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Bioaugmentation as a tool to enhance the removal of refractory compound in coke plant wastewater

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Abstract

Pollution caused by coal conversion wastewater has been a severe problem for decades in China due to the use of coal as the main energy source. An aerobic–anoxic–oxic (A1–A2–O) system was developed for treating coke plant wastewater and good results were obtained. GC/MS analysis indicated that the main ingredients of the effluent were aromatic and heterocyclic compounds, alkanes and phthalic acid esters etc. Bioaugmentation with specialized microorganism could be a powerful tool to improve the wastewater treatment processes. In this study, quinoline, which was poorly removed by the A1–A2–O system, was chosen as a target pollutant, and a quinoline-degrading bacterium, identified as *Burkholderia pickettii* was used as bioaugmentation microorganism. The feasibility of bioaugmentation in combination with A1–A2–O system was investigated. The performance of the A1–A2–O system and the contribution of each stage to COD removal were investigated. The contribution of anaerobic, anoxic and oxic reactors to COD removal was 25, 16 and 59%, respectively. The results of bioaugmentation experiments showed that the oxic reactor, to which the aerobic microorganism was added, was the best location of bioaugmentation in the A1–A2–O system. Bioaugmentation could be used as an efficient and effective method of improving the removal efficiency of recalcitrant organic compounds from industrial wastewater.

Keywords: Bioaugmentation; Coke plant wastewater; Quinoline; Refractory compound; Anaerobic–anoxic–oxic system

1. Introduction

Coke plant wastewater is generated in the coal coking, coal gas purification and by-product recovery processes of coke plants. The wastewater contains ammonia, thiocyanate, phenolics and other organic compounds, such as mono- and poly-cyclic nitrogen-containing aromatics, oxygen- and sulfur-containing heterocyclics and polynuclear aromatic hydrocarbons (PAHs) [1,2]. These wastewaters are very harmful due to their high ammonia content and the presence of inhibitory or toxic organic compounds. Most of the heterocyclic compounds and PAHs have been reported to be carcinogenic. There are many coke plants and coal gasification units throughout China because coal is the main energy source. Therefore, pollution caused by coal conversion wastewater has been a severe problem for decades.

Conventional treatment of coke plant wastewater includes solvent extraction, steam stripping and biological treatment. Due to the presence of refractory and inhibitory compounds, the conventional biological treatment is not efficient in removing COD to meet the effluent standard. In recent years, our laboratory has developed anoxic–oxic (A–O) and anaerobic–anoxic–oxic (A1–A2–O) processes for treating coke plant wastewater and achieved good results [1]. However, it was found that the effluent COD is difficult to reduce to less than 200 mg/l. GC/MS analysis indicated that the main ingredients of the effluent are aromatic and heterocyclic compounds, alkanes and phthalic acid esters etc.

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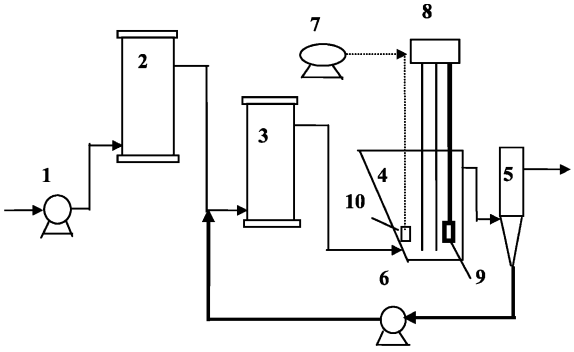
Bioaugmentation of activated sludge systems with specialized microorganism could be a powerful tool to improve the wastewater treatment processes, for example, to improve the flocculation of activated sludge and to enhance the removal efficiency of recalcitrant compounds. The specialized microbes include indigenous or genetically modified organisms. Bioaugmentation has been reported to enhance removal of 3-chlorobenzoate, 4-methyl benzoate, toluene, phenol, and chlorinated solvents [3–6]. However, the addition of specialized strains to activated sludge to enhance the removal of pollutants present in the influent is not yet widely applied. This is due to the fact that bioaugmentation of activated sludge is less predictable and controllable than direct physical or chemical destruction of pollutants.

Quinoline, a heterocyclic compound, which was poorly removed in the A1–A2–O system, was chosen as a target pollutant. Several research works have been done on the biodegradation of quinoline [7,8]. The objectives of this study were (1) to test the effects of bioaugmentation on quinoline removal; (2) to determine the best location at which the specialized microorganism was added in combination with A1–A2–O system.

2. Materials and methods

2.1. Microorganism

A quinoline-biodegrading microorganism was isolated from activated sludge from a coke-oven wastewater treatment plant using quinoline as sole carbon and nitrogen source. It is a Gram negative, rod-shaped and aerobic strain, which was identified as *Burkholderia pickettii*.



1—Influent pump; 2—Anaerobic reactor; 3—Anoxic reactor; 4—Oxic reactor; 5—Sedimentation tank; 6—Recycling pump; 7—Air compressor; 8—pH controller; 9—pH probe; 10—Air diffuser.

Fig. 1. Experimental set-up for A1–A2–O system.

2.2. Experimental set-up

The experimental set-up is shown in Fig. 1. The volumes of anaerobic, anoxic and oxic reactors made of Perspex were 2.5, 2.5 and 7 l, respectively.

The anaerobic reactor was packed with semi-soft media, which were constructed by plastic ring and synthetic fiber string. The anoxic reactor is a completely mixed reactor while the oxic reactor is a hybrid bioreactor in which polyurethane foam carriers were added. The characteristic of the polyurethane foam carriers carrier were described in a previous publication [9].

2.3. Bioaugmentation experimental method

Sample water was taken from the anoxic reactor, oxic reactor and the sedimentation tank. A certain amount of specialized microorganism, that is, a quinoline-degrading bacterium, was mixed with sample water and quinoline was added into the mixed solution, to make the final quinoline concentration 300 mg/l. The final biomass concentration of the quinoline-degrading bacteria in the mixed liquor was 0.75 and 1.5 g/l, respectively. Bioaugmentation experiments were carried out at 28 °C. Samples were taken at intervals and quinoline concentrations analyzed by HPLC.

2.4. Analytical methods

COD was analyzed according to Standard Methods for Water and Wastewater Examination [10]. TOC was measured using a Shimadzu TOC-5000 analyzer. Biomass was measured by a weighing method. For quinoline quantification, a HPLC system (Hewlett–Packard model 5050 with an UV detector) was used. Twenty microliter samples were injected after centrifugation and filtration. Separation was carried out in a C^{18} reverse-phase column, 250 × 4.6 mm, 5 μm (Hewlett–Packard Zorbax SB-C^{18}, USA). The elution solvent, which consisted of a mixture of methanol and water (60:40, v/v), was introduced to the column at a flow rate of 1 ml/min. Quinoline was detected at 275 nm. For GC/MS analysis, samples were extracted by CH_2Cl_2 into neutral, basic and acid phase (repeated three times for each phase) and then concentrated by evaporating in a water bath at 39–41 °C. The prepared sample was used for GC/MS analysis. The analytical conditions were as follows: a capillary column made of quartz with inner diameter of 0.25 mm and length of 50 m was packed with OV-101; temperature for the gasification compartment was maintained at 280 °C; the temperature control program was followed by retaining at 70 °C for 3 min and then increasing to 280 °C with an increment of 3 °C/min; temperature for MS ion source was 200 °C and electron energy was 70 eV [1].

3. Results and discussion

3.1. Isolation and identification of quinoline degrader

A quinoline-degrading microbe was isolated from the activated sludge of a coke oven wastewater treatment plant by enrichment shaking culture at 30 °C. The strain was purified by successive streak transfers an agar-plate medium and maintained as slant cultures on tryptone–glucose extract agar. This quinoline degrader was capable of using quinoline as the sole source of carbon, nitrogen and energy. It was identified as *B. pickettii* using a Biolog Microstation System (ID = 0.733). It was a Gram-negative rod-shaped aerobe (6 μm long and 2 μm wide). Colonies were mucoid and grey when grown on solid quinoline–mineral salt medium (MSM).

3.2. Performance of A1–A2–O system

In recent years, this laboratory has developed the A1–A2–O system for coke plant wastewater treatment [1]. In this system, the anaerobic process acts as a pretreatment for partial degradation of some refractory and inhibitory compounds [2]. The possible mechanism for anaerobic degradation of refractory organic compounds in coke plant wastewater was partial scissions of polycyclic and heterocyclic rings, forming some long chain organics and low molecular weight fatty acids [2,11]. Previous study showed that the anaerobic phase played an important role in the A1–A2–O system. Organic compounds with complicated structures and large molecules in coke plant wastewater could be partially converted into more biodegradable organic compounds, which resulted in formation of more suitable and available carbon source for denitrifiers in anoxic phase, and furthermore reduced the inhibitory substances to nitrifiers in the oxic phase. Therefore, nitrification and denitrification performances could be enhanced. The following anoxic–oxic processes play the main role in reducing COD and nitrogen through the performance of predenitrification–nitrification.

The operational parameters of each reactor during steady-state period, such as temperature, dissolved oxygen, hydraulic retention time and flow rate are listed in Table 1.

Table 1
The operational parameters of A1–A2–O system during steady-state period

Parameters	Anaerobic	Anoxic	Oxic
Flow rate (l/h)	0.2	0.2	0.2
HRT (h)	12.5	12.5	35
DO (mg/l)	–	–	4.0–6.0
pH	–	–	7.2–8.0
Temperature (°C)	28–30	28–30	28–30

The biomass concentrations in each reactor, including suspended and attached growth were measured during the steady operation period, the results are shown in Table 2.

The results in Table 2 indicated that the total biomass in the anaerobic reactor had a high concentration of 8.1 g/l of which a large part of the biomass was in attached growth, because the semi-soft media packed in the anaerobic reactor retained the microbial sludge quite well, that is to say, the media had a good performance to attach the biomass. In the oxic reactor, the total biomass achieved was 4.7 g/l, and the attached biomass accounted for 3.4 g/l. The anoxic reactor is a completely mixed reactor, containing only suspended-growth biomass and the sludge concentration was 2.8 g/l.

The contribution of each stage, that is A1, A2 and O, to the removal efficiency of COD was investigated. The results were calculated based on the system influent concentrations and depicted in Fig. 2. It was evident that most of the influent COD was removed by the oxic reactor.

The influent and effluent organic composition was analyzed by GC/MS. The results indicated that several organic compounds, including naphthol, naphthonitrile, methylimidazole, isocarbostyril, benzofuran, benzoquinoline and anthrylnitrile could be completely removed in the A1–A2–O system. Many of these compounds are large molecules and have complicated structures that are unfavorable to the environment. The complete removal of these compounds from coke plant wastewater reduced the harmful effect on the receiving watercourse. Phenolic compounds, such as phenols, cresol, methylphenol, dimethylphenol, could be removed by more than 95%. Pyridines, such as pyridine, methylpyridine, C_2alkylpyridine, C_3alkylpyridine, C_4alkylpyridine, phenylpyridine, could be removed by more than 94%. However quinolines, such as quinoline, isoquinoline methylquinoline were poorly removed by this system.

3.3. Removal of quinoline by bioaugmentation

Quinoline, a heterocyclic nitrogen-containing aromatic compound, which was poorly biodegraded in the A1–A2–O system, was chosen as a target pollutant. The combination of the A1–A2–O system with bioaugmen-

Table 2
Biomass concentrations at steady-state operation

	Anaerobic	Anoxic	Oxic
Suspended-growth (g/l)	2.4	2.8	1.3
Attached-growth (g/l)	5.7	0	3.4
Total biomass (g/l)	8.1	2.8	4.7

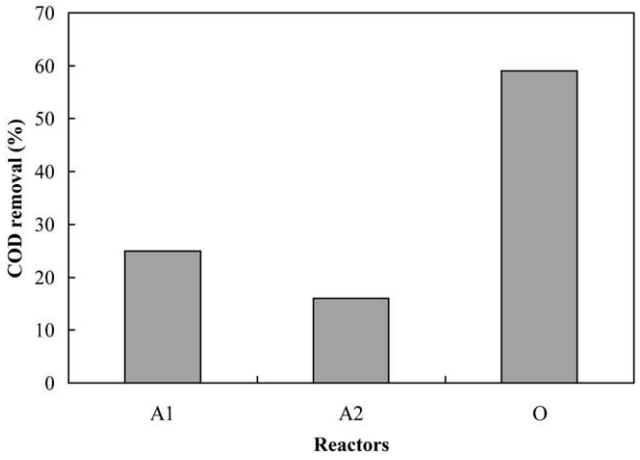


Fig. 2. The contribution of each stage to COD removal.

tation for improving the removal efficiency of recalcitrant organic compounds, quinoline, was investigated. The specialized microorganism was added to the different locations of A1–A2–O system in order to optimize the addition point of bioaugmenting biomass. The result of quinoline degradation through bioaugmentation at different locations of A1–A2–O system is demonstrated in Fig. 3.

When the specialized microorganisms were added in different locations, they all could play a bioaugmentation role (Fig. 3). This indicated that the added microorganism, that is, *B. pickettii* could tolerate the refractory and inhibitory organic compounds existing in coke plant wastewater and played the degradative role. However, the locations at which microorganisms were added had an influence on the bioaugmentation effect. When the concentration of added microorganisms was 0.75 g/l, the degradation rate of quinoline was highest if the microorganisms were added into the oxic phase. After 7 h, the removal efficiency of quinoline in the oxic phase, anoxic phase and sedimentation tank effluent were 34, 25 and 20%, respectively. When the concentration of added biomass increased to 1.5 g/l, the biodegradation rate of quinoline increased at all three different locations. Within the 4 h after the bioaugmentation started, the degradation rate of quinoline was as follows: oxic phase > anoxic effluent > sedimentation tank effluent. After 4 h, however, the degradation rate changed as follows: anoxic effluent > oxic phase > sedimentation tank effluent.

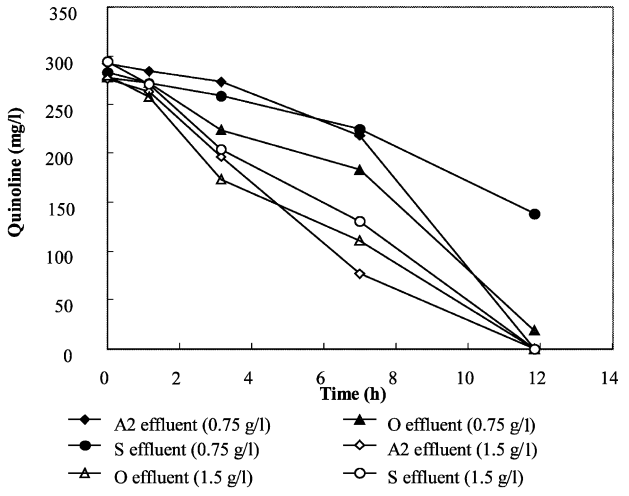


Fig. 3. Degradation of quinoline through bioaugmentation at different locations.

From the point of view of overall degradation of quinoline, the oxic phase was the best location for bioaugmentation. This is because many kinds of microorganism species, including many heterotrophic microorganisms, nitrifying bacteria existed in the oxic reactor. When the specialized microorganism was added, a new microbial community could be formed, in which indigenous microorganisms could degrade the inhibitory compounds existing in coke plant wastewater, such as pyridines, phenolic compounds etc. Therefore, the possible inhibitory effect of such compounds to the added microorganisms could be reduced. On the other hand, the indigenous heterotrophic microorganisms in the oxic phase could convert many large molecular compounds existing in coke plant wastewater into smaller compounds, which were simple in structure and easy for microbial uptake, that is to say, forming more biodegradable organic compounds, they therefore could act as carbon and energy source for added microorganisms and stimulated their growth.

Although the difference of water quality between the anoxic effluent and the oxic reactor was small, the microbial community was different. In the effluent of anoxic phase the main microorganisms were denitrifiers. In the effluent of sedimentation tank, there were few microorganisms.

The above results revealed that bioaugmentation could be used as a tool for the removal of recalcitrant organic compounds, which is poorly removed by conventional biological treatment system. Bioaugmentation is a powerful tool when combined with A1–A2–O system for the treatment of coke plant wastewater. The further investigation is under way in our laboratory.

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