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Petrochemical wastewater treatment with a pilot-scale bioaugmented biological treatment system*

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Abstract: In solving the deterioration of biological treatment system treating petrochemical wastewater under low temperatures, bioaugmentation technology was adopted by delivering engineering bacteria into a pilot-scale two-stage anoxic-oxic (A/O) process based on previous lab-scale study. Experimental results showed that when the concentrations of COD and NH_4^+-N of the influent were 370~910 mg/L and 10~70 mg/L, the corresponding average concentrations of those of effluent were about 80 mg/L and 8 mg/L respectively, which was better than the Level I criteria of the Integrated Wastewater Discharge Standard (GB8978-1996). According to GC-MS analysis of the effluents from both the wastewater treatment plant (WWTP) and the pilot system, there were 68 kinds of persistent organic pollutants in the WWTP effluent, while there were only 32 in that of the pilot system. In addition, the amount of the organics in the effluent of the pilot system reduced by almost 50% compared to that of the WWTP. As a whole, after bioaugmentation, the organic removal efficiency of the wastewater treatment system obviously increased.

Key words: Bioaugmentation, Petrochemical wastewater, Pilot-scale study, GC/MS
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INTRODUCTION

Xenobiotics such as petroleum hydrocarbon, aniline, nitrobenzene, organchlorines, volatile phenols and their analogies in petrochemical wastewater are highly toxic and inhibitory to microbial activity, especially under the low temperature condition of winter, which would lead to a series of problems such as poor effluent quality and unfavorable operational system. Accordingly, conventional activated sludge processes are incompetent for treating petrochemical wastewater (SEPAC, 1992). By delivering engineering bacteria, bioaugmentation technology could effectively improve the biological treatment efficiency to refractory organics, which had been shown in some small-scale activated sludge process (Boon *et al.*, 2000; 2003; Quan *et al.*, 2004) as well as other bioreactors (Dhouib *et al.*, 2003; Kennes *et al.*, 1997; Venkata Mohan *et al.*, 2005). However, concerned researches and applications were almost focused on certain pollutants (Head and Oleszkiewicz, 2004; Ro *et al.*, 1997), while its application and research in full-scale petrochemical wastewater had not been reported. Based on the selection and breeding of special bacteria and the use of engineering bacteria, pilot study was conducted and the removal efficiency and the following system stability after bioaugmentation were investigated, with the expectation to provide reliable data and technical support for the application of bioaugmentation.

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EXPERIMENTAL SETUP AND METHODS

Experimental setup

The pilot-scale system was a two-stage A/O (anoxic/oxic) process (Fig.1 and Table 1), namely, wastewater from the distributing box flowed into the No. 1 anoxic tank (A1), then to No. 1 oxic tank (O1) followed by the No. 2 anoxic tank (A2) and finally the No. 2 oxic tank (O2). Each anoxic stage was 2.2 m^3 in volume, with the oxic stage being 2.8 m^3, while the total available capacity was 10 m^3. Wastewater was fed in the mode of gravity flow and there was no sludge recirculation.

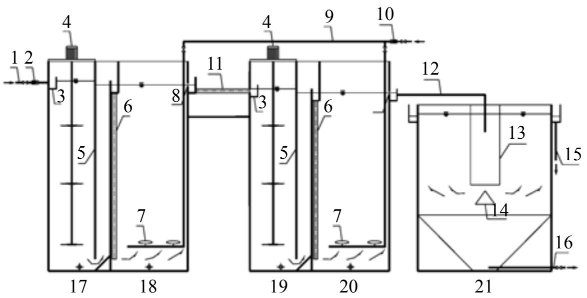

Fig.1 Schematic diagram of the pilot-scale experimental setup
1: Influent pipe; 2: Liquid flowmeter; 3: Water distribution gutter; 4: Agitator; 5: Baffle; 6: Water distribution pipe; 7: Aerator; 8: Water distribution hole; 9: Air lines; 10: Gas flow meter; 11: No. 1 water collecting pipe; 12: No. 2 water collecting pipe; 13: Water distributing system; 14: Baffle-board; 15: Effluent pipe; 16: Waste sludge pipe; 17: A1 tank; 18: O1 tank; 19: A2 tank; 20: O2 tank; 21: Settling basin

Table 1 Specification of the pilot-scale setup

No.	Setup	Specification and type	Number
1	Liquid flow meter	LZB-40 (0.16~1.6 m^3/h)	1
2	Agitator	J250 (0.35~0.7 m)	4
3	Aerator	ZH215	8
4	Gas flow meter	LZB-25 (0.16~1.6 m^3/h)	1
5	A1 tank	0.8 m×1.2 m×2.67 m	1
6	O1 tank	1.0 m×1.2 m×2.67 m	1
7	A2 tank	0.8 m×1.2 m×2.67 m	1
8	O2 tank	1.0 m×1.2 m×2.67 m	1
9	Settling basin	D×H=1.8 m×2.2 m	1

Wastewater quality and quantity

The pilot system (two-stage A/O stage) was set up in parallel with the petrochemical wastewater treatment system (A/O process had 4 sections, the first section was anoxic stage, and the other three were oxic stages). Petrochemical wastewater flowed into the pilot system following primary settling basin and the retention basin of the WWTP, and the designing wastewater flow rate was 0.5 m^3/h. The water quality is shown in Table 2.

Table 2 Water quality of influent in petrochemical WWTP

Parameter*	Value	Level I criteria**
COD	400~900	100
BOD	150~350	30
NH_4^+-N	10~80	15
SS	≤150	70
Oil and grease	≤80	10
pH	7~9	6~9

COD, BOD, SS, NH_4^+-N are chemical oxygen demand, biochemical oxygen demand, suspended solids and ammonium nitrogen, respectively; * Parameters except for pH are in mg/L; ** Integrated Wastewater Discharge Standard of China (SSB, 1997)

Bioaugmentation method

1. Construction of engineering bacteria

The engineering bacteria are defined as novel bacteria with multiple degrading functions and could degrade various organics by separating target genes which have different degrading abilities with gene engineering technology, while general engineering bacteria are described as highly-efficient degrading flora which are rationally combined after being isolated from natural environments, polluted sites or treatment systems and could decompose numerous organics. However, these two descriptions do not have substantial differences.

According to the specific persistent organic pollutants determined in the effluent of the petrochemical WWTP, specific degrading bacteria were selected and bred. Based on the design parameter of the plant configurations and the microorganisms' niche, the efficient bacteria obtained were preliminarily grouped according to their niche and self-adaptive strategy was applied for the breeding of engineering bacteria. The recombination strategy of the bacteria is shown in Table 3. After then, these engineering bacteria were inoculated into oxic tanks containing different organics and would adhere on the suspending carrier, and further self-adaptive construction was conducted. Engineering bacteria C1 (containing petroleum hydrocarbon degrading bacteria and bio-flocculants-producing bacteria) were added into O1 tank, while engineering bacteria C2 (containing phenol degrading bacteria and NH_4^+-N degrading bacteria) and engineering bacteria C3 (containing benzene degrading bacteria and decoloring bacteria and other microorganisms) were added into O2 tank.

Table 3 The recombination of bacteria

No.	Function	Species
Cl	Petroleum hydrocarbon degrading bacteria	*Plesiomonas*
		Pseudomonas
		Enterobacter
		Bacillus cereus
		Bacillus subtilis
		Acinetobacter calcoaceticus
		Bacillus
		Brevibacterium
		Pseudomonas
		Micrococcus
		Flavobacterium
	Bio-flocculants-producing bacteria	*Enterococcus*
		Streptococcus
		Klebsiella
		Arthrobacter
		Bacillus
C2	Phenol degrading bacteria	*Pseudomonas*
		Brevibacterium
		Bacillus
		Micrococcus
	NH_4^+-N degrading bacteria	*Nitrobacter*
		Nitrococcus
		Nitrosococcus
		Nitrosomonas
C3	Benzene degrading bacteria	*Pseudomonas*
		Streptococcus
		Bacillus
		Pseudomonasaeruginasa
		Pseudomonasfluorescens
		Arthrobacter
		Acinetobacter
	Decoloring bacteria	*Klebiella*
		Ochrobactrium
		Aeromonas
		Paenibacillus
		Xanthomonas
		Bacillus
	Others	*Bacillus cereus*
		Alcaligenes sp. IS-67
		Alcaligenes sp. IS-92

2. Immobilization of engineering bacteria

Instead of the conventional high-molecular compounds, the internal material of carrier was utilized in our study was made of volcanic pumice and artificial cotton with large specific surface area, high porosity, good hydrophilic-hydrophobe balance, rough surface and sufficient mechanical intensity and the positive charge carried on the surface (shown in Fig.2). By adjusting the proportion of volcanic pumice and artificial cotton, the density of the carrier is between 1.04×10^3~1.08×10^3 kg/m^3. The quantity added was 30% of that of the effective volume of the oxic tank (1.68 m^3). The carrier was activated for the better adhesion and growth of the engineering bacteria by using solution prepared with $MgSO_4$, $CuSO_4$, $Fe_2(SO_4)_3$, $CoCl_2$ and so on (The details of the activating solution are shown in Table 4). Then the bacteria were inoculated at concentration of 10^{12} CFU/ml at intervals until the bacteria in the system reached 1×10^9~2×10^9 CFU/ml.

Fig.2 The morphology of the suspending carrier

Table 4 The composition of activating solution

Chemicals	Content (%)	Chemicals	Content (%)
SiO_2	48.12	TiO_2	0.08
CaO	7.46	K_2O	2.86
MgO	3.76	Na_2O	3.47
Fe_2O_3	8.90	SO_3	0.74
FeO	1.43	Mn_3O_4	1.87
Al_2O_3	12.16		

Analysis methods

Samples were collected every three hours and then combined in order to obtain a daily composite sample. The analytical methods (SEPAC, 2002) and different parameters are listed in Table 5. All of these parameters analyses were performed in triplicate.

RESULTS AND DISCUSSION

Start-up of the pilot system and its performances

The pilot system began to operate on Sept. 10, 2005 with the initial flow rate being 0.5 m^3/h. On Sept. 12, engineering bacteria and suspending carrier were delivered into the system and then bacteria began self-adaptive breeding while the flow rate was

Table 5 Methods and equipments used for examining different parameters

Parameters	Analytical method	Equipments
COD	Dichromate method	HANA COD detector
BOD	Dilution and seeding method	YSI2MODEL95 dissolved oxygen meter
NH_4^+-N	Nessler's reagent colorimetric method	755 ultraviolet-visible spectrophotometer
SS	Gravimetric method	ALC-210.4 electronic balance
Oil	Ultraviolet spectroscopy	755 ultraviolet-visible spectrophotometer
pH	–	PHS-25 pH meter
Organic pollutants	Gas Chromatography-Mass Spectrometry (GC/MS)*	MP5890GC/MS chromatography-mass machine

* The chromatography conditions were as follows: SE-54 type capillary column made of quartz with inner diameter 0.32 mm and length 25 m; column temperature: the temperature retained at 40 °C for 2 min and then increased to 250 °C with an increment of 3~5 °C/min and kept at 250 °C for 30 min. The mass conditions were as follows: temperature for MS ion source was 250 °C, the multiplier voltage was 2400 V and electron energy was 70 eV. Sample feeding amount: 0.2 µl

0.2 m^3/h. After Sept. 14, the flow rate was adjusted to 0.35 m^3/h and increased gradually until Sept. 18 when designed loading 0.5 m^3/h was reached. The effective hydraulic retention time was 17.5 h.

The daily removal efficiency changes of COD and NH_4^+-N in the start-up phase are shown in Fig.3a and Fig.3b respectively. From Fig.3a we can see that while the concentration of COD fluctuated between 480~900 mg/L, the average concentration of COD in the effluent stabilized below 100 mg/L only 7 d after the addition of engineering bacteria, which was under the national wastewater Level I discharge standard (SSB, 1997), and the system displayed super resistant ability to the fluctuation of wastewater quality and quantity after the addition of engineering bacteria. As shown in Fig.3b, while the concentration of NH_4^+-N in the influent varied from 18 to 45 mg/L, the average concentration of NH_4^+-N in the effluent was about 29 mg/L. Various factors would affect denitrification, such as DO (dissolved oxygen), temperature and pH value, besides, the generation time of nitrobacteria is relatively long, so NH_4^+-N concentration in the effluent could not meet Level I discharge standard yet. However, in the later phase of start-up period, NH_4^+-N concentration in the effluent exhibited a declining trend, which indicated that the microorganism's denitrifying ability began to be exerted gradually, especially for O2 tank where denitrifying bacteria had been delivered, where approximately 50% NH_4^+-N had been degraded. Therefore, compared to the conventional process, by delivering high efficiency bacteria, bioaugmentation technology would dramatically reduce start-up time for wastewater treatment system and the engineering bacteria would start steady degradation of COD and NH_4^+-N rapidly.

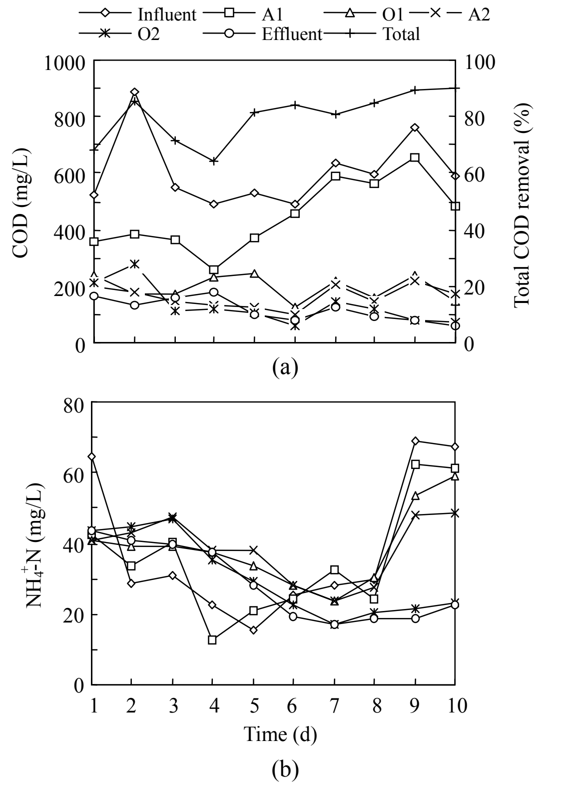

Fig.3 The daily concentration changes of COD (a) and NH_4^+-N (b) in the start-up phase

Performances of the pilot system in production resuming phase

When partial installations were stopped for examination and repair from Sept. 19, until all installations stopped producing on Sept. 24, the quality of the influent was good and could totally meet national standard. After Sept. 29, parts of the setup began to operate, the daily concentration changes of COD and NH_4^+-N in the production resuming phase are shown in Fig.4a and Fig.4b.

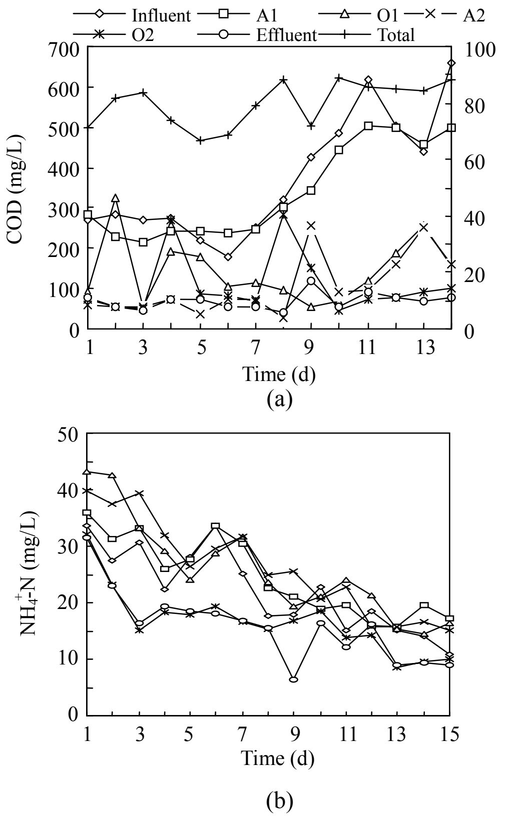

Fig.4 The daily concentration changes of COD (a) and NH_4^+-N (b) in production resuming phase

As shown in Fig.4a and Fig.4b, although the concentration of COD and NH_4^+-N was slightly lower than that of the regular production phase, due to the organics discharged from the outlet during repairing operation and rudimental organics in the inlet pipes, the concentration of COD and NH_4^+-N fluctuated in the range of 180~660 mg/L and 11~34 mg/L respectively, with the maximum value being 3.7 times and 3.1 times that of the minimum value respectively. Though sometimes the quality of the effluent could not meet the national standard, the efficient engineering bacteria immobilized on the carrier could adjust rapidly to water quality loading shock and show their super degrading ability, accordingly, the effluent quality gradually increased to that of steady phase and the system performed in a good condition.

Also from Fig.4a and Fig.4b, the influent quality in resuming phase was not quite stable, the changes of COD concentration in effluent was in accord with that of the influent. After the treatment of A1 tank and O1 tank, about 65% COD was removed, which enhanced the stability of A2 tank and O2 tank and ensured the removal efficiency of the high efficient bacteria to the residual persistent organic pollutants and NH_4^+-N, resulting in 80% total removal efficiency of COD with concentration all below 100 mg/L. Meanwhile, as the time gone by, the function of denitrifying bacteria began to display, and the NH_4^+-N concentration in each unit decreased, indicating that removal of NH_4^+-N was achieved in different units, nevertheless, the average removal efficiency of NH_4^+-N was about 57% in O2 tank, where denitrifying bacteria had been added, demonstrating the advantages of the delivery of engineering bacteria. As a result, the average concentration of NH_4^+-N in the effluent was 17 mg/L with the minimum value 6 mg/L and could completely meet national standard in the latter phase. In addition, compared to A1 tank, concentration of NH_4^+-N in the effluent from O1 tank was a little higher; this may be because after the anaerobic metabolism of A1 tank, macromolecular nitrogen containing organics were decomposed into the form of NH_4^+-N. Comparison of the quality of influent and effluent showed that the present process was appropriate for treating wastewater containing relatively low amount of NH_4^+-N. Consequently, from the concentration changes of COD and NH_4^+-N in the production resuming period, although the wastewater quality varied in a large range, bioaugmentation process can rapidly resume its disposal ability and lead to the stabilization of the process, which would reduce the cost needed for start-up and is prospectively useful in the emergency disposal.

Performance of pilot system in the regular production phase

The performance of pilot system in the regular production phase is shown in Fig.5a and Fig.5b. Fig.5a indicates that after the factory returned to its regular condition from Oct. 15, the concentration of COD in the influent was extremely unstable, and fluctuated between 370 mg/L and 910 mg/L. While the ambient temperature in the pilot field was already below 10 °C and the water temperature was below 13 °C, the pilot system entered low temperature operation period. However, the average concentration of COD in the effluent retained at about 77 mg/L, especially in the later phase when the system stabilized; the minimum value of COD concentration was close to 30 mg/L and the total rate meeting national stan-

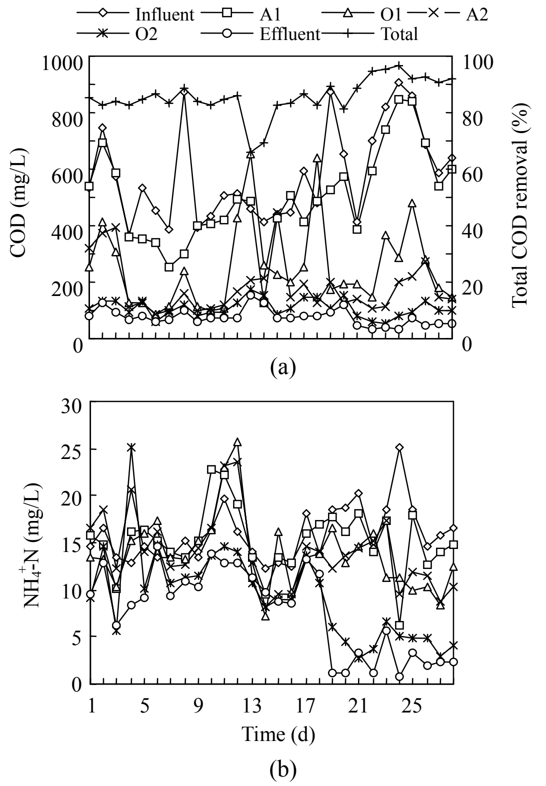

Fig.5 The daily concentration changes of COD (a) and NH_4^+-N (b) in regular production phase

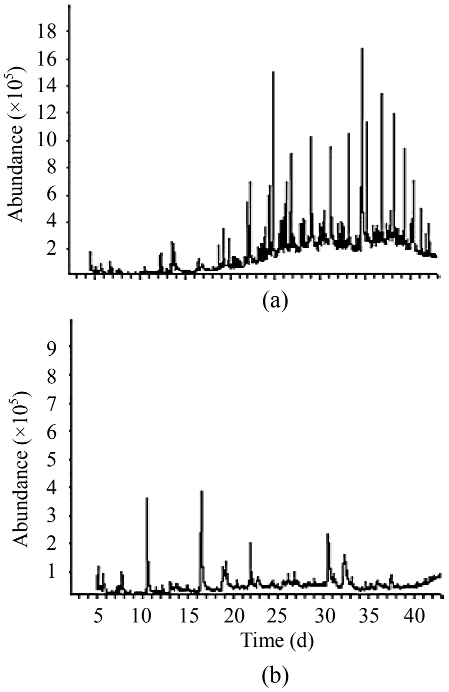

Fig.6 Effluent from secondary settling basin of the WWTP (a) and the pilot system (b)

dard was above 85%. On the other hand, the concentration of NH_4^+-N in the influent was not high, and varied from 16 mg/L to 25 mg/L, yet by microorganisms' degradation, quite an amount of organic nitrogen transformed into NH_4^+-N, leading to obvious increasing trend of NH_4^+-N concentration in the subsequent units. Although the water temperature was quite low, in cooperation with the high efficiency bacteria, the concentration of NH_4^+-N in the effluent wholly conformed to the national standard of lowest value 0.84 mg/L. The above phenomena showed that low temperature had little influence on effluent quality, and that after very short period of adjusting, the engineering bacteria could still retain their efficient degrading ability at low temperature condition.

GC/MS analysis of effluent quality

GC/MS analysis of organics contained in the effluent from the secondary settling basin of the WWTP and the pilot system was conducted (Fig.6).

Fig.6 showed that there were obvious changes in the number and amount of organics before and after bioaugmentation. The number of the refractory organics reduced to 32 after bioaugmentation compared to 68 before bioaugmentation technology was adopted. Without bioaugmentation, large amount of long-chain alkane, halogenated hydrocarbon, benzene and its analogies, esters as well as aldehyde ketone existed in the effluent, of which the content of long-chain alkane was the highest; after bioaugmentation, the concentration of long-chain alkane decreased dramatically, with 70%~100% removal efficiency, while benzene and its analogies, esters as well as aldehyde ketone were almost removed, the total amount of organics reduced by about 50%. Therefore, the bioaugmented two-stage A/O process performed well in removing refractory organics in petrochemical wastewater.

DISCUSSION

Mechanisms of bioaugmentation

Due to their metabolism and adaptation to extreme environments, microorganisms could degrade various organics; however, their degrading ability may be adversely influenced by several factors such as the pollutants' chemical characteristics, concentration, biodegradability as well as the physicochemical characteristics of the environment. In the original A/O process, the A stage operated in facultative condition; due to the limited oxygen supply, highly active microorganisms had to decompose organics that could hardly be degraded in aerobic condition in order to meet their energy need for metabolism, then the long chain of macromolecules would break off and turn them into micromolecules which were easier for degradation and thus be removed in the following aerobic units. In addition, microorganisms in O section were mainly eukaryotes, which would insure the effluent quality. Consequently, the sludge recirculation in the original A/O process would lead to the instability of the microbial community structure; thus, the assimilation of degrading function would be serious and thus result in poor purifying efficiency. The original process had been adjusted into two-stage A/O process in the pilot study, and by delivering suspending carrier for microorganism immobilization, the facultative and anaerobic bacteria would again display their ability to degrade organics and enhance the biodegradability of the wastewater for secondary aerobic degradation. By this step-by-step biodegradation, microorganisms in different biological tanks would display their individual functions and improve their ability to remove organics efficiently in biological system. After adjusting, the aerobic stages became the main functional unit of bioaugmentation; most of the COD and NH_4^+-N was removed in O stage, since many microorganisms including heterotrophic bacteria and nitrobacteria exist in aerobic environment. After the addition of functional bacteria into O tank, a new microbial community would form, while the original indigenous flora would degrade the relatively biodegradable organics such as phenols and alkanes, which would in turn create favorable condition for the display of engineering bacteria's function. In addition, the heterotrophic bacteria that exist in A tank and O tank would turn macromolecular substances into micromolecular substances that are easier for microorganisms' uptake. Therefore, it is sensible for delivering the engineering bacteria selected and bred according to specific pollutants into two O tanks, with the performance of the wastewater treatment system being the cooperative action of indigenous flora and bioaugmentation bacteria.

Two-stage A/O processes and bioaugmentation

The full-scale treating system applied A/O process, and the laboratory setup for bioaugmentation was two-stage reactor. The configuration and the inoculation of the two reactors were different, thus the data obtained were not sufficient to prove that their different performances were the result of bioaugmentation. However, from the designing view of the experiment, the function of the two stages of the A/O process were different: in the first stage, most of the easier biodegradable carbohydrates were decomposed, while most of the refractory organics were removed in the second stage, thus two-stage A/O process is better than the original A/O process (Zakkour *et al.*, 2001) even the inoculation was not conducted (Elmitwalli *et al.*, 2002). Yet the limited field condition and time inhibited further proceeding with the experiment, which was the shortcoming of our research. The following experiment will focus on the influences of different configuration of reactors on the performances of bioaugmentation.

Influences of exotic microorganisms on bioaugmentaion

Without doubt the invasion of exotic organisms definitely existed because the influent of the pilot setup was not sterilized, and so would certainly have effects on the efficiency of bioaugmentation. However, the engineering bacteria we delivered into the reactor were all indigenous organisms from the biological tanks of the other WWTP and had high adaptability. Once they became predominant in the reactor, they would be quite stable (Yu and Mohn, 2002). Although the influent was not sterilized, influences of the invasion of new microorganisms were quite minor and the performances of the reactor could also prove this. In addition, the purpose of the pilot experiment was for engineering application while the invasion of the new organisms was inevitable. Consequently, the engineering bacteria should have comprehensive adaptability. In short, the invasion of exotic organisms was certain, but had little influences on the efficiency of bioaugmentation and would not threaten the biological system.

CONCLUSION

By delivering high efficient engineering bacteria, the application of bioaugmentation technology in a pilot two-stage A/O process treating petrochemical wastewater under low temperature was investigated, the results from this work led to the following conclusions:

(1) It is feasible to treat complex petrochemical wastewater by using two-stage A/O processes. When the concentrations of COD and NH_4^+-N of the influent were 370~910 mg/L and 10~70 mg/L respectively, the corresponding average concentrations of those of the effluent were about 80 mg/L and 8 mg/L. In addition, when wastewater temperature was below 13 °C in the later phase of the pilot study, the stability and disposal ability of the system was not affected; the process showed great ability resist the fluctuation of water quality and quantity as well as temperature.

(2) The analysis of GC/MS demonstrated that after bioaugmentation, the concentration of long-chain alkanes decreased dramatically, the removal efficiency was more than 70%, and almost all of the benzenes and their analogies, esters as well as aldehyde ketones had been removed. The number of refractory organics reduced to 32 after bioaugmentation compared to 68 while bioaugmentation technology had not been adopted, while the amount of the organics reduced by almost 50% after bioaugmentaton, which indicated that bioaugmentation technology is high efficiency in removing refractory organics.

(3) Under low temperature conditions, the present process was efficient in removing pollutants, especially the refractory organic pollutants in petrochemical wastewater, and stayed stable throughout all the time. With almost no changes in the original wastewater treatment facilities, by adding engineering bacteria and suspending carriers and altering the aeration amount, the purification efficiency and the stability of the process would be greatly enhanced, which was the optimum strategy for the reconstruction of the A/O in petrochemical WWTP.

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