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Treatment of short chain nonylphenol polyethoxylates and its microflora structure with an anoxic-oxic activated sludge process

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ABSTRACT

An anoxic-oxic activated sludge process (AOASP) was carried out to treat shortchain nonylphenol polyethoxylates (SCNPEOs). The operation parameters, including temperature, hydraulic retention time (HRT) and solids retention time (SRT), were optimized by an $L_9(3^3)$ orthogonal experiment. Effluent concentrations and effluent removal efficiency of each SCNPEOs component were selected as the evaluation index for the orthogonal experiment. The optimum operation parameters were achieved as temperature of 40 °C, HRT of 12 hr and SRT of 15 d. The largest influence factor to the degradation of mixed nonylphenol diethoxylate (M-NP2EO) was temperature, followed by HRT and SRT. The molecular biology analysis results supported the conclusions of orthogonal experiment. The highest ratio of Proteobacteria achieved 40% in anoxic tank and 50% in oxic tank. These results showed that the AOASP might be an effective method to degrade SCNPEOs.

Keywords: anoxic-oxic activated sludge process; degradation; denaturing gradient gel electrophoresis; fluorescence in situ hybridization; microflora structure; orthogonal design; shortchain nonylphenol polyethoxylates

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INTRODUCTION

Shortchain nonylphenol polyethoxylates (SCNPEOs, n=1, 2) and nonylphenol (NP) are the degradation products of nonylphenol polyethoxylates (NPEOs), which are the most commonly used nonionic surfactants [1-3]. SCNPEOs and NP have strong lipophilicity, toxicity, cumulative property and estrogenic effect, and they are difficult to be further degraded [4-6]. Thus, the final degradation of SCNPEOs and NP is the crux to the harmless degradation of NPEOs.

Biodegradation method was widely used to treat organic pollutants [7,8]. Ma *et al.* reported that 174 nmol/L of NPEOs (including 144 nmol/L of SCNPEOs and NP) in influent and 43.9 nmol/L of SCNPEOs and NP in effluent were detected respectively in a domestic wastewater treatment plant [9]. Okayasu *et al.* performed an experiment to degrade NPEOs by activated sludge [2]. It was observed that SCNPEOs could be degraded to NP effectively in anoxic environment and NP showed a higher degradation rate than SCNPEOs in oxic environment. These researches indicated that an anoxic-oxic activated sludge process (AOASP) with appropriate operation parameters might degrade SCNPEOs and NP effectively.

Operation temperature is very important for the biodegradation of NPEOs. Chang *et al.* found that NP biodegradation efficiency increased at higher temperature (<50 °C) in oxic activated sludge [10]. Normally longer hydraulic retention time (HRT) means better biodegradation effect. However, overlong HRT might waste the degradation ability, especially for the well domesticated activated sludge [11]. Longer solids retention time (SRT) brought benefit to the complete biodegradation of NPEOs due to the growing microflora quantity and microflora diversity [12]. Nevertheless, long-term operation without sludge disposal could result in sludge aging and the

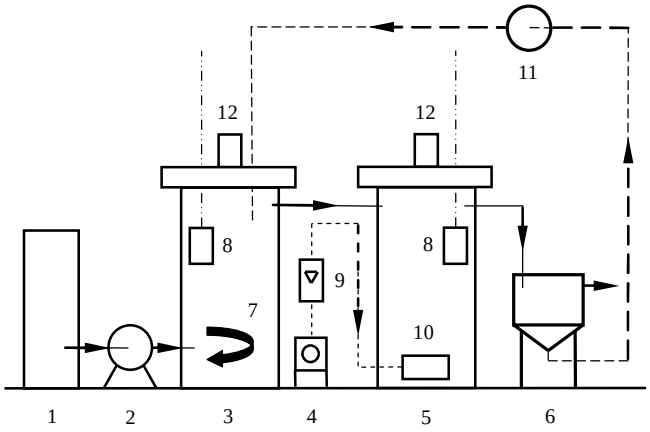
accumulation of harmful substance [13].

In this study, an $L_9(3^3)$ orthogonal experiment was carried out to degrade mixed nonylphenol diethoxylate (M-NP2EO) and investigate the optimum operation parameters of temperature, HRT and SRT for M-NP2EO degradation. High performance liquid chromatography (HPLC) technology was a matured method to measure the concentrations of microcomponent [14,15]. The concentrations of M-NP2EO and NP were measured by HPLC and were used to evaluate the degradation efficiencies. Chemical oxygen demand (COD), ammonia and mixed liquor suspended solids (MLSS) concentrations were measured to monitor the operation conditions of the AOASP. The molecular biology methods, including polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE) and fluorescence in situ hybridization (FISH), are very were applied to analyze the microflora structure of activated sludges.

EXPERIMENTAL SECTION

Experimental system

Fig. 1 was the schematic diagram of the AOASP. The effective volumes of anoxic tank 3, oxic tank 5 and sedimentation tank 6 were 3 L, 2 L and 0.6 L, respectively. Influent pump 2 pumped influent into anoxic tank 3, and decided the influent flow. The mixed liquor streamed from anoxic tank 3 to oxic tank 5 and sedimentation tank 6 by gravity. Sludge returning pump 11 returned activated sludge from sedimentation tank 6 to anoxic tank 3 and the circulation rate was 2. Stirrer 7 was used to keep the activated sludge in anoxic tank 3 suspended. The oxygen for oxic tank 5 was supplied by air pump 4 and its flow was controlled by air flow meter 9. The temperatures of anoxic tank 3 and oxic tank 5 were controlled by heater 8.



1 influent tank; 2 influent pump; 3 anoxic tank; 4 air pump; 5 oxic tank; 6 sedimentation tank; 7 stirrer; 8 heater; 9 air flow meter; 10 air diffuser; 11 internal circulating pump; 12 sludge returning pump; 13 exhaust pipe

Fig. 1 Schematic diagram of the AOASP

Table 1 Influent of synthetic domestic sewage

Category	Composition	Concentration (mg/L)
C source	Glucose	500
N source	Urea	54
P source	KH_2PO_4	35
Buffer solution	$NaHCO_3$	120
Mineral salts	$CaCl_2$	6
	$FeSO_4 \cdot 7H_2O$	0.55
	$MgSO_4 \cdot 7H_2O$	6
	$MnSO_4 \cdot H_2O$	6

Operation methods

The experiment carried out for 150 d and could be divided into 3 periods as sludge cultivation period (Run1), sludge domestication period (Run2) and orthogonal experiment period (Run3). Run1 started with MLSS of 2,000 mg/L, temperature of 25 $^{\circ}$C, HRT of 10 hr and influent of synthetic domestic sewage (Shown in Table 1). It continued for 60 d until the MLSS concentration remained stable about 4,000 mg/L. Run2 was carried out from the 61st day to the 90th day. M-NP2EO was added into influent to partly replace glucose at the step of 20, 40, 60 and 80 mg/L. The influent theoretical COD concentration was kept as a constant of 500 mg/L. An orthogonal experiment of $L_9(3^3)$ was carried

out in Run3. Temperature, HRT and SRT were selected as the factors and each factor has 3 values. The detailed values of these factors were shown in Table 2.

Table 2 Factor's value of orthogonal experiment

Value	Factor		
	A-Temperature (°C)	B-HRT (hr)	C-SRT (d)
1	20	6	15
2	30	9	25
3	40	12	40

Analytical methods

MLSS, COD and NH_4^+-N were measured by the standard methods [16]. Temperature was measured by thermometer directly. The concentrations of SCNPEOs and NP in influent and effluent were determined by HPLC (Waters 600, America, with normal mobile Hypersil APS-2 column) and were used to evaluate the orthogonal experiment. The chromatograph standard of M-NP2EO and NP were bought from Tokyo Chemical Industry Co., LTD, Japan.

The DNA of all microflora in activated sludges was extracted by the method of Petersen et al firstly [17]. Then the DNA fragment was sublimated by the Agarose Gel DNA Purification Kit from TaKaRa, Dalian, China. Sublimated DNA was used for the PCR amplification (TL988, Xian Tianlong, China) to get the 16S rDNA fragments of microflora. The amplification product of PCR was used for DGGE (DGGE-1, Nanjing Xinxiaoyuan, China) [18]. The result of DGGE was photographed by a UV Gel Imaging System (WV-BP334CH, Jiangsu Jieda, China) and was analyzed by Quantity One software. The FISH result was observed with a fluorescence microscope (BA400, Xiamen Motic, China) and was analyzed by MoticFluo1.0 software.

RESULTS AND DISCUSSION

Mixed liquor suspended solids concentration

Fig. 2 was the daily changes in MLSS concentrations. Activated sludge flowed out from sedimentation tank due to its imperfect design on the 12th day and the 35th day, and then led to sharp reductions in MLSS concentrations. From the 35th day, MLSS concentrations increased steadily and achieved about 4,000 mg/L on the 58th day. With the addition of M-NP2EO into influent, MLSS concentrations presented a clear downward trend and then increased slowly in Run2. It indicated that M-NP2EO was toxic to the microflora, but the microflora could adapt to it after domestication. MLSS concentrations in Run3 were stable, and it showed that the AOASP could degrade M-NP2EO effectively.

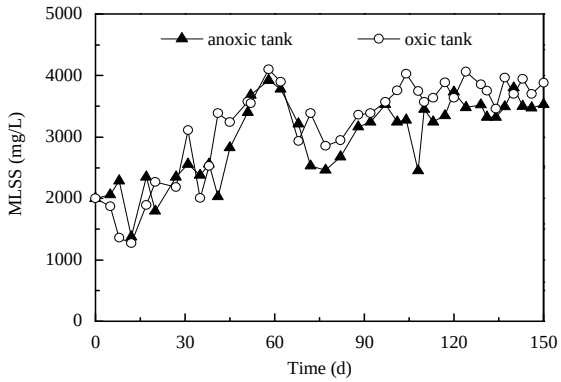


Fig. 2 Daily changes in MLSS concentrations

Chemical oxygen demand removal

The daily changes in COD concentrations and removal efficiencies were shown in Fig. 3. COD concentrations in anoxic tank (from 70.31 mg/L to 136.52 mg/L) were higher than those in oxic tank (from 32.57 mg/L to 79.29 mg/L) in Run1. Effluent COD concentrations (from 21.94 mg/L to 65.09 mg/L) were slightly lower than those in oxic tank, and effluent COD removal efficiencies increased gradually from 86.44% to 94.98% during the cultivation. In Run2, the addition of M-NP2EO and its toxicity to microflora resulted in higher COD concentrations in oxic tank (from 54.05 mg/L to 95.56 mg/L), higher COD concentrations in effluent (from 46.51mg/L to 86.06mg/L) and lower effluent COD removal efficiencies (from 83.39% to 89.56%). COD concentrations and effluent COD removal efficiencies presented sudden variation tendencies after the addition of M-NP2EO, and then recovered step by step. COD concentrations and COD removal efficiencies in Run3 changed slightly and were near to that at the end of Run2. This showed that the activated sludge in AOASP had adapted to M-NP2EO and degraded them stably.

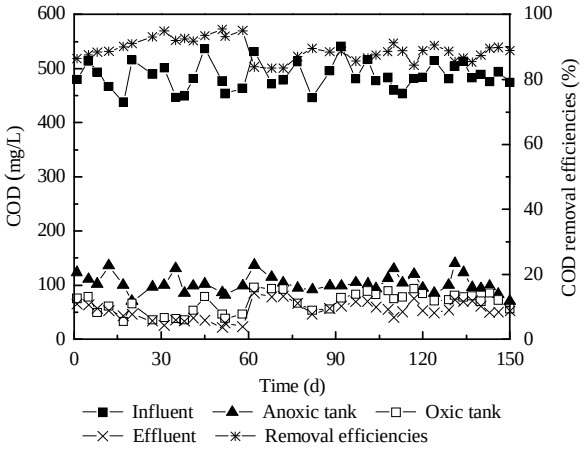


Fig. 3 Daily changes in COD concentrations and removal efficiencies

Ammonia removal

Fig. 4 showed the daily changes in NH_4^+-N concentrations. The NH_4^+-N in influent generated by the hydrolyzation of urea and was varied from 3.10 to 4.98 mg/L. More NH_4^+-N was produced in anoxic tank by further hydrolysis and was oxidized to NO_3^--N in oxic tank. The NH_4^+-N concentration in anoxic tank decreased from 12.25 mg/L to 7.16 mg/L in Run1, increased to 11.35 mg/L after the addition of M-NP2EO in Run2 and finally kept stable about 9.50 mg/L in Run3. The NH_4^+-N concentration in oxic tank and effluent was lower than that in anoxic tank, but also presented the same variation trends. The NH_4^+-N concentration in oxic tank and effluent were stable about 1.00 mg/L in Run3. These variation trends also indicated that the activated sludge in AOASP could adapt to M-NP2EO and finally degraded them.

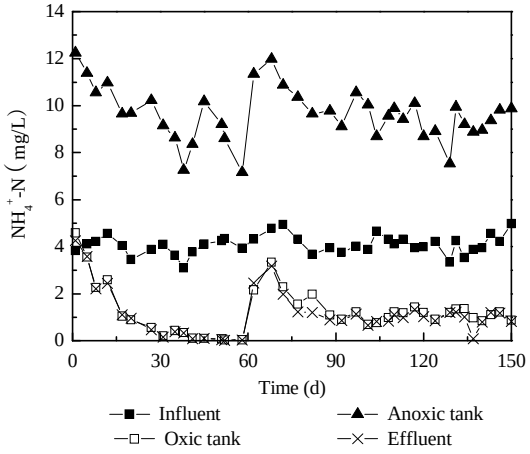


Fig. 4 Daily changes in NH_4^+-N concentrations

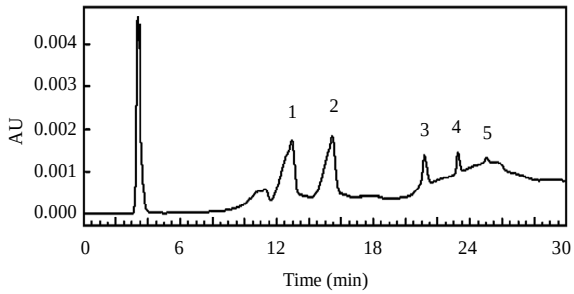


Fig. 5 The chromatogram of influent

Shortchain nonylphenol polyethoxylates and nonylphenol removal

Fig. 5 was the chromatogram of influent with M-NP2EO concentration of 80 mg/L. The M-NP2EO in influent was composed of NP1EO, NP2EO, NP3EO, NP4EO and NP5EO, with the concentrations of 27.01, 30.16, 8.05, 1.61 and 0.35 mg/L, respectively. The measured total amount of NP1EO, NP2EO, NP3EO, NP4EO and NP5EO was 67.18

mg/L in influent, and was not equal to 80 mg/L. This could be resulted from the measurement error or the impurities in M-NP2EO. Table 3 showed the concentrations and removal efficiencies of M-NP2EO and NP in influent and effluent under different operation parameters.

Table 3 The concentrations and removal efficiencies of M-NP2EO and NP under different operation parameters

Component		NP1EO	NP2EO	NP	NP3EO	NP4EO	NP5EO	Total M-NP2EO[a]
Influent (mg/L)		27.01	30.16	—[b]	8.05	1.61	0.35	67.18
Effluent (mg/L)	1	3.042	2.199	1.103	0.339	0.125	—	6.808
	2	1.505	1.882	1.771	1.028	0.107	—	6.293
	3	1.013	2.307	1.869	0.714	0.037	—	5.94
	4	1.216	1.250	1.178	0.152	—	—	3.796
	5	1.934	1.065	0.434	—	—	—	3.433
	6	0.791	1.128	0.382	—	—	—	2.301
	7	1.945	0.613	0.272	—	—	—	2.83
	8	0.736	1.003	0.488	—	—	—	2.227
	9	0.534	0.730	0.086	—	—	—	1.35
Removal Efficiencies (%)	1	88.74	92.71	—	95.79	92.23	100	89.87
	2	94.43	93.76	—	87.23	93.35	100	90.63
	3	96.25	92.35	—	91.13	97.70	100	91.16
	4	95.49	95.85	—	98.11	100	100	94.35
	5	92.84	96.46	—	100	100	100	94.89
	6	97.07	96.26	—	100	100	100	96.57
	7	92.80	97.97	—	100	100	100	95.79
	8	97.28	96.67	—	100	100	100	96.68
	9	98.02	97.58	—	100	100	100	97.99

a. The total amount of NP1EO, NP2EO, NP, NP3EO, NP4EO and NP5EO;
b. Not detected.

Removal efficiencies of total M-NP2EO (*K*) under different operation parameters were used as the evaluated index to analyse the optimum operation parameters, as shown in Table 4. Fig. 6 showed the relationship between the average removal efficiencies of total M-NP2EO ($\overline{K}$) and the different operation parameters. From Table 4 and Fig. 6, we could find that temperature had the largest effect on removal efficiencies of total M-NP2EO, followed by HRT and SRT. The optimum operation parameters were temperature of 40 °C, HRT of 12 hr and SRT of 15 d.

As shown in Table 3, NP3EO, NP4EO and NP5EO could be completely degraded to NP2EO, NP1EO and NP after the long-term operation. But NP2EO, NP1EO and NP were still remained under each operation parameters. Therefore, it was necessary to analyse the relationship among NP1EO removal, NP2EO removal, NP residual and the different operation parameters. The analysis methods were similar with Table 4 and Fig. 6, only replacing total M-NP2EO removal efficiencies by NP1EO removal efficiencies, NP2EO removal efficiencies and NP residual concentrations, respectively.

Table 4 Analysis of orthogonal experiments based on removal efficiencies of total M-NP2EO (*K*)

No.	A-Temperature (°C)	B-HRT (hr)	C-SRT (d)	*K* (%)
1	20	6	15	89.87
2	20	9	25	90.63
3	20	12	40	91.16
4	30	6	25	94.35
5	30	9	40	94.89
6	30	12	15	96.57
7	40	6	40	95.79
8	40	9	15	96.68
9	40	12	25	97.99
*K*1	271.66	280.01	283.12	
*K*2	285.51	282.20	282.97	
*K*3	290.46	285.72	281.84	
$\overline{K}$ 1	90.55	93.33	94.37	
$\overline{K}$ 2	95.27	94.06	94.32	
$\overline{K}$ 3	96.82	95.24	93.95	
R	6.27	1.90	0.43	

For NP1EO removal, HRT had the largest effect on removal efficiencies, followed by temperature and SRT. The optimum operation parameters were temperature of 40 °C, HRT of 12 hr and SRT of 25 d. For NP2EO removal, temperature had the largest effect on removal efficiencies, followed by SRT and HRT. The influence of SRT and HRT was very slight. The optimum operation parameters were temperature of 40 °C, HRT of 9 hr and SRT of 25 d.

For NP residual, temperature had the largest effect on removal efficiencies, followed by SRT and HRT. The optimum operation parameters were temperature of 40 °C, HRT of 12 hr and SRT of 15 d.

When considered all the factors, the largest influence factor was temperature, followed by HRT and SRT. The optimum operation parameters were temperature of 40 °C, HRT of 12 hr and SRT of 15 d.

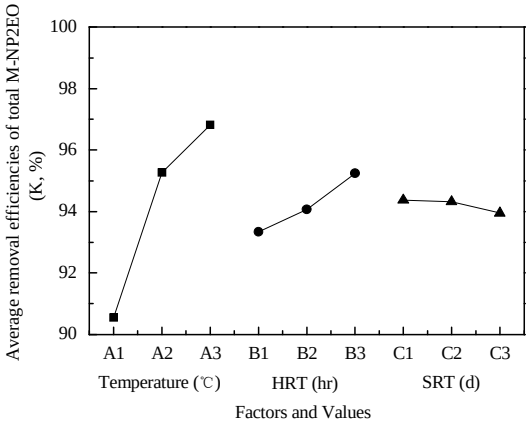


Fig. 6 Relationships between average removal efficiencies of total M-NP2EO (K) and the different operation parameters

Analysis of molecular biology methods

9 activated sludge samples from anoxic tank and 9 activated sludge samples from oxic tank under different orthogonal parameters (Shown in Table 4) were used for the DGGE analysis. Fig. 7 was the DGGE fingerprints of the 18 samples. The strips of sample 7, sample 8 and sample 9 were obvious more than those of other samples. It agreed that 40 °C was the best parameter of temperature and temperature had the largest influence to M-NP2EO degradation.

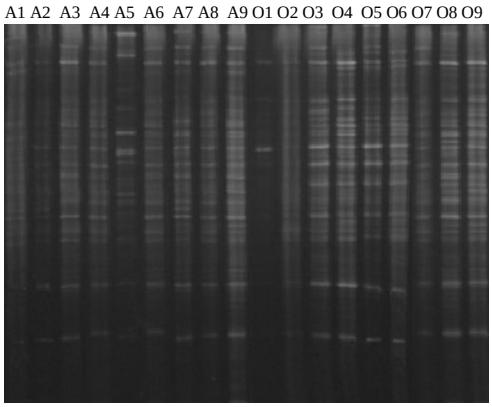
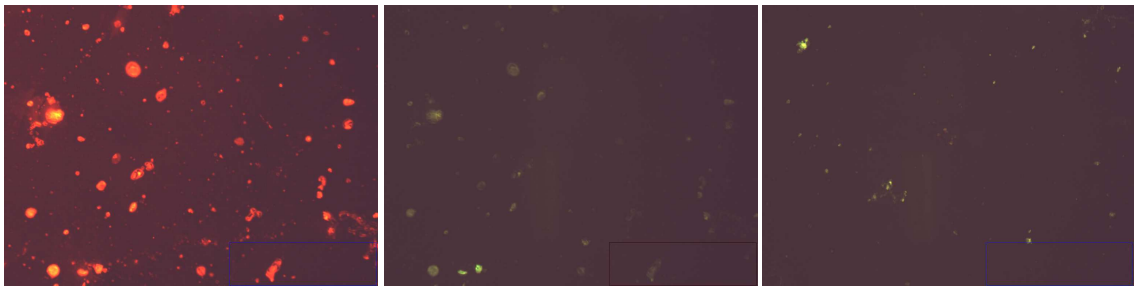


Fig. 7 DGGE fingerprints of 18 samples under different operation parameters



(a) all microflora **(b) β-Proteobacteria** **(c) γ-Proteobacteria**

Fig. 8 Fish photos of activated sludge sample

β-Proteobacteria and *γ-Proteobacteria* were the dominant bacteria to degrade M-NP2EO. 18 samples were also analyzed by FISH to measure the ratio of *β-Proteobacteria* and *γ-Proteobacteria.* Fig. 8 was the detailed FISH photos of activated samples. FISH results showed that the highest ratio of *β-Proteobacteria* (25% in anoxic tank, 30% in oxic

tank) and *γ-Proteobacteria* (15% in anoxic tank, 20% in oxic tank) were occurred in Sample 8 (temperature of 40 °C, HRT of 9 hr and SRT of 15 d).

CONCLUSION

An AOASP was carried out to treat M-NP2EO and an orthogonal experiment was performed to optimize the operation parameters (temperature, HRT and SRT), the following results were achieved:

(1) The AOASP could adapt to M-NP2EO gradually after sufficient cultivation and could degrade M-NP2EO effectively.
(2) MLSS concentrations decreased obviously with the addition of M-NP2EO into influent, but then increased slowly and kept stable finally after sufficient cultivation.
(3) The effluent COD concentration and COD removal efficiency presented clear downward trend with the addition of M-NP2EO into influent, but achieved about 60 mg/L and 90% respectively after sufficient cultivation.
(4) Taking into account NP residual and the removal efficiencies of NP1EO, NP2EO, M-NP2EO, the largest influence factor to M-NP2EO degradation was temperature, followed by HRT and SRT.
(5) Taking into account NP residual and the removal efficiencies of NP1EO, NP2EO, M-NP2EO, the optimum operation parameters for M-NP2EO degradation were temperature of 40 °C, HRT of 12 hr and SRT of 15 d.
(6) The results of molecular biology analysis supported the conclusions of orthogonal experiment. The highest ratio of *β-Proteobacteria* and *γ-Proteobacteria* were occurred in Sample 8 (temperature of 40 °C, HRT of 9 hr and SRT of 15 d).

Acknowledgements
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