow at a range of aniline concentrations between 50 and 500 mg/L. The optimal pH and temperature for aniline degradation were determined to be 7.0 and 30, respectively. The presence of NH_4NO_3 as an additional nitrogen source (100-500 mg/L) had no adverse effect on bacterial growth and aniline degradation. The presence of heavy metal ions, such as Co^{2+}, Zn^{2+}, Ni^{2+}, Mn^{2+} and Cu^{2+} had an inhibitory effect on aniline degradation. **Conclusions** The isolated bacterial consortium can degrade aniline up to 500 mg/L effectively and tolerate some heavy metal ions that commonly exist in chemical wastewater. It has a potential to be applied in the practical treatment of aniline-containing wastewater.

Key words: Aniline; Priority pollutant; Biodegradation; Wastewater treatment

INTRODUCTION

Aniline is an important industrial chemical used as an intermediate in the production of a wide range of synthetic organic chemicals and polymers, such as pesticides, dyes and pharmaceuticals. Wide-scaled production and use of aniline make it present in many effluents from the chemical industries. Its industrial manufacture and application could discharge aniline-containing wastes into the environment. In addition, aniline can also enter the environment as a result of partial biodegradation of xenobiotic compounds including certain azo dyes. Aniline can also be formed as a product of initial microbial transformation from a number of agro-chemicals, such as carbamates, ureas and aromatic amides[1], and therefore aniline is ubiquitous in the environment.

Aniline is a toxic pollutant, which has been listed as a priority pollutant by China Environmental Monitoring Center, the U.S. Environmental Protection Agency and other similar authorities. It is known to be carcinogenic and very harmful to aquatic life. Aniline is

[1] The work was supported by the National Natural Science Foundation of China (Grant No. 29637010).

[2] Correspondence should be addressed to Dr. Jian-Long WANG, Tel: 86-10-62784843. Fax: 86-10-62771150. E-mail: wangjl@tsinghua.edu.cn

Biographical note of the first author: Jian-Long WANG, born in 1964, Ph. D. degree, professor of Tsinghua University, majoring in the field of water pollution control as well as environmental biotechnology.

0895-3988/2003
CN 11-2816

not biodegraded as easily as phenol and bezonate. We have investigated the removal of aniline from aqueous solution by macro-porous adsorptive resin. To date a few studies have been done on the biodegradation of aniline-like xenobiotic compounds. Sheng *et al.* investigated the solution of a bacterium for degrading high concentration of aniline[2]. Liu *et al.* studied the isolation and characterization of a bacterial strain for the degradation of aniline[3]. O'Neil *et al.* studied the bacterial growth on aniline[4]. Recently Bengtsson and Carlsson[5] studied the contribution of suspended and adsorbed groundwater bacteria to degradation of dissolved and adsorbed aniline.

This research was aimed at addressing the biodegradation of aniline by an isolated stable bacterial consortium.

MATERIALS AND METHODS

Inoculum and Medium

The activated sludge was taken from aeration tank treating chemical wastewater and used as inoculum. The composition of medium used for acclimation is shown in Table 1.

TABLE 1

Medium for Acclimation of Activated Sludge

Chemicals	Concentration (mg/L)
KH_2PO_4	6000
$CaCl_2 \cdot 2H_2O$	30
NaCl	500
$MgSO_4 \cdot 7H_2O$	50
$FeCl_3 \cdot 6H_2O$	1.0
$ZnSO_4 \cdot 7H_2O$	1.0
$CuSO_4 \cdot 5H_2O$	1.0
$CoCl_2 \cdot 6H_2O$	1.0
Aniline	50-500

Note. The pH was adjusted to be 6.0-7.0.

Analytical Methods

Biomass was measured as the optical density (OD) at 510 nm using a spectrophotometer. Aniline concentrations of all samples in this work were determined by spectrophotometer. The absorbance of the colored complex of aniline with N-(1-Naphthyl)-ethylendiamine-dihydro-chloride was read at 545 nm by a UV-spectrophotometer (UV-250 Model Spectrometer, Shimadzu Co., Japan).

RESULTS AND DISCUSSION

Acclimation of Sludge

The enriched activated sludge was used as seed sludge. The acclimation was carried out by gradually increasing the aniline concentration from 100 mg/L to 500 mg/L. After two-month operation, a stable bacterial consortium capable of degrading aniline was obtained. The bacterial growth on aniline at different concentrations was investigated and the results are shown in Fig.1.

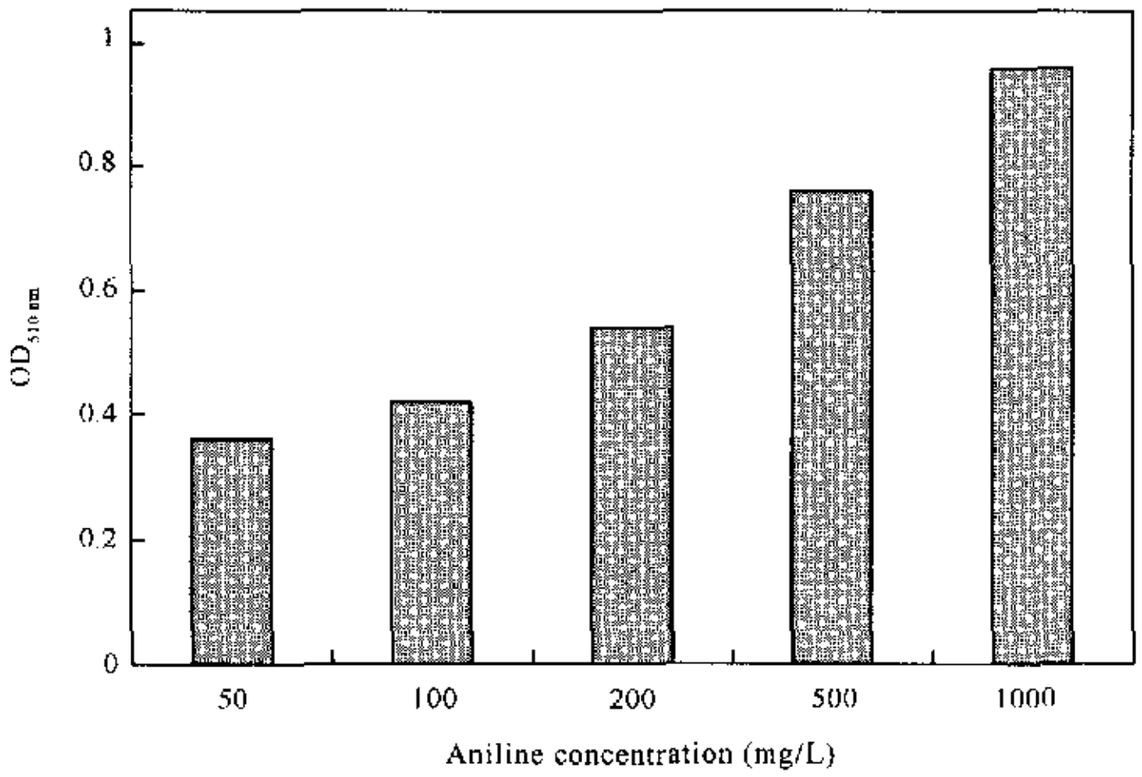

FIG. 1. The bacterial growth on aniline at different aniline concentrations.

It was indicated that the bacterial consortium isolated in this study could grow at a range of aniline concentrations (50-500 mg/L), which suggested that the bacterial consortium was adapted to aniline and could be used as the sole source of carbon and nitrogen.

Bacterial Growth and Aniline Degradation

During the bacterial growth of aniline, the measurement of bacterial biomass (as OD at 510 nm) and aniline degradation were performed. In this experiment, aniline was used as the sole source of carbon and nitrogen, and its initial concentration was 500 mg/L. The pH was adjusted to be 7.0. The bacterial growth curve is depicted in Fig. 2. Meanwhile, the variation of aniline during bacterial growth was also measured in order to study the aniline degradation, and the result is shown in Fig. 2.

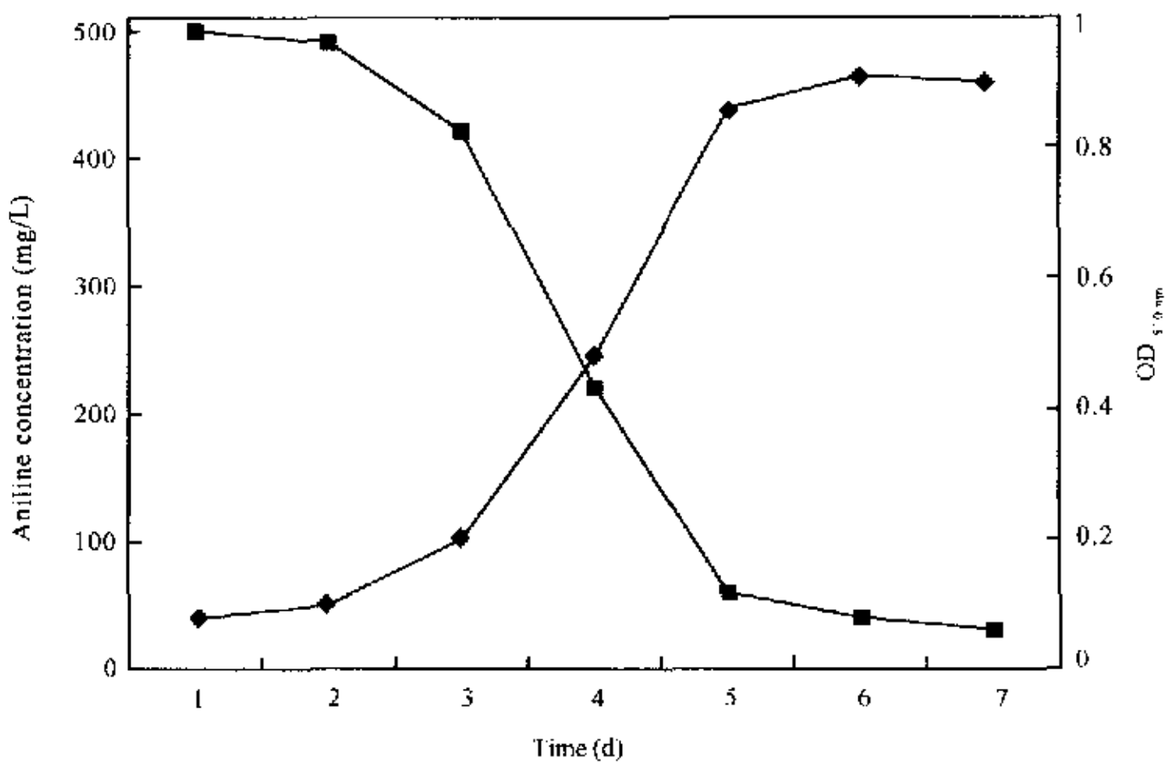

FIG. 2. Variation of aniline degradation and bacterial growth with time.
Symbol (◆)representing the biomass concentration and (■) representing the aniline concentration.

It was evident that the bacterial consortium could use aniline as a substrate to grow. The bacterial growth corresponded with aniline degradation quite well. It was accepted that ammonium was released from aromatic ring during the biodegradation of aniline. Consequently, analysis of ammonia variation could be used to give an indication of aniline degradation. In this study, we also analyzed the variation of ammonia concentration during bacterial growth, which revealed that a high degree of aniline mineralization was achieved (data not shown).

The results also indicated that aniline could be completely removed from the growth medium. During aniline degradation, formation of a yellow pigment in the growth medium was observed, which suggested that some intermediates were produced and accumulated. O'Neill *et al.* [4] also reported the production of a pigment in the medium during bacterial growth on aniline; they suggested that the formation of 2-hydroxymuconic semi-aldehyde was resulted from meta cleavage of catechol, but it had to be further confirmed.

Effect of pH

The pH of the medium was adjusted to be 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. The effect of pH on aniline degradation was investigated and the results are shown in Fig. 3.

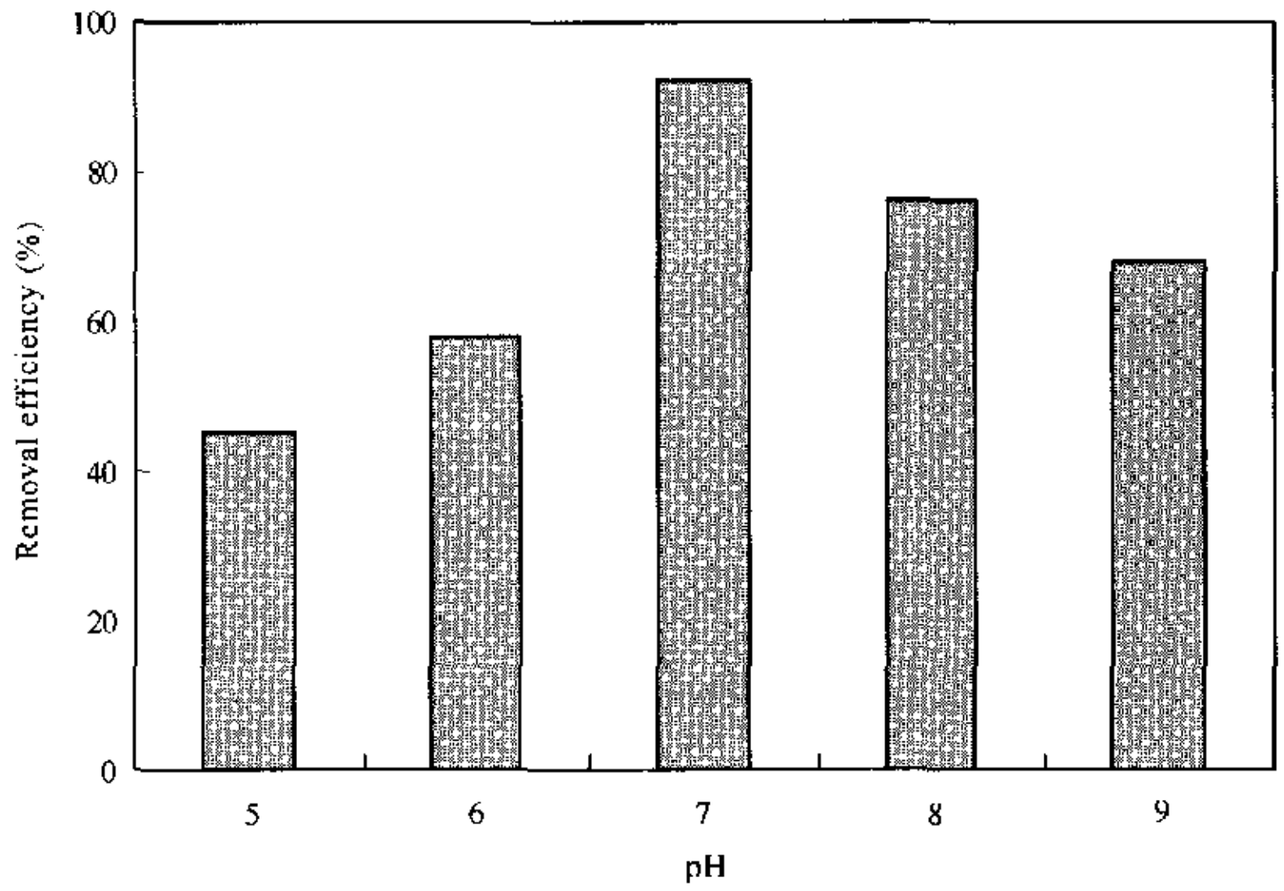

FIG. 3. Effect of pH on aniline degradation.

The optimal pH for aniline degradation was 7.0, and when the pH value was either increased from 7.0 to 9.0 or decreased from 7.0 to 5.0, the removal efficiency of aniline would decrease.

Effect of Temperature.

The effect of temperature on aniline degradation was investigated at 15℃, 20℃, 30℃, 40℃ and 50℃, and the results are shown in Fig. 4.

We could see that the optimal temperature for aniline degradation was 30℃. However, the bacterial consortium could degrade aniline at all degrees of temperature. When the temperature changed from 20℃ to 40℃, the removal efficiency of aniline was above about

90%, which suggested that this isolated bacterial consortium had a good adaptation to temperature change.

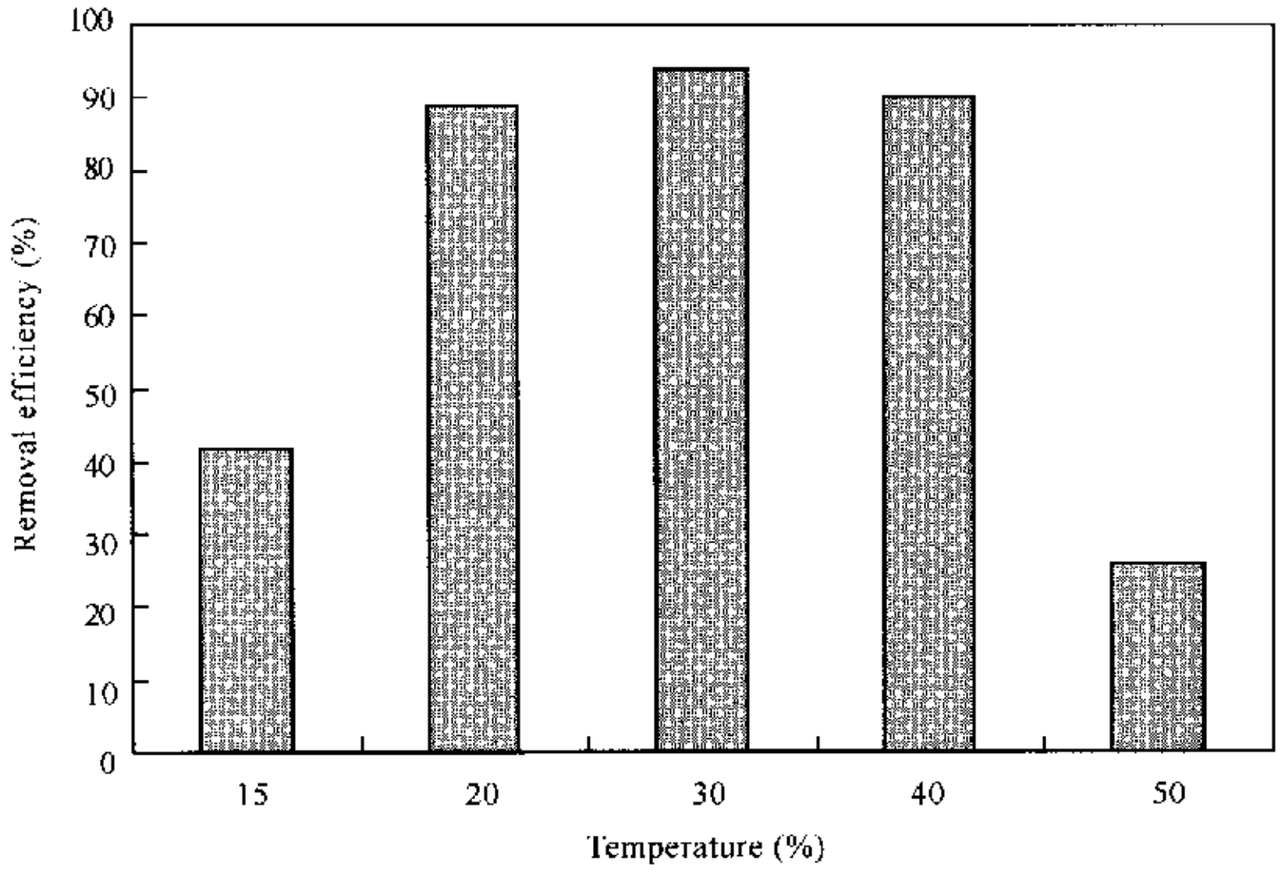

FIG. 4. Effect of temperature on aniline degradation.

Effect of Nitrogen Source

The above study indicated that the isolated bacterial consortium could use aniline as the sole source of carbon and nitrogen, unless as stated otherwise, aniline was not used as the sole source of carbon and nitrogen. However, industrial wastewater was likely to contain alternative source of nitrogen. Consequently, the effect of an additional source of nitrogen on aniline degradation was studied. Ammonium nitrate was used as an additional nitrogen source, its concentration varied from 100 mg/L to 500 mg/L. The aniline concentration was kept at 500 mg/L, and the pH was adjusted to be 7.0. The results are shown in Table 2.

TABLE 2

Effect of Additional Nitrogen Source on Bacterial Growth and Aniline Degradation

NH_4NO_3 (mg/L)	OD_{510nm}	Aniline Removal (%)
100	0.78	100
200	0.79	100
300	0.76	100
400	0.80	100
500	0.79	100

The presence of an additional nitrogen source had no effect on bacterial growth and aniline degradation. O'Neill *et al.*[4] also studied the bacterial growth on aniline in the presence of NH_4Cl as an additional nitrogen source. The results revealed that the mean generation time of bacteria, aniline degradation and ammonia release was not adversely affected by the presence of NH_4Cl. Sheng *et al.* [2] also investigated the effect of the ratio of carbon to nitrogen on bacterial growth. They concluded that when C/N ratio (mol/mol) was

2:1, the biomass in the mixed liquor achieved its maximal value. The increase or decrease in C/N ratio would result in reduction of bacterial growth and lower degradative ability of bacteria. The reason might be that the presence of excess nitrogen source could inhibit the release of ammonium from aromatic ring of aniline, which is generally regarded as the initial step in aniline degradation.

Effect of Heavy Metal Ions

Industrial wastewater is likely to contain some heavy metal ions that usually have adverse impacts on treatment efficiency. Therefore the effect of several heavy metal ions on bacterial degradative ability was investigated. The tested metal ions included $CoCl_2$, $ZnSO_4$, $NiSO_4$, $MnCl_2$, $CuSO_4$ at a concentration of 1.0 mmol/L. The initial aniline concentration was 500 mg/L, with the pH of the medium adjusted to be 7.0. The results are shown in Fig. 5.

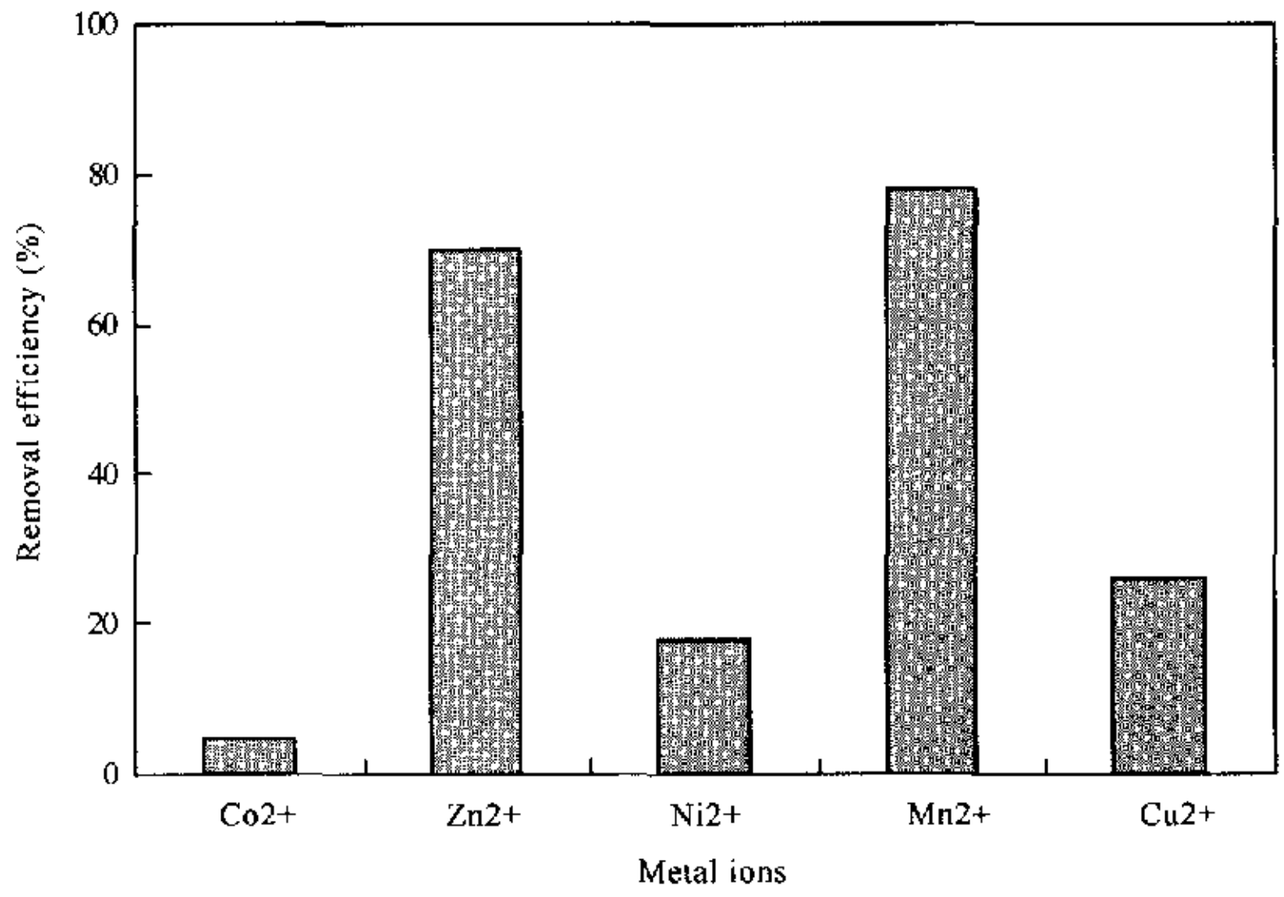

FIG. 5. Effect of heavy metal ions on aniline degradation.

It was demonstrated that Co^{2+} had a strong inhibitory effect on aniline degradation. The presence of Mn^{2+} and Zn^{2+} in the medium had less inhibition on the degradative ability of bacterial consortium.

The inhibitory order was $Co^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+}$.

Identification of Metabolites

Metabolites of aniline degradation by isolated bacterial consortium were extracted using dichloromethane at different time intervals and identified by GC/MS method. Some of the intermediates were isolated and identified. The mass spectral analysis indicated that the main compounds isolated during aniline degradation were catechol and 2-hydroxymuconic semi-aldehyde. Therefore, the pathway of aniline degradation by isolated bacterial consortium could be tentatively proposed as follows (Fig. 6).

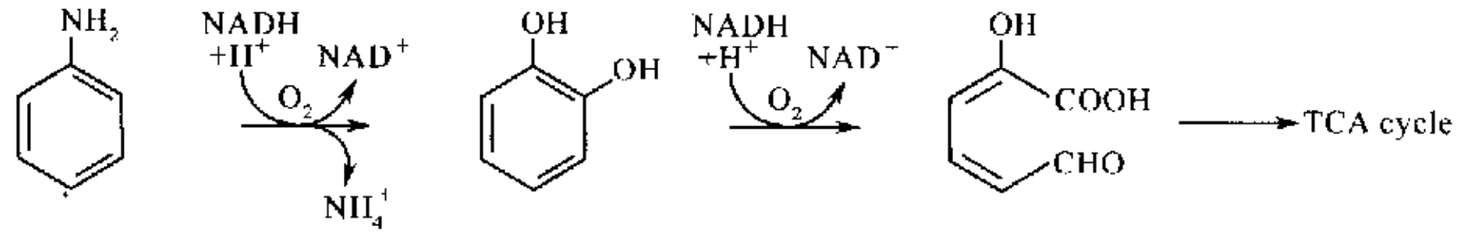

FIG. 6. Proposed pathway of aniline degradation by isolated bacterial consortium.

CONCLUSION

Through a period of acclimation, a stable bacterial consortium capable of degrading aniline can be obtained. It could use aniline as the sole source of carbon and nitrogen. The isolated consortium can grow at different aniline concentrations. The factors influencing aniline degradation, such as pH, temperature, additional nitrogen source and heavy metal ions can be determined. The optimal pH and temperature are 7.0 and 30, respectively. The bacterial growth and aniline degradation are not affected by the presence of additional nitrogen source. Heavy metal ions have adverse effects on bacterial degradative ability.

REFERENCES

1. Neilson, A.H. (1994). Organic chemicals in the aquatic environment: distribution, persistence, and toxicity. Lewis, Boca Raton. Fla.
2. Sheng, D.L., Zhang, S., and Liu, H. (1999). Isolation of a bacterium for depreding high concentration aniline. *Chin. J. Appl. Environ. Biol.* **50**, 18-22. (In Chinese)
3. Liu, Z., Yang, H., and Zhou, P. (1999). Isolation and characterization of a bacterial strain for the degradation of aniline. *Acta Scientiae Circumstantiae* **19**, 174-179. (In Chinese)
4. O'Neill, F.J., Bromley-Challenor, K.C.A., Greenwood, R.J., and Knapp, J.S. (2000). Bacterial growth on aniline: implications for the biotreatment of industrial wastewater. *Water Research* **34**, 4397-4409.
5. Bengtsson, G. and Carlsson, C. (2001). Contribution of suspended and sorbed groundwater bacteria to degradation of dissolved and sorbed aniline. *Applied Miocrobiology and Biotechnology* **57**, 234-241.

(*Received March* 26, 2003 *Accepted June* 28, 2003)