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Phenol Inhibition and Restoration of the Bioactivity of Anaerobic Granular Sludge

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Abstract Inhibition and restoration of different concentrations of phenol on the bioactivity of anaerobic granular sludge were investigated with laboratory-scale equipment. It indicated that phenol concentration lower than 50 mg/l show no inhibitory effect on bioactivities of granular sludge. However, methane productivity, extracellular polymeric substances (EPS) content, and coenzyme F_{420} activity were decreased by varying degrees when phenol concentration adopted for inhibition ranged between 50 and 400 mg/l. Noticeably, methane productivity could be fully or partly restored in case the phenol was removed after 24 h of phenol inhibition.

Keywords Anaerobic granular sludge · Phenol · Inhibition · Restoration · Bioactivity

Introduction

As one of the common contaminants in wastewater generated from petroleum and petrochemicals, coal conversion, and phenol-producing industries, phenols have been classified as a kind of hazardous pollutants owing to their potential toxicity to human health and aquatic life [1], and the removal of phenols could be conducted with physical, chemical, and biological processes [2].

Since it is either environment-friendly or highly efficient, biodegradation of phenol in wastewater would be more preferred than any other physiochemical treatment process. Additionally, anaerobic treatment process appeared to be more acceptable than that of

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aerobic treatment process as it was cost-effective, less sludge yielding, and methane-producing [3]. However, as always used as a kind of disinfectant, phenol was toxic to a variety of microorganisms since it is inhibitive to oxidative phosphorylation and enzyme biosynthesis of microbial cells. The inhibitory effect of phenol on methane production has already been investigated using anaerobic biogranules in an upflow anaerobic sludge blanket (UASB) bioreactor. Other than inhibition, biogranules might regain 100% of bioactivity gradually once the concentration of phenol was lowered below the so-called "threshold toxicity level" (varying from 850–1,700 mg/l according to different types of biogranules), for the phenol toxicity to anaerobic granular sludge was neither cumulative nor permanent [4]. However, there was few detailed research on the restoration of bioactivity till now.

In this study, in order to further understand the inhibitory and restorative mechanism of phenol to biogranules, inhibition of phenol on methane-producing capacity of anaerobic granular sludge, as well as the restoration of biogranular activity after inhibition, especially the contents of both the extracellular polymeric substances (EPS) and the activity of methanogenesis related coenzyme, F_{420}, were investigated.

Materials and Methods

Experimental Apparatus

Reaction bottle (500 ml) containing anaerobic granular sludge and wastewater was connected with Smith fermentation tube through a silicone tube. The reaction bottle was firstly aerated with nitrogen to maintain the anaerobic environment, then the Smith fermentation tube was filled with NaOH (2 mol/l) to absorb biogas. The reaction temperature was kept constant at 35 °C with a water bath.

Wastewater and Anaerobic Granular Sludge

Synthetic wastewater with the COD:N:P proportion of 200:5:1 was used throughout this study, and the synthetic wastewater contains (mg/l): COD 4,000, peptone 800, glucose 2,800, beef extract 500, NH_4Cl 400, KH_2PO_4 90, $CaCl_2{\cdot}2H_2O$ 60, $MgSO_4{\cdot}7H_2O$ 50, $FeSO_4{\cdot}7H_2O$ 40, $NaHCO_3$ 5,000, H_3BO_3 0.1, $ZnCl_2$ 0.1, $CuCl_2$ 0.06, $MnSO_4{\cdot}H_2O$ 0.1, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.1, $AlCl_3$ 0.1, $CoCl_2{\cdot}6H_2O$ 0.1, $NiCl_2$ 0.1, and phenol 0–400. The anaerobic granular sludge used in this study was from DSM Citric Acid (Wuxi) Ltd., China.

Operating Procedure

Eighty milliliter of synthetic wastewater and different concentrations of phenol (0, 25, 50, 100, 200, 300, and 400 mg/l, respectively) were added into the reaction bottle. Anaerobic granular sludge was then added into the reaction system to keep the volatile suspension solids (VSS) at about 15 g/l. The pH was adjusted to 7 using NaOH. Methane accumulation, contents of EPS and the activity of F_{420} were determined as the inhibition and restoration process went along.

Of the phenol inhibition and restoration process, the first 24 h and the following 72 h were the inhibitory period and the restoration period, respectively. During the inhibition period, in addition to nutrient solution and anaerobic granular sludge, there were also different concentrations of phenols ranging from 0 to 400 mg/l in the reaction bottle.

During the restoration period, anaerobic granular sludge was firstly removed from phenol-containing supernatant through centrifugation, then the biogranules was washed with water twice, and finally the separated biogranules and synthetic wastewater without phenol were refilled into the reaction bottle. Noticeably, due to the better sedimentation trait of anaerobic granular sludge, duration of the washing and refilling procedure might be negligible in comparison with the whole inhibition and restoration process, as it took only several minutes.

Extraction of EPS

EPS extraction in the supernatant and anaerobic sludge was conducted as reference [5].

Analytical Methods

COD, VSS, and phenol were determined according to the State Environmental Protection Administration of China (SEPA) standard [6]. Coenzyme F_{420} was determined as reference [7]. The extracted EPS was analyzed for total exopolysaccharides [5], protein [8], and nucleic acid [9]. The sum of total carbohydrates, proteins, and DNA were considered to represent the total amount of EPS. The biogas composition was assayed with a Gas Chromatograph (GC910, Kechuang, China) equipped with a stainless packed column (with Porapak N 60–80 as carrier) connected to a thermal conductivity detector. The column temperature was isothermic at 90 °C. The carrier gas was argon and the flow rate was 15 ml/min.

Results and Discussion

Inhibition and Restoration of Methanogenic Activity by Anaerobic Granular Sludge

As indicated on Fig. 1, the methane production within the first 24 h decreased to various extents as the phenol concentration was increased. The bioactivity of anaerobic granular sludge got inhibited immediately after the addition of phenol concentrations into wastewater over 50 mg/l.

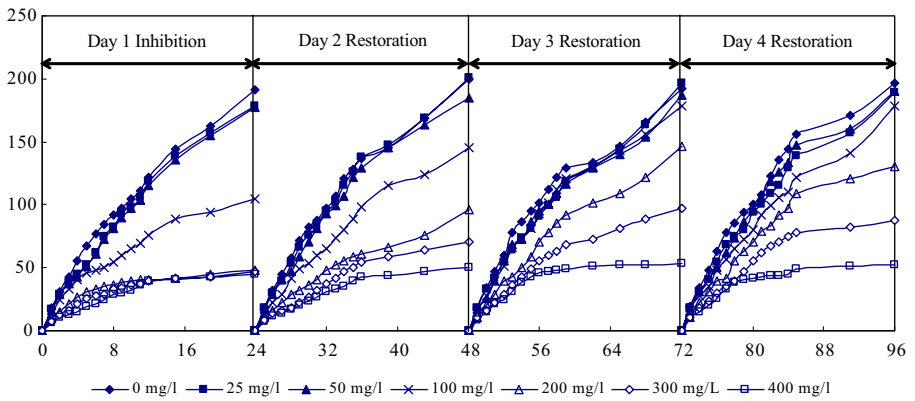

Fig. 1 Inhibition and restoration of methanogenic activity of anaerobic granular sludge

During the second day (from 24th to 48th hour) of the restoration period, methane production kept almost constant as that of the control group when 25 and 50 mg/l of phenol were used to perform the inhibition test, respectively. Therefore, bioactivity of the anaerobic granular sludge was not impacted by low concentration (less than 50 mg/l) of phenol. In contrast, methane production increased from 105 ml (24th hour) to 145 ml (48th hour) when 100 m/l of phenol was used during the inhibition period. It indicated that the bioactivity of anaerobic granular sludge can be gradually recovered after inhibition with a phenol dosage less than 100 mg/l. At the same time, methane production was only 50% (95.8 ml) and 35% (70.4 ml) of the control group (199.4 ml) at the 48th hour when 200 and 300 mg/l of phenol were added to the reaction system during the first 24 h, respectively. It suggested that the bioactivity of anaerobic granular sludge might be inhibited acutely under this condition. Remarkably, granule bioactivity did not seem able to recover, as methane production at 48th hour was nearly the same as that at 24th hour with 400 mg/l of phenol.

During the third day (from 48th to 72nd hour) of the inhibition and restoration process, methane production almost fully recovered when 50 and 100 mg/l of phenol were added to the reaction bottle during the inhibition period, respectively. In contrast, methane production was only partly recovered from 95.8 ml (48th hour) to 146.8 ml (72nd hour) and from 70.4 ml (48th) to 96.9 ml (72nd hour), when 200 and 300 mg/l of phenol were used during the inhibition period, respectively. However, bioactivity of the anaerobic granular sludge could not be recovered when 400 mg/l of phenol was adopted for the inhibition test, for the methane production of which kept almost unchanged. During the fourth day (from 72nd to 96th hour) of the restoration process, methane production somewhat decreased from 146.8 ml (72nd hour) to 130.6 ml (96th hour) and from 96.9 ml (72nd hour) to 88.1 ml (96th hour), when 200 and 300 mg/l of phenol were used for inhibition. And when 400 mg/l of phenol was adopted for inhibition, methane production was still not restored even after 96 h of restoration.

Granules Inhibition by Phenol and Bioactivity Restoration as Indicated by the F420 Content of Biogranules

As a low potential electron carrier in both the hydrogenase and dehydrogenase systems, F_{420} could be found in members of three distantly related groups of prokaryotes, namely the archaea, the aerobic actinomycetes, and the cyanobacteria. Moreover, methanogenic bacteria are the only coenzyme F_{420}-containing microorganisms in anaerobic digesters, for these microorganisms could obtain energy for cell growth from the reduction of carbon dioxide to methane and use electrons from the oxidation of hydrogen and formic acid [7]. Thus, by means of fluorescent assay, sometimes the F_{420} content could be used to indicate the methane production capability of granular sludge during the wastewater treatment process [10, 11].

F_{420} activity was determined at the 24th, 48th, 72nd, and 96th hour of the inhibition and restoration process, respectively. As shown in Fig. 2, activity of F_{420} decreased gradually as phenol concentration increased from 0 to 400 mg/l. In contrast with that of the control group (0.105 μmol/g VSS), F_{420} content was decreased by 75%, as it was only 0.026 μmol/g VSS at the 24th hour when phenol concentration increased to 400 mg/l.

Along with the restoration period, the F420 content rapidly (within 48 h) increased back to nearly the same level as the control, at phenol concentrations of 25 and 50 mg/l. In addition, F_{420} content could be increased close to that of the control group when 100 mg/l of phenol was used for inhibition, and F_{420} content could be increased to about 70% and 44% of that of the control group when 200 and 300 mg/l of phenol were used for inhibition within

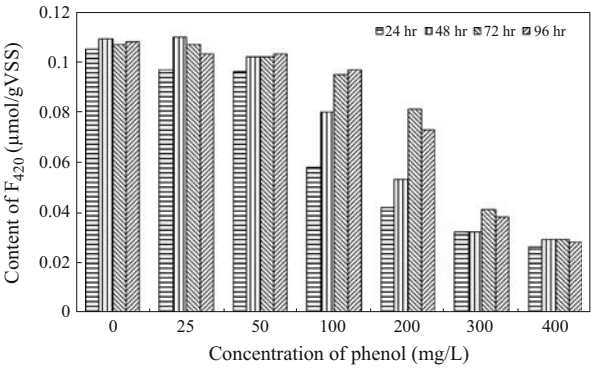

Fig. 2 Inhibition of phenol to the activity of F_{420} of anaerobic granular sludge

96 h. It appeared that bioactivity of the anaerobic granular sludge could be fully or partly recovered in case the phenol concentration adopted for inhibition was less than 300 mg/l in terms of the F_{420} level. On the contrary, bioactivity might not be recovered at all when phenol concentration was over 400 mg/l, as F_{420} content of which did not changed at all throughout the inhibition and restoration process. It suggested that high concentration of phenol in the reaction bottle could not be well assimilated and then degraded by anaerobic granular sludge during the restoration process.

Effect of Phenol Inhibition on Contents of EPS of Anaerobic Granular Sludge

As a metabolic products accumulating on the surface of bacterial cells, extracellular polymeric substances (EPS) consist of a variety of organic substances such as exopolysaccharide, protein, DNA, humic acid, lipids, and glycoprotein [5]. EPS could not only protect the cells from harsh external environments and provide energy and carbon when nutrients are in short supply, but also EPS were involved in the formation of microbial aggregates and adhesion to surface. Hence, for anaerobic granules, their EPS content might represent an indirect bioactivity indicator [12].

Each component of EPS, namely the content of exopolysaccharide, protein, and nucleic acid was determined at the 24th hour when inhibition period was ended. As shown from Fig. 3, when concentration of phenol adopted was 25 mg/l, content of exopolysaccharides

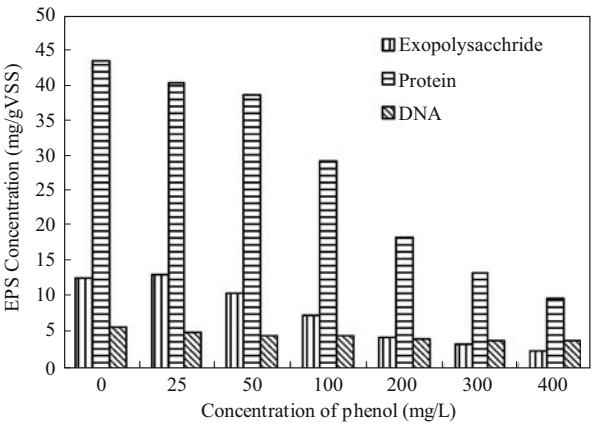

Fig. 3 Inhibitory effects of phenol to EPS concentration of anaerobic granular sludge

(13.50 mg/g VSS) was nearly the same as that of the control group (12.85 mg/g VSS), which indicated that low concentration of phenol had little effect on the exopolysaccharide accumulation of anaerobic granular sludge. However, concentration of exopolysaccharide decreased sharply when the phenol concentration was over 50 mg/l. Moreover, content of EPS protein also decreased rapidly as phenol concentration increased. As shown on Fig. 3, protein content was already decreased to 10.15 mg/g VSS when phenol concentration was 400 mg/l, while which was as high as 43.49 mg/g VSS of the control group. In contrast, it appeared that phenol showed little inhibitory effect to the nucleic acid, as the concentration of which decreased only from 5.89 to 4.05 mg/g VSS when phenol concentration increased from 0 to 400 mg/l. It was found that not only no harsh extraction process adopted, but the phenol concentration up to 400 mg/l might not lead to the substantive disruption of microbial cells in this study, for DNA contained in EPS was usually found in small quantities as which were released from the dead cells after lysis other than carbohydrate and protein [13].

Therefore, inhibition of the bioactivities of anaerobic granules by phenol would be enhanced with the increase of phenol dosage used, for content of exopolysaccharide and protein within the EPS decreased sharply (Fig. 3) other than that of the DNA, and which may contribute to the decrease of methane accumulation.

Conclusion

Phenols are growth inhibitory to microorganisms in biological treatment processes and regarded as priority pollutants in the USEPA list as they are toxic, carcinogenic, mutagenic, and teratogenic. On the other hand, bioactivities could be regained from phenol inhibition since phenol was biodegradable [14]. As indicated before, bioactivity of the biogranules sludge by phenol inhibition could be fully recovered within 72 h when less than 100 mg/l was used during the inhibition period, and the bioactivity of anaerobic granular sludge could be partly recovered within 96 h when less than 300 mg/l was adopted for inhibition. In contrast, inhibition effect by phenol could be observed obviously when more than 300 mg/l was adopted in the inhibition period, especially when more than 400 mg/l of phenol was used, bioactivity of the anaerobic granular sludge might not be recovered at all even after 96 h of restoration process. However, to better understand the phenol inhibition and restoration mechanism of the bioactivities of the anaerobic granular sludge, more detailed research should be conducted on how the phenol inhibition and restoration process go along from both microbiological and enzymatic aspects.

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References

1. Collins, G., Foy, C., Mchugh, S., Mahony, T., & Flaherty, V. O. (2005). Anaerobic biological treatment of phenolic wastewater at 15–18. *Water Research*, *39*, 1614–1620.
2. Fang, H. H. P., Chen, T., Li, Y. Y., & Chui, H. K. (1996). Degradation of phenol in wastewater in an upflow anaerobic sludge blanket reactor. *Water Research*, *30*(6), 1353–1360.

3. Fang, H. H. P., Liang, D. W., Zhang, T., & Liu, Y. (2006). Anaerobic treatment of phenol in wastewater under thermophilic condition. *Water Research*, *40*, 427–434.
4. Fang, H. H. P., & Chan, O. C. (1997). Toxicity of phenol towards anaerobic biogranules. *Water Research*, *31*(9), 2229–2242.
5. Wang, Z. P., Liu, L. L., Yao, J., & Cai, W. M. (2006). Effects of extracellular polymeric substances on aerobic granulation in sequencing batch reactors. *Chemosphere*, *63*, 1728–1735.
6. State Environmental Protection Administration of China (SEPA) (2002). *Water and wastewater monitoring methods* (4th ed.). Beijing: Chinese Environmental Science Publishing House (in Chinese).
7. Dolfing, J., & Mulder, J. W. (1985). Comparison of methane production rate and coenzyme F_{420} content of methanogenic consortia in anaerobic granular sludge. *Applied and Environmental Microbiology*, *49* (5), 1142–1145.
8. Frølund, B., Griebe, T., & Nielsen, P. H. (1995). Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology Biotechnolology*, *43*, 755–761.
9. Yu, Y. X., Wu, Q. G., & Meng, X. T. (1990). *Manual of the determination of environmental microbiology*. Beijing: China Environmental Science Press (in Chinese).
10. Zabransk, J., Schneiderov, K., & Dohanyos, M. (1985). Relation of the coenzyme F_{420} to the activity of methanogenic microorganisms. *Biotechnology Letters*, *7*(8), 547–552.
11. Schulze, D., Menkhaus, M., Fiebig, R., & Dellweg, H. (1988). Anaerobic treatment of protein-containing waste waters: correlation between coenzyme F_{420} and methane production. *Applied Microbiology and Biotechnology*, *29*, 506–510.
12. Liao, B. Q., Allen, M. D. G., Droppo, I. G., Lepard, M. G. G., & Liss, S. N. (2001). Surface properties of sludge and their role in bioflocculation and settleability. *Water Research*, *35*(2), 339–350.
13. Liu, H., & Fang, H. H. P. (2002). Extraction of extracellular polymeric substances (EPS) of sludges. *Journal of Biotechnology*, *95*, 249–256.
14. Veeresh, G. S., Kumar, P., & Mehrotra, I. (2005). Treatment of phenol and cresols in upflow anaerobic sludge blanket (UASB) process: A review. *Water Research*, *39*, 154–170.