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Application of bioaugmentation to improve the activated sludge system into the contact oxidation system treating petrochemical wastewater

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ABSTRACT

In this paper, bioaugmentation was applied to upgrade a full-scale activated sludge system (S2) into a contact oxidation system (S1). Results showed that when chemical oxygen demand (COD) and ammonia nitrogen (NH_4^+-N) concentration of the petrochemical wastewater were 320–530 mg/L and 8–25 mg/L, respectively, the bioaugmented process (S1) took only 20 days when they were below 80 mg/L and 10 mg/L, respectively. However, the unbioaugmented conventional activated sludge process (S2) spent 30 days to reach the similar effluent quality. As the organic loading rate (OLR) increased from 0.6 to 0.9 and finally up to 1.10 kg COD/m^3 d, S1 showed strong resistance to shock loadings and restored after three days compared to the seven days required by S2. Based on the results of this paper, it shows that bioaugementation application is feasible and efficient for the process upgrade due to the availability of the bioaugmented specialized consortia.

1. Introduction

Bioaugmentation is the application of indigenous or allochthonous or genetically modified organisms to polluted hazardous waste sites or bioreactors in order to accelerate the removal of undesired pollutants (Chong et al., 1997; Fantroussi and Agathos, 2005; Head and Oleszkiewicz, 2004; Reberto et al., 2003). By inoculating strains which are efficient in degrading target pollutants, bioaugmentation could effectively remove the refractory organics involved in wastewater. Previous studies indicated that bioaugmentation was feasible for the treatment of waste streams produced from pharmaceutical factories (Saravanane et al., 2001), coke plants (Park et al., 2008; Wang et al., 2002), pulp mills (Yu and William, 2001), dye (Chen et al., 2006) and other industries. However, those researches on bioaugmentation were limited to lab-scale reactors or target organic substances such as 2-chlorophenol, 2,4-dichorophenol, EDTA and dichloroethene (Boon et al., 2000; Chen et al., 2005; Farrell and Quilty, 2002; Kyoung et al., 1997; Olaniran et al., 2006; Quan et al., 2004; Saravanane et al., 2001; Wang et al., 2002; Yu and William, 2001). The efficiency of the bioaugmentation depends on many factors, which include the chemical property and concentration of the pollutants, and the activity of the bioaugmented bacteria. Therefore, process performances were unpredictable and the full-scale applications of bioaugmentation to the existing industrial wastewater treatment facilities were rarely reported.

The petrochemical wastewater treatment plant (WWTP) studied in this research was located in northeast China. Its influent was a mixed waste stream from an oil refinery factory and various petrochemical industries producing dyestuff, chemical fertilizers, calcium carbide, glycol, oxirene, acrylon, synthetic resin and pesticides. The wastewater contains numerous refractory organics such as petroleum hydrocarbons, benzene hydrocarbons, aniline, nitrobenzene, phenols as well as their derivatives. These organics are highly toxic and inhibitory to microbial activity and would lead to a series of problems, such as poor effluent quality and unstable operation. Therefore, as both the amount and type of petrochemical products increased, the existing anoxic–oxic (A/O) activated sludge process can not meet the demands of the increasingly complicated petrochemical wastewater. It is urgent to develop and apply innovative technologies for the proper treatment of petrochemical wastewater.

Based on the achievements acquired in the pilot study (Zhao et al., 2007), bioaugmentation was applied in the full-scale petrochemical WWTP to improve the existing activated sludge process by upgrading it to a contact oxidation process. Successful bioaugmentation depends mainly on the behavior of the inoculated

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strains in the environment where they are introduced. Therefore, the growth rate of the organisms must be higher than decreasing rate of washout and predation (Bouchez et al., 2000; Fantroussi and Agathos, 2005). To avoid this, repeated inoculation of highly competent pollutant-degrading specialized bacteria was applied (Gilbert and Crowley, 1998; Kyoung et al., 1997; Loperana et al., 2006, 2007; Singer et al., 2005). Although periodic addition could provide the system with sufficient biomass, it could not justify the high cost and complex operation. For this reason, the present application of bioaugmentation was combined with immobilization technology through the contact oxidation process. It proved to be a good solution towards the prevention of the microorganisms from being washed out or grazed by other microorganisms such as protozoa (Danne and Häggblom, 1999; Fantroussi and Agathos, 2005; Moselmy et al., 2002, 2003). Meanwhile, it was proved that the immobilized cells were more efficient than free-living cells. Therefore, the immobilized bacteria required a less lag period before the biodegradation could take place (Moselmy et al., 2002, 2003; Wang et al., 2002). In addition, immobilized microorganisms can withstand pH, temperatures and high concentrations of pollutants, which are lethal to free-living cells (Hadjiev et al., 2007).

Compared to the previous applications of bioaugmentation which mainly involved lab-scale systems (Friis et al., 2006; Hu et al., 2008; Semprini et al., 2007), the present study was unique for its full-scale biological treatment system with genuine process variability. The main objectives of this research were: (1) to evaluate the feasibility of bioaugmentation application for the rapid upgrade of the activated sludge process to the contact oxidation process, (2) to verify the performances of the bioaugmented system, (3) to investigate the differences of the bacterial community structure between the upgrade system and the original system, (4) to explore feasible and reliable strategies for successful bioaugmentaion.

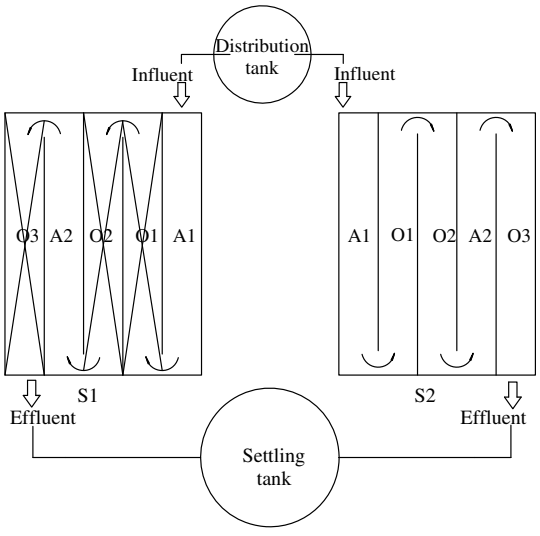

Fig. 1. The layout of S1 (A/O contact oxidation process with bioaugmentation and S2 (A/O conventional activated sludge process without bioaugmentaion).

2. Methods

2.1. Full-scale A/O contact oxidation process

The parallel biological systems in the petrochemical WWTP were investigated in the present study. The layouts of S1 (the bioaugmented contact oxidation upgrading system) and S2 (the conventional activated sludge system without bioaugmentation) were shown in Fig. 1. During our study, another conventional activated sludge system (S3) was shut down for the maintenance and repair purpose. This S3 system was used to study the comparison of start-up time between the bioaugmented contact oxidation system and the conventional activated sludge system without bioaugmentation. The schematic diagram of the A/O tank for S1 was presented in Fig. 2. The difference of S1 to S2 was the polyurethane foams packed within S1. The A/O tank had a size of 60 m × 40 m × 8 m ($L \times W \times H$) and the effective depth of water was 7.2 m. The tank was made up of five compartments. The first and the fourth compartments (A_1 and A_2) without aeration facilities acted as anoxic tanks. The other three aeration compartments (O1, O2, and O3) packed with polyurethane foams as the carriers were contact oxidation tanks. Agitators and vertical baffles were installed in anoxic tanks for the adequate mixture of the wastewater and to avoid the accumulation of suspended solids in the biological system. Thus, under the same effluent and environmental conditions, the existing activated sludge system without bioaugmentation was operated in parallel with the bioaugmented contact oxidation upgraded process with the purpose to investigate their different performances.

2.2. Characteristics of petrochemical wastewater

Before entering the biological systems, the petrochemical wastewater mentioned above was pretreated by neutralization and primary sedimentation. The temperature of the wastewater during the upgrading phase was 27–32 °C. Characteristics of the petrochemical wastewater entering the biological system were listed in Table 1.

2.3. Upgrading procedures

After carriers were installed in the contact oxidation tank, biological system S1 was bioaugmented with mixed cultures of specialized bacteria targeting to various refractory organics. These

Table 1
The characteristics of petrochemical wastewater

Parameters	Value	Level I criteria[a]
COD	300–600	100
BOD	150–350	30
NH_4^+-N	10–30	15
SS	⩽150	70
Oil and grease	⩽50	10
pH	7–9	6–9

[a] *Note:* Integrated wastewater discharge standard of China (State Environmental Protection Administration of China, 1996); parameters except for pH are in mg/L.

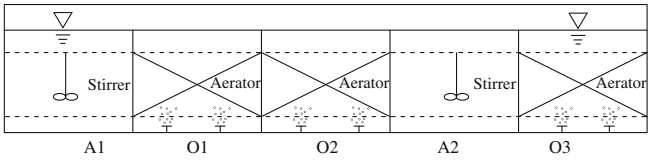

Fig. 2. The schematic diagram of S1.

bacteria, mainly consisting of *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Flavobacterium* and *Micrococcus*, were enriched from the activated sludge of various petrochemical WWTP through isolation and acclimation. Details for the isolation and acclimation process of the specialized bacteria were presented elsewhere (Zhao et al., 2007). Meanwhile, certain necessary organic substrates and inorganic trace elements were added to stimulate the growth of these microorganisms. Batch cultivation was adopted in a way that the partial wastewater in the tank was discharged and fresh petrochemical wastewater was introduced. Through this, suspended biomass was washed out to avoid competing with the fixed microorganisms for substrates (Tijhuis et al., 1994; Zhan et al., 2006). The organic loading rate (OLR) was increased stepwise from 0.04 to 0.5 kg COD/m^3 d at the end of the upgrading period as the flow rate reached the design value of 700 m^3/d. The preliminary cultivation and acclimation were finished twelve days later.

Metabolic rate is the amount of energy expended in a given period. Oxygen serves as an electron acceptor in the metabolism of the aerobic bacteria. Thus, the metabolic rate of microorganisms in each compartment of S1 could be limited through the adjustment of DO concentration. Then, unique bacterial community structure would form in different locations of the biological system (Gelda and Effler, 2002). The average DO concentrations in three oxic tanks were 1.45, 2.40, and 6.0 mg/L, respectively.

2.4. Shock loading experiments

After continuous flow and steady-state were realized, shock loading experiments were carried out to investigate the performances of bioaugmented system under perturbation conditions. The shock loadings were generated by increasing the inflow rate of the biological system. The corresponding OLR for the system was elevated and the hydraulic retention time (HRT) of the petrochemical wastewater was reduced. This suggested that the biological system should remove more pollutants during less time. Otherwise, the effluent quality would deteriorate. The experimental design conditions were described in Table 2.

2.5. Bacterial community structure analysis

Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) had been developed to analyze bacterial community structures without the inherent biases of cultivation (Lapara et al., 2006). Thus it becomes one of the most efficient molecular biotechnologies in monitoring the microbial communities of the environmental samples (Lapara et al., 2002). Biomass samples were collected from each compartment of S1 and S2. The gene fragments of mixed bacteria were first extracted from the above biomass samples and then the V3 region of 16S rRNA was amplified by polymerase chain reaction (PCR). The PCR products were then analyzed by denaturing gradient gel electrophoresis (DGGE). The specific steps were as follows.

2.5.1. Extraction of genomic DNA

Biomass samples of the full-scale contact oxidation process were collected by washing the biofilm attached on the carrier with sterilized water. The biomass of the activated sludge process was collected directly in the activated sludge form. All these sampling were performed in a steady operational state. One milliliter of suspended samples was washed with 500 μL sodium phosphate and then the mixture was centrifuged at 12,000 rpm for 10 min. Genomic DNA was extracted from the above supernatant by a bacterial Genomic DNA Extraction Kit (TaKaRa, Dalian, China) according to the supplier instructions.

Table 2
Shock loading experiments schedule

Test	Inflow rate (m^3/h)	OLR (kg COD/m^3 d)	HRT (h)	Duration (d)
1	800	0.6	21.6	6
2	1200	0.9	14.44	2
3	1500	1.10	11.52	0.125

2.5.2. PCR amplification

The V3 region of 16S rDNA genes were amplified by using universal primers F_{338}GC (5'-CGCCCGCCGCGCGCGGCGGGCGGGGC-GGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3') and R_{518} (5'-ATTACCGCGGCTGCTGG-3'). The final PCR mixture (50 μL) contained 100 ng DNA extract, 2 μL of each primer, 4 μL deoxynucleoside triphosphates, 5 μL 10 × PCR buffer (Mg^{2+} plus), 0.5 μL *Taq* polymerase, and 0.5 μL BSA. The touchdown PCR protocol included 8 min of initial denaturation at 94 °C, 30 cycles of 94 °C for 40 s (denaturation), 55 °C for 40 s (annealing) and 72 °C for 30 s (extension). PCR products were stored at 4 °C and detected by electrophoresis on a 2% agarose gel stained with ethidium bromide. All biochemical reagents were purchased from TaKaRa, Dalian, China.

2.5.3. DGGE analysis

DGGE was performed on a D-Code Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). Five microliter of PCR products and 10 μL of 10 × loading buffer were loaded onto 8% (w/v) polyacrylamide gels using a denaturing gradient ranging from 35% denaturant at the top of the gel to 60% denaturant at the bottom (100% denaturant contains 7 M urea and 40% (v/v) formamide). Electrophoresis was performed at 60 °C, initially at 20 V for 30 min and then at 150 V for 9 h. Finally, gels were stained with SYBR Green 1 and visualized and photographed by a transillumination scanner. Bacterial community structures were analyzed by visually identifying DNA bands that migrated at different distance in each lane on the denaturing gels.

2.6. Analytical methods

Effluent from each compartment and influent from the distribution tank were regularly collected for the off-line testing of ammonia nitrogen (NH_4^+-N) and chemical oxygen demand (COD) according to standard methods (State Environmental Protection Administration of China, 2002). The DO concentration and temperature of the wastewater were measured by a DO sensor. Organic pollutants contained in the influent and effluent of S1 and S2 were detected by gas chromatography-mass spectrometry (GC–MS) machine (GC-6890N/MS-5973N, Agilent, USA). The chromatography conditions were described elsewhere (Zhao et al., 2007).

3. Results and discussion

3.1. COD and NH_4^+-N removal efficiency at steady-state

It took the bioaugmented A/O contact oxidation system (S1) 20 days to meet the national discharge standards. For the unbioaugmented activated sludge system (S3), it required 30 days to reach the same effluent quality as S1. This demonstrated that bioaugmentation was a powerful tool to shorten the adaptation time of the biological system. As shown in Fig. 3, when the COD of the influent varied between 320–530 mg/L, the average effluent COD concentrations were 70 mg/L for S1 and 79 mg/L for S2. Though the difference was small, it was still quite encouraging considering the low biodegradability and great quantity of the petrochemical wastewater. Although the NH_4^+-N contained in the influent was lower than 25 mg/L, the average concentration of NH_4^+-N in the

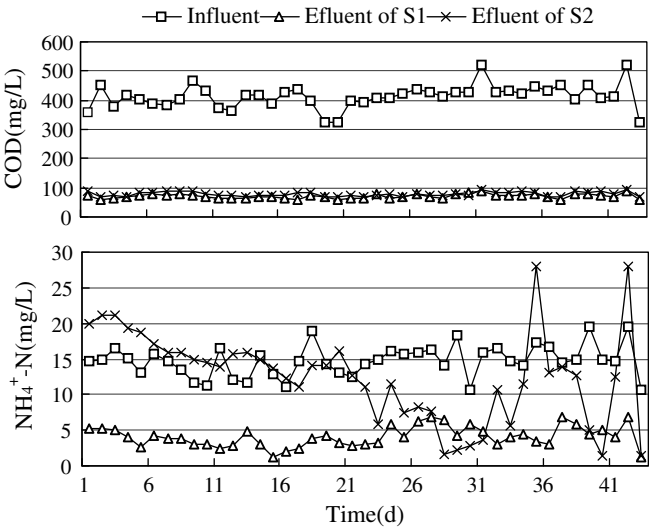

Fig. 3. Effluent COD and NH_4^+-N concentration of the bioaugmentd contact oxidation system (S1) and the activated sludge (S2) without bioaugmentation.

effluent of S2 was 12.4 mg/L. As for S1, despite the generation of NH_4^+-N by nitrogen-containing organics, its effluent NH_4^+-N concentration was 4.1 mg/L and the average removal efficiency was 72%. Thus, under the same working conditions, the bioaugmented system performed better than the unbioaugmented system, especially for nitrification. This may be the action of the bioaugmented specialized bacteria and the formation of the biofilm in the contact oxidation process. Biofilm could retain sufficient slow-growing bacteria with special metabolic capabilities.

3.2. Shock loading resistant ability

As described in Table 2, the performances of the bioaugmented contact oxidation process (S1) and the activated sludge process without bioaugmentation (S2) with shock loadings were shown in Fig. 4. Along with the shock loadings, both S1 and S2 suffered effluent quality perturbations, whereas the variation of S1 was much smaller than that of S2, especially for nitrification efficiency. It took S2 about one week to return to the normal states, while S1

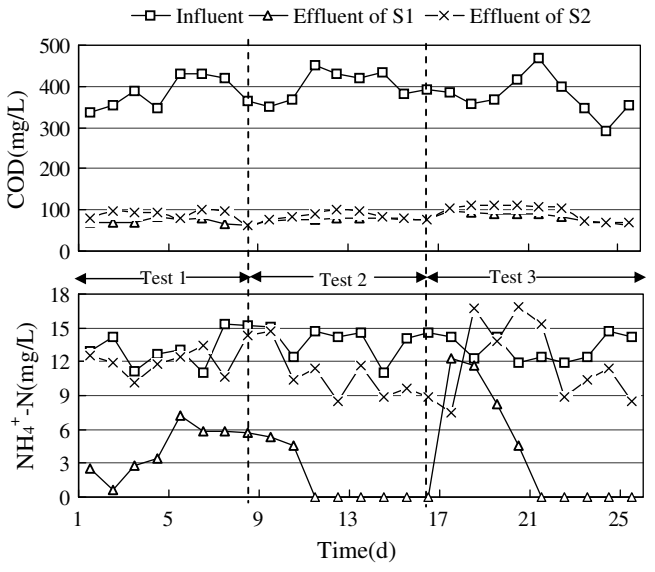

Fig. 4. Performance of the S1 and S2 during shock loading period.

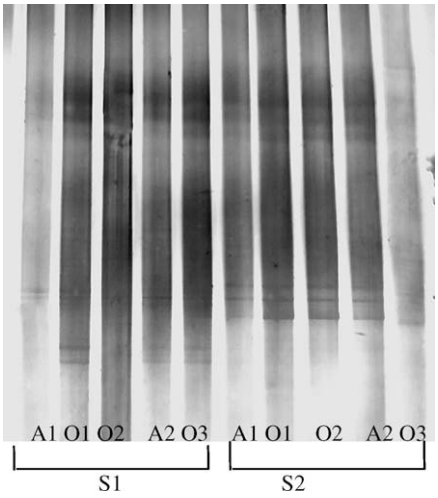

Fig. 5. PCR-DGGE fingerprints in each stage of S1 and S2.

restored only 3 days later when the OLR increased to 0.6 and then to 0.9 kg COD/m^3 d. The effluent NH_4^+-N of S1 was even undetectable in the later phase of Test 2. When short-time shock loading occurred with OLR rising to 1.1 kg COD/m^3 d, the effluent quality of S1 was only slightly influenced and still conformed to discharge standards. S1's effluent quality began to improve 24 h later rather than five days later for S2. The average COD removal efficiencies of S2 when OLR stayed at 0.6, 0.9 and 1.1 kg COD/m^3 d were 76.8%, 77.8% and 75.6%, respectively, while those of S1 were 80.9%, 81.0% and 77.8%. As for NH_4^+-N, the conversion efficiencies of S1 were 67.2%, 94.9%, and 69.2% in the three serial tests, which were obviously higher than S2 with 8.6%, 27.2% and 17.3% conversion efficiencies. Thus, under normal working conditions, the bioaugmenented S1 performed just slightly better than the unbioaugmented S2. However, S1 showed better resistance to shock loadings than S2. Thus, S1 was much potential when wastewater volume and organic contents increased followed the enhancement of production or the inevitable accidental wastewater discharge (see Fig. 5).

3.3. Degradation and removal to refractory organics

The GC/MS results of the influent and the effluent from the bioaugmented contact oxidation process (S1) and the activated sludge process without bioaugmentation (S2) were presented in Table 3. The number of organics was reduced to 21 in the bioaugmented system compared to 46 when bioaugmentation was not adopted. Certain refractory hydrocarbons (including alkanes, alkenes, alkynes and aromatic hydrocarbons), ketones, phenols, heterocyclic compounds, amines were removed in the bioaugmented system.

Table 3
Organics numbers comparison of influent and effluent

Organics	Influent	Effluent of S1	Effluent of S2
Hydrocarbons	37	8	13
Ketones	14	3	8
Phenols	13	1	6
Heterocyclic compounds	11	1	3
Esters	11	5	8
Amines	7	ND	1
Ethanols	6	ND	1
Nitrobenzene	1	ND	ND
Others	14	3	6
Total	114	21	46

Note: "ND" not detected.

Table 4
Contribution of each reactor to the pollutants removal

Stage		A1	O1	O2	A2	O3	Total
COD removal	S1	32.5	29.1	12.5	7.0	3.1	84.2
Efficiency (%)	S2	28.5	15.4	19.5	6.2	4.8	74.4
NH_4^+-N conversion	S1	–	–	4.8	14.9	49.7	69.4
Efficiency (%)	S2	–	–	–	–	19.6	19.6

Note: "–" more NH_4^+-N was observed in the effluent compared to the influent.

Although the organics were only a small portion of the total organic pollutants, they are hazardous if discharged to the environment.

3.4. Contribution of each stage to pollutants removal and bacterial community analysis

As petrochemical wastewater passed through each stage of the A/O process, pollutants were removed through the combined functions of each compartment. By monitoring the steady-state COD and NH_4^+-N concentration of wastewater sampled at each end of the stage, the role of each compartment in pollutants removal was investigated. As presented in Table 4, for COD removal, the O1 stage of the bioaugmented system (S1) performed much better than that of the unbioaugmented system (S2), while the O2 stage of S2 was slightly better than that of S1. However, the overall COD removal efficiency of S1 was 84.2%, which was higher than S2 with 74.4%. As for NH_4^+-N, 19.6% nitrification efficiency was achieved mainly in the O3 stage of S1. In S2, more NH_4^+-N was converted by nitrogen-containing organics. As nitrifiers failed to perform their functions, the NH_4^+-N was accumulated in the former four stages of S2. For S1, NH_4^+-N accumulation appeared in the first two stages, and then it began to decrease in the O2 stage. Most of NH_4^+-N was converted in the O3 stage with a 49.1% conversion efficiency.

From the data presented in Table 4, it could be inferred that the pollutants in S1 were decomposed gradually through cooperative action of each stage, rather than the random behavior of each stage contained in S2. It was hypothesized that the specialized bacteria inoculated in S1 may lead to its different performances from S2. Therefore, bacterial community analysis was conducted through PCR-DGGE technology to provide evidence for this hypothesis. The PCR-DGGE fingerprints were presented in Fig. 3. It was obvious that the lanes of samples collected from different locations in S2 appeared in almost the same bands. Thus, no detectable shift of the bacterial community was observed in different stages of the conventional activated sludge system (S2). The possible explanations were the impacts of sludge recirculation and the deficiency of specialized bacteria for the removal of target recalcitrant organics, especially for nitrobacteria which would convert ammonia nitrogen to nitrate. For S1, both the diversity and particularity (represented by the unique bacterial bands) of the bacterial community were better than that of S2. This might attribute to the control of the metabolic rate through the adjustment of DO concentration in its three oxidation tanks (Zhang et al., 1998). By controlling the DO concentration of O1 and O2, there was still a sufficient amount of biodegradable organics left after the decomposition of O1 and O2. This would provide a relative favorable nutritional environment for the proliferation and domestication of the specialized bacteria inoculated in O3. As a result, specialized bacteria that performed different pollution removal tasks were formed in each stage. A significant amount of organic pollutants was lost in O1 and O2, while the majority of NH_4^+-N was converted in the last stage. As a result, the removal of organic substances and the conversion of NH_4^+-N were not synchronous. The unique bacterial community structure and predominant bacteria in different stages might be the causes.

4. Conclusions

The results of this work lead to the following conclusions:

(1) Bioaugmentation with specialized bacteria targeted to various refractory organics was successful in the full-scale upgrade to a five-stage A/O oxidation contact process. For the start-up time, the upgraded process spent only 20 days when its effluent COD and NH_4^+-N were below 80 mg/L and 10 mg/L, respectively, compared to 30 days for the activated sludge system. Besides, the rapid upgrade period, the bioaugmented system also proved to be a powerful tool in improving the degradation efficiency of recalcitrant compounds and the resistance to shock loadings.
(2) Organic pollutants were removed gradually in the bioaugmented system, which was un-isochronous with the nitrification process due to the diverse bacterial community and unique predominant bacteria presented in each stage of the bioaugmented system. Thus, real temporal and spatial multiple stages were accomplished by the collaborate functions of the unique bacterial communities formed in each compartment.
(3) Successful bioaugmentation relies on various factors. Among these factors, the survival of consortia inoculated into the system was the most significant factor. Possible strategies, such as the adjustment of DO concentration in the biological tank, should be considered to create the optimum operational conditions for the growth and reproduction of the bacteria inoculated. Thus, bioaugementation application is successful due to the availability of the bioaugmented specialized consortia.

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