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Bioaugmentation combined with biofilim process in the treatment of petrochemical wastewater at low temperatures

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Abstract

Three sets of lab-scale reactors, which applied activated sludge process, bioaugmented activated sludge process and bioagumented biofilm process, respectively, were operated parallel to explore the optimum process for the treatment of petrochemical wastewater at low temperatures (13-15℃). Though being inoculated twice with enriched specialized bacteria, the bioaugmented activated sludge reactor (R2) didn’t show significant overall improvement on effluent quality when compared with the unbioaugmented reactor (R1) (average removal efficiency, COD: R1=65.02%, R2=70.39%; NH4+-N: R1=42.07%, R2=52.49%), except for increased levels of enzyme activity as described by dehydrogenase activity (DHA) and slightly better performance at the early stage of inoculation. Microscopic observation indicated that free-living cells were scarce in R2 and the main explanation was the grazing of protozoa to the bioaugmented cells. However, the application of porous polyurethane foam as carrier in the bioaugmented biofilm reactor (R3) could retain sufficient biomass within the reactor, and the COD (75.80%) and NH4+-N (70.13%) removal efficiencies were enhanced with more stable performances. In conclusion, massive inoculation couldn’t always warrant successful bioaugmentation due to predation to the inoculated specialized bacteria, and biofilm process was promising when combined with bioaugmentation technology in the treatment of petrochemical wastewater at low temperatures.

Keywords: *Bioaugmentaion; Low temperature; Activated sludge; Bioflim; Specialized bacteria*

1. Introduction

Traditionally, activated sludge process is widely used in dealing with industrial wastewater owing to its simplicity and relatively low cost. Petrochemical wastewater is heterogeneous organic compound mixtures, which contains quantity of organic compounds that possess some degree of either toxicity or activity inhibition to the microorganisms in the biological unit [1]. Moreover, the microbial activity would be further inhibited under low temperature conditions [2-4], when the adsorption and settling ability of the activated sludge would be influenced. Thus, microorganisms in the activated sludge system, even well acclimatized, are inefficient in dealing with petrochemical wastewater containing relatively high concentration of organic compounds due primarily to low biodegradability and inhibitory effects of these organic compounds [5], especially under low temperature conditions.

Bioaugmentation is the application of indigenous or allochthonous wild-type or genetically modified organisms to polluted hazardous waste sites or bioreactors in order to accelerate the removal of undesired pollutants [6]. It had been widely used in enhancing the removal ability of biological system to nitrogen [7] and phosphorous [8] as well as various organic refractory chemicals contained in industrial wastewater [9,10]. Although bioaugmentation of activated sludge system with the introduction of specialized bacteria was successful in some cases with significant improvement to the removal of target compounds[11,12], it is not yet widely applied due to several factors concerning unfavorable environmental conditions and the competition between the inoculums and other microorganisms existed in the system[5,11]. However, these limitations can be solved by replacing the suspending biomass system by attached biomass process [13]. Immobilization of mixed populations of microorganisms, predominantly bacteria, on or within inert supports has the following advantages [14,15]: (1) high reactor biomass concentrations, (2) strong capacity to handle shock loadings, and (3) low excess sludge production.

The objective of this research is to investigate the effectiveness of bioaugmentation technology and to explore an optimum bioaugmentation strategy for the treatment of petrochemical wastewater under low temperatures (13-15 ℃). Therefore, three lab-scale reactors, which applied activated sludge process (R1), bioaugmented activated sludge process (R2) and bioagumented biofilm process (R3), respectively, were operated parallel under low temperatures to compare their performances in treating petrochemical wastewater.

2. Materials and methods

2.1. Stand-up and operation of the A/O process

Three identical plexiglass anoxic-oxic (A/O) set-ups (shown in Fig.1), with effective volumes of anoxic tank, oxic tank and clarifier were 1.5L, 3.5L and 1.25L, respectively, were adopted. Each aerobic tank was inoculated with the same amount (0.5L) of activated sludge (MLSS=4000mg/L) taken from the aerobic tank of the petrochemical wastewater treatment plant (WWTP). Polyurentane foams were added as carrier in R3. Under steady-state, specialized bacteria were bioaugmened into the oxic tank of R2 and R3, while R1 was operated under the similar condition without bioaugmentation. Wastewater collected from the primary settling basin of petrochemical WWTP (Table1 shows its quality) was fed into these systems with flow rate increased stepwise to 0.5m3/h. The activated sludge was recycled at a 100% ratio and the excess sludge was discharge at a 10% ratio per day from R1 and R2. The hydraulic retention time (HRT) for anoxic stage and oxic stage was 3h and 7h, respectively. The dissolved oxygen (DO) in oxic tank was 4.0-6.0 mg/L. The wastewater was 13-15℃ during the whole process.

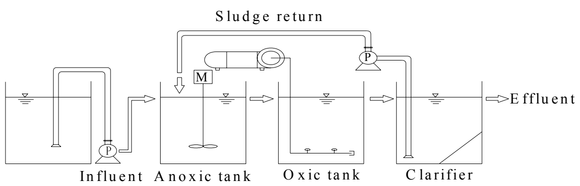


Figure 1. The schematic diagram of the experimental A/O process

Table 1. Influent quality of the biological systems

Parameters	Value	Level Ⅰ Criteria[a]
COD	400~600	100
	200~300	30
NH_4^+-N	30-50	15
SS	70-200	70
Oil and grease	≤80	10
pH	7-9	6-9

a.Integrated wastewater discharge standard of China [16]; Values are in mg/L except for pH.

2.2. Bioaugmentation method

Specialized bacteria previously isolated from various environments, which mainly consisted of Pseudomonas, Bacillus, Acinetobacter, Flavobacterium and Micrococcus, were functioned as COD degrading bacteria (mainly consist of oil and grease, phenol and aniline degrading bacteria), bioflocculant-producing bacteria and denitrifier. They were primarily acclimated with petrochemical wastewater after being taken from the refrigerating chamber and then inoculated into R2 and R3 with a total dry mass 150 mg/L 4 days after steady-state was achieved. The second bioaugmentation in R2 was performed 13 days later in the same way while the addition amount rising up to 700mg/L. Systems were hermetically isolated from each other to avoid cross-contamination.

2.3. Immobilization on polyurethane foams

Polyurethane foam is considered as a suitable carrier for cell immobilization for its easy control of the pore size, stable maintenance of quantity of cells and large-scale application at low price. It was widely used as a carrier in the biodegradation of organic compounds [17,18]. Spontaneous adhesion immobilization strategy was adopted in the present study as it is simple, cheap and allows significant biomass immobilization [13]. Physical characteristics of the polyurethane foam are summarized in Table2. Strip polyurethane foams were stuffed in spherical polythene plastic (D=80mm) frame to protect the carrier from being washed out from the system. The carrier hold-up was 30% in R3.

Table 2. Physical characteristics of the polyurethane foam

Items	Values
Pore size	150-500μm
Specific densiy	0.2-0.95
Specific area	$2.0\times10^4 m^2/m^3$
Acid and alkali resistence ability	5≦pH≦11
Service life	10 years

2.4. Analytical methods

Daily composite samples of influent and effluent were obtained by mixing samples collected every 6 hours. COD, NH4+-N were analyzed according to standard methods [19]. Biomass attached on the polyurethane foam was removed by microwave agitation, while the biomass of the activated sludge process was collected directly. Biomass concentration was determined by filtering the samples through 0.45 μm millipore filter and then drying at 105℃ until constant weight. The free-living bacteria were counted as colony forming unit (CFU) using culture method. Protozoa was observed by electronic microscope. For dehydrogenase activity (DHA) quantification, TTC-DHA method [20] was used. Polyurethane foam with biofilm for scanning electron microscope (SEM) observation was fixed in 4% glutaraldehyde buffer (2.5%, pH=6.8) for 1.5h at 4℃, then rinsed three times in 0.1M phosphate buffer (pH=6.8), dehydrated using an ethanol series (50%, 70%, 80%, 90% once for 10-15min and 100% thrice for 10-15min), died overnight in the desiccator and then were fixed on metal supports and sputter coated with gold (10nm) (k550x, EMITECH, England). Finally, biofilm samples were observed with a Philips XL30 SEM (Quanta 200, FEI, the Netherlands) and photographed.

3. Resluts and disscusions

3.1. Performances of each reactor

The daily influent and effluent COD and NH4+-N of each reactor were shown in Fig.2. When the influent COD and NH4+-N were 309.00-548.71mg/L and 32.08-49.26mg/L, respectively, the performances of R2 were slightly better than that of R1 (average removal efficiency, COD: R1=65.02%, R2=70.39%; NH4+-N: R1=42.07%, R2=52.49%). However, the average effluent COD and NH4+-N of R2 was up to 133.97mg/L and 20.52mg/L respectively, and the improvement on pollutants removal efficiency only lasted for about 2 days after bioaugmentation. The nitrification behavior of R1 was even better than R2 before the second inoculation, while slightly better performance was detected in R2 after the second bioaugmentation. Thus, even R2 was inoculated twice with the specialized consortia, improvment induced by bioaugmentation was still unfavorable.

Therefore, under low temperatures, by inoculating specialized bacteria into the activated sludge set-up, bioaugmentation technology failed for the treatment of petrochemical wastewater as the effluent quality couldn't meet the national wastewater discharge standards [16]. The previous literatures indicated that bioaugmentation would be a useful tool for the removal of recalcitrant organic compounds and the enhancement of the wastewater treatment systems' stability under extreme environments [21], such as low and high temperatures, saline environments, acidic and alkaline environments as well as deep-sea environments. Results herein may due to the difficulty in the ecological control of the added specialized strains and the other microorganisms involved in the activated sludge system, thus the availability of the specialized consortia added was reduced.

With the application of polyurethane foam as carrier, the average effluent COD and NH4+-N of R3 were 91.69mg/L and 20.52 mg/L, and it's quite promising since the average removing efficiency to COD and NH4+-N reached to 75.80% and 70.13% respectively. Meanwhile, in the later period, the effluent concentration COD and NH4+-N were below 90mg/L and 12 mg/L, respectively. The results suggested that bioaugmented system with inert support performed better than both the conventional and the bioaugmented activated sludge system. The environment created by polyurethane foam provided a favorable condition for the growth and proliferation of the inoculums, i.e, the degrading capability of microorganisms had a better chances to display with their immobilization on polyurethane foams. Moreover, compared to R2, since high concentration of biomass and high cellular retention time were achieved by biofilm, only one inoculation was conducted..

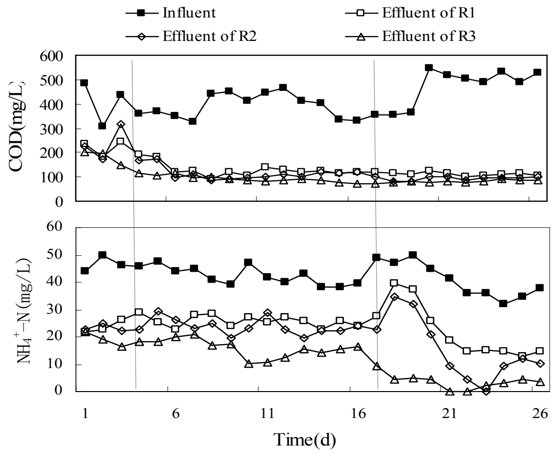


Figure 2. Influent and effluent characteristics of each reactor

3.2. Biomass Concentrations and Enzyme Activities

The average DHA and biomass in each reactor during one month operation were detected The DHA of the activated sludge in R2 (1.83mgTF/gMLSS.h) was much higher than that of the R1 (0.99mgTF/gMLSS.h), while the average concentration of MLSS in R1 (1100mg/L) was slightly higher than that of R2 (900mg/L), which demonstrated that with the addition of specialized bacteria, the activity of the activated sludge increased compared to the unbioaugmented one, however, the inoculums failed to exhibit their capability to decompose the target pollutants and there was no notable correlation between pollutants removal efficiency and enzyme activity, which differed from the conventional concept that the higher the enzyme activity, the lower organics remaining in the effluent[20]. This phenomenon may be particular to low temperature as the microorganisms needs long period for its lag phase until the degradation began to display. For biofilm reactor, sufficient biomass (2000mg/L) was retained with even higher activity (2.08mgTF/gMLSS.h), which performed best with the microorganisms bioaugmented in the system. These results indicated that massive inoculation didn't always coupled with favorable performances, and the availability of inoculums was crucial for successful bioaugmentation.

3.3. Microscope observations

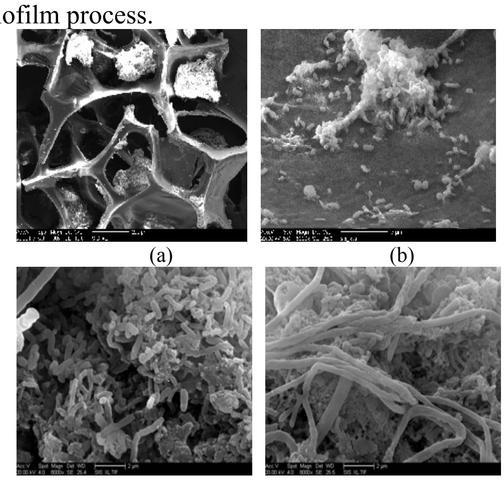
Obvious proliferation of protozoa after the inoculation of specialized consortia was observed in R2, while free-living bacteria were scare. In order to determine whether the lack of free bacteria was mainly caused by washout or by protozoa, average free-living bacteria in aeration tank and secondary tank was counted as colony forming unit (CFU). Under the same operational conditions, free-living bacteria in R1 was higher than that of the R2, while the proportion of free-living cells that lost along with the effluent were 75% and 30% for R1 and R2, respectively. It could be inferred that the main reason for the rapid disappearance of the numerous bacteria inoculated in R2 was not washout but the grazing of the protozoa which overgrew with the addition of large amount of specialized bacteria. Conventionally, the proliferation of protozoa was considered to be an indication for favorable water quality, however, it was not the case after bioaugmentation with massive specialized bacteria in the system, where the ecosystem equilibrium was disturbed. Thus, it would be advisable to take measures to prevent the inoculated bacteria from being phagocytized by overgrowing protozoa.

3.4. SEM observation of biofilm

Microorganisms immobilized on polyurethane foam were observed by SEM. Images are showed in Fig.3. There were three kinds of immobilization forms for the bacteria on polyurethane foam, that is: (a) cells entrapped in the pores; (b) individual cells distributed randomly on the on the surface of polyurethane foam; (c) and (d) Cells congregated together on the surface or in the pores of polyurethane The numerous microorganisms immobilized on the porous carrier performed well in the pollution degradation and were against from the predation of protozoa and the washing out along with the effluent. Many previous study pointed out that compared to conventional free cell systems, the bioreactors with immobilized cells showed better results including greatly improved reactor productivity and enhanced withstand ability to extreme environment such as low temperature due to its high cell density and optimum microbial community structure [13,18,19].

However, from Fig.3(a), it was obvious that there was large percentage polyurethane foam hadn't been utilized both for the pores and the surface, possible reasons are the relatively short operational time or the washing force of the flow or the unfavorable condition for cells' immobilization. Thus, certain modification may be required for the wide application of porous polyurethane foam s as a carrier.

The results obtained above demonstrated that under low temperatures, bioaugmentation of the activated sludge process with domesticated specialized bacteria induced a slightly better performance in treating petrochemical wastewater than the system without bioaugmentation, while bioaugmentation combined with biofilm process performed effectively in pollutants removal with high microbial activity. Thus, bioaugmentation was optimal when combined with biofilm process.



(a) (b) (c) (d)

Figure3. SEM images of the biofim

4. Conclusion

Under low temperatures, compared to bioaugmened activated sludge process, since it could retain sufficient biomass, bioaugmentation combined with biofilm process performed more effectively in removing pollutants contained in petrochemical wastewater with high microbial activity, while bacterial species were disappeared due to the strong increase in the grazing pressure exerted on the inoculums in the activated sludge system. Thus, the application of polyurethane foam as carrier in the bioaugmentation practice is promising for the retention of sufficient biomass and prevention mechanisms to the immobilization cells. Further researches on the ecological relationships in bioaugmented system by adopting advanced molecular microbial techniques are necessary for better understanding to the bioaugmentation mechanism.

5. Acknowledgment

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