Biodegradation of Biomass Gasification Wastewater by Two Species of *Pseudomonas*Using Immobilized Cell Reactor

Shen Tian, Cheng Qian, and Xiushan Yang*

College of Life Science, Capital Normal University, 100037 Beijing, P.R. China, E-mail: cnu_yangxs@yahoo.com

Received January 18, 2005; Revised August 29, 2005; Accepted September 7, 2005

Abstract

An immobilized cell bioreactor with granular activated carbon as the inert material inoculated two species, *Pseudomonas* sp1 and *Pseudomonas* sp2, to degrade chemical oxygen demand (COD) and benzene, naphthalene, phenanthrene, pyridine, quinoline, and isoquinoline in the wastewater discharging from a biomass gasification power-generation plant. The results indicated that these toxic compounds were removed efficiently. The course of the 66-d experiment was divided into three phases mainly in accordance with different influent COD concentrations: microbial adaptation and proliferation phase (from 1 to 23 d), stable metabolic phase (from 24 to 57 d), and high efficient reaction phase (from 58 to 66 d). The high removal rates of COD and some toxic compounds with a 24-h hydraulic retention time were accomplished.

Index Entries: Biomass gasification wastewater; *Pseudomonas* species; granular activated carbon; immobilized cells; chemical oxygen demand; toxic compound removal.

Introduction

Power-generation plants of biomass gasification were built in Guangdong, Jiangsu, and Hilongjiang provinces in China. Wastewater produced in the plants contains a high concentration of chemical oxygen demand (COD) and polyaromatic hydrocarbons (PAHs), which are a major class of environmental pollutants and have toxic, mutagenic, and carcinogenic

^{*}Author to whom all correspondence and reprint requests should be addressed.

properties. The China Environmental Protection Agency has listed several of these hydrocarbons as priority pollutants because of their potential impact on humans and the ecosystems (1). Although the plants supply power from biomass, the wastewater discharged from the gasification process should be treated and reused in order to avoid pollution.

The potential for biodegradation of COD and PAHs presenting in biomass gasification wastewater is not well understood, but biodegradation would be very useful for the removal of COD and PAHs (2). Many of the PAHs are known to be biodegradable by bacteria and fungi. Researchers have demonstrated the degradability of PAHs by microorganisms (3–6). Few demonstrations have been made illustrating the biodegradabilities of COD and benzene, naphthalene, phenanthrene, pyridine, quinoline, and isoquinoline presenting in biomass gasification wastewater. In our previous study, microorganisms were able to degrade COD of biomass gasification wastewater (7,8), and two species of the genus *Pseudomonas* satisfactorily removed COD and benzene, naphthalene, phenanthrene, pyridine, quinoline, and isoquinoline by using a biofilm reactor with a 24-h hydraulic retention time (HRT) (9,10).

The aim of the present study was to investigate the possibility of improving COD removal rate and enhancing degradation of some toxic compounds in the wastewater by two species of *Pseudomonas* using an immobilized cell reactor packed with granular activated carbon.

Materials and Methods

Characteristics of Wastewater

The biomass gasification wastewater was obtained from the Energy Institute of Guang Zhou, China Academy of Science, Guang Dong province. Table 1 provides the characteristics of the wastewater.

Cultures

Two species, *Pseudomonas* sp1 and *Pseudomonas* sp2, preserved in our laboratory were used. The enrichment cultures were conducted at 120 rpm, 30° C, and 24 h in Luria-Bertani medium, and then 120 rpm, 30° C, and 96 h in modified HP medium was used for adaptation. The adapted cultures were inoculated with an amount of 6% (v/v).

Reactor

An immobilized cell reactor was made of a Plexiglas column with an active liquid volume of 0.3 L. After the wastewater was aerated, it was pumped into the reactor from the bottom. The effluent was aerated again to supplement dissolved oxygen and pumped into the reactor. The ratio of aeration to liquid was 114:1. The reaction was conducted at 40°C at pH 7.0–7.5. The time course of the experiment was divided into three phases: adaptation and proliferation phase, stable metabolic phase, and high efficient reaction phase.

 $Table\ 1$ Characteristics of Biomass Gasification Wastewater (mg/L)

рН	COD	Benzene	Naphthalene	Phenanthrene	Quinoline	Isoquinoline	Pyridine
6.8–7.5	1500-4100	12.31-36.06	58.74-69.78	0.24-0.49	6.11-9.69	0.27-6.448	2.334-7.02

Analytical Methods

The sample was centrifuged at 5°C and 9167g for 10 min. The supernatant was used to determine COD concentration according to standard methods (10). The analysis of benzene, naphthalene, phenanthrene, pyridine, quinoline, and isoquinoline was determined by SP-3420 gas chromatography at the following conditions: Flame ionization detector, nitrogen gas phase of 30 mL/min, column of 0.25 mm \times 50 m \times 0.25 μ m, gasification temperature of 250°C, detector temperature of 80°C with an increase of 70°C \times 3 min $\xrightarrow{0.25^{\circ}\text{C/s}}$ 190°C \times 0.1 min $\xrightarrow{0.13^{\circ}\text{C/s}}$ 250°C \times 5 min.

Results and Discussion

Degradation of COD

The course of the experiment lasted 66 d, during which three phases were defined as adaptation and proliferation phase (from 1 to 23 d), stable metabolic phase (from 24 to 57 d), and high efficient reaction phase (from 58 to 66 d). At the beginning of the adaptation and proliferation phase (from 1 to 9 d), the COD removal rate was 100%, owing to the adsorption of granular activated carbon. From 10 to 15 d, an effluent COD concentration of 182.99 mg/L with a 144-h HRT was reached, and from 16 to 23 d, an effluent COD concentration of 90.45 mg/L with a 96-h HRT was obtained. During this phase, the bacteria were attached on the surface of the granular activated carbon, resulting in a color change of the granular activated carbon from black to brown. Moreover, the brown gradually became fuscous with the prolonged operation time of the reactor.

After 23 d of operation, HRT was shortened to 48 h in stable degradation of the wastewater. Figure 1 shows the effluent COD concentration and COD removal rate during the stable metabolic phase.

Effluent COD concentrations of 234.4, 78.1, 77.6, and 0 mg/L with COD removal rates of 83.33, 94.45, 96.3, and 100% on 1, 2, 3, and 4 were accomplished in the 48-h HRT, respectively. It was obvious that COD degradation efficiency was increased and stabilized with operation time. The results indicated that the capacities of decomposing COD and toxic compounds were enhanced as a result of further adapted bacteria.

Figure 2 shows the effluent COD concentrations and COD removal rates with a shortened HRT of 36 h. Effluent COD concentrations of 316.2, 197.63, and 158.1 mg/L with COD removal rates of 77.51, 86.19, and 88.95% on d 1, 2, and 3–5, respectively, were achieved. Furthermore, the COD removal rate was stabilized at 91.57–94.44% by the end of the phase.

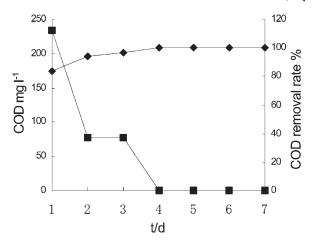


Fig. 1. Effluent COD concentration (\blacksquare) and COD removal rate (\spadesuit) with 48-h HRT.

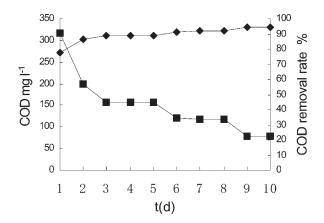


Fig. 2. Effluent COD concentration (■) and COD removal rate (♦) with 36-h HRT.

The results clearly imply that the bacteria of degrading COD and toxic compounds were adapted significantly which led to high COD removal, even at an HRT of 36 h.

Figure 3 shows the effluent COD concentrations and COD removal with an HRT of 24 h. Effluent concentrations of 198.8, 159.04, and 115.38 mg/L on d 1, 2 to 3, and 4 with COD removal rates of 86.11, 88.89, and 91.94%, respectively, were achieved. The COD removal rates were stabilized at 94.63% during d 5–8, and COD removal rates of 97.3 and 100% were obtained on d 9 and 10, respectively. The phenomenon indicated that better adaptation of the bacteria was accomplished, resulting in higher COD removal efficiency.

In the high efficient reaction phase, the influent COD was increased from 1400–2000 to 3500–4100 mg/L with a 24-h HRT. An effluent COD concentration of 142–158.08 mg/L with a COD removal rate of 95.56–96.15%

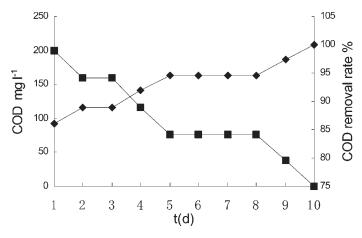


Fig. 3. Effluent COD concentration (■) and COD removal rate (♦) with 24-h HRT.

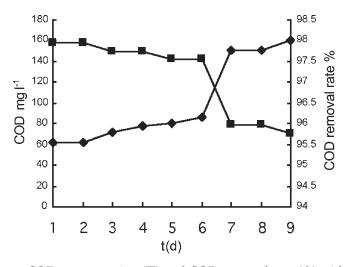


Fig. 4. Effluent COD concentration (■) and COD removal rate (♦) with 24-h HRT.

was steadily obtained on operation d 1–6, and on d 7 to 8, an effluent COD concentration of 79.04 and 71.136 mg/L and a COD removal rate of 97.78 and 98% was accomplished, respectively (Fig. 4).

Table 2 illustrates the means of influent COD concentration and COD removal rate in different phases during the time course of the experiment. In the phase of adaptation and proliferation, a high average COD removal rate of 96.74% was reached (Table 2), which was partially owing to the adsorption of granular activated carbon, and in the phase of stable metabolism, a slightly decreased COD removal rate of 92.58% was detected. In the phase of high efficient reaction, however, a COD removal rate of 96.51% was obtained, owing to the accumulation of efficient adapted bacteria in the reactor.

III Different i nabeb					
	Influent COD concentration	COD removal rate			
Phase	(mg/L)	(%)			
Adaptation and proliferation	1500-2100	96.74			
Stable metabolism	1500-2100	92.58			
High efficient reaction	3500-4100	96.51			

Table 2
Average Influent COD Concentration and COD Removal Rate in Different Phases

Table 3 Concentration of Some Toxic Compounds in Phase of High Efficient Reaction $(mg/L)^a$

Tir	me	Benzene	Naphthalene	Phenanthrene	Quinoline	Isoquinoline	Pyridine
In	fluent	12.31	69.78	0.24	9.69	0.27	7.02
Effluent	9.19 9.20	7.12 UD	53.73 22.58	0.05 UD	UD UD	UD UD	UD UD
	9.21 9.22	UD UD	4.85 UD	UD UD	0.75 UD	UD UD	UD UD
	9.23 9.24	UD UD	17.96 UD	UD UD	1.48 1.45	UD UD	UD UD
	_9.25	UD	UD	UD	UD	UD	UD

^aUD, undetectable.

Degradation of Benzene, Naphthalene, Phenanthrene, Pyridine, Quinoline, and Isoquinoline

In the phase of adaptation and proliferation, the concentration of these toxic compounds in effluent during d 1–13 was undetectable for the same reason of adsorption by granular activated carbon. From d 14, however, quinoline was detected until d 23 and then quinoline disappeared in the effluent with a 96-h HRT. In addition to quinoline, the other five toxic compounds were undetectable during this period.

In the phase of stable metabolism, all of the toxic compounds except quinoline were undetectable with a 48-h, 36-h, and 24-h HRT, and a quinoline removal rate of 88.03 and 87.95% was detected with a 36- and a 24-h HRT, respectively.

Table 3 shows the effluent concentration of the toxic compounds in the phase of high efficient reaction.

Conclusion

In the adaptation and proliferation phase of the immobilized cell reactor with granular activated carbon as the inert material, a COD removal rate of 96.74% was reached, owing to the adsorption of granular activated carbon, and the time of reactor startup was shortened efficiently. In the

phase of stable metabolism of the reactor, an average COD removal rate of 92.58% was obtained. In the high efficient reaction phase, although a higher influent COD concentration was loaded than for the former two phases and the same 24-h HRT as the former two phases was used, the COD removal rate and degradation of some toxic compounds in the wastewater were highly efficient. Therefore, the results indicate through 66 d of operation of the reactor that the immobilized *Pseudomonas* sp1 and *Pseudomonas* sp1 reactor was a satisfactory approach to degrade COD and some toxic compounds in biomass gasification wastewater.

Acknowledgments

We gratefully acknowledge the support of the Ministry of Science and Technology (2002BA40302), the Nature Science Foundation of Beijing (5022003), and the Education Committee of Beijing municipality.

References

- 1. Nam, K. and Kukor, J. J. (2000), Biodegradation 11, 1–9.
- Eriksson, M., Sodersten, E., Yu, Z., Dalhammar, G., and Mohn, W. W. B. (2003), Appl. Environ. Microbiol. 69, 275–284.
- 3. Field, J. A., Jong, E. D., Costa, G. F., and de Bont, J. A. (1992), *Appl. Environ. Microbiol.* **58**, 2219–2226.
- 4. MacLeod, C. T. and Daugulis, A. J. (2003), Appl. Microbiol. Biotechnol. 62, 291–296.
- 5. Alcalde, M., Bulter, T., and Arnold, F. H. J. (2002), Biomol. Screen 7, 547–553.
- 6. Feitkenhauer, H., Müller, R., and Maumlrkl, H. (2003), Biodegradation 14, 367–372.
- 7. Yang, X. S., Zhao, J., Luo, H. P., Qian, C., and Dong, X. (2001), S. China Environ. Sci. **21**, 109–111 (in Chinese).
- 8. Zhang, W. H., Tian, S., Li, J., Cao, Y. L., and Yang, X. S. (2002), *Acta Energiae Solaris Sinica* 24, 459–461 (in Chinese).
- 9. Tian, S., Qian, C., Liu, Y., and Li, X. (2003), *Acta Energiae Solaris Sinica* **24**, 866–869 (in Chinese).
- 10. Tian, S., Qian, C., Liu, Y., Li, X., and Yang, X. S. (2004), *Acta Scientiae Circumstantiae* **24**, 281–285 (in Chinese).