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Research Article

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Study on bioaugmentation in rapid startup of BF-BFB and enhancing effect of coke plant wastewater treatment

Yingjun Haoa, Suqin Lia,*, Xiaolei Wub, Zongna Lia, Yuan Lia and Jieyu Zhaob

a*School of Metallurgical and Ecological Engineering, University of Science and Technology Beijing, Beijing, China*
bDepartment of Energy& Resources Engineering, College Engineering, Peking University, Beijing, China

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ABSTRACT

Because of its high concentration of toxic and complicated composition, the coke plant wastewater is very difficult to meet the discharge standards by normal activated sludge process in practical production. A bioaugmentation technology was applied in biofilm startup of bio-filter and bio-fluidized bed (BF-BFB) system to improve membrane performance and enhance the treatment effect. As a new bacteria cultivated, comamonas testosterone has better synergism and higher COD & NH3-N removal rate. Based on experiments, the system`s rapid startup and stable performance were investigated in this paper. The results showed that the biofilm was mature and stable in 25 days and the removal efficiency of COD and NH3-N were up to more than 95%. By SEM and gene library technology, the dominant strains were identified. The Proteobacteria was the largest bacteria group, accounting for 55.00% of the total library. A stable micro ecosystem formed ultimately to improve the removal effect of recalcitrant organic compounds from industrial coke plant wastewater.

Keywords: The coke plant wastewater; Systems startup; Bioaugmentation; Gene library technology

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INTRODUCTION

Coke plant wastewater contains a lot of phenol, cyanide, benzene, aromatic, heterocyclic, fat and other refractory organic pollutants and has great toxicity and poor biodegradability. High removal rate of COD & NH3-N at a low cost are the key problems and hot focus. By adding bacteria genus, nutrients, microorganisms, or matrix analogues which have specific function to the biofilm bio-system, bioaugmentation technology can be used to start up biofilm quickly and to degrade refractory organic pollutants efficiently.

In recent years, bioaugmentation technology with specialized microorganisms had been applied to coke plant wastewater treatment, and successfully improved COD and NH3-N removal efficiency. A quinoline-degrading bacterium, identified as Burkholderia pickettii was used as bioaugmentation microorganism in A2 /O system to treat coking wastewater. The results proved that bioaugmentation could be used as an efficient and effective method for improving the removal efficiency of recalcitrant organic compounds from industrial wastewater[1]. Bioaugmention and PCR-DGGE method were developed to monitor structure changes of Thauera sp. in coke wastewater treatment plant responding to operational perturbations. The analysis data showed concomitant shift of Thauera composition and the system's COD removal function[2]. A cyanide-degrading yeast, Cryptococcus humicolusMCN2, and unidentified microorganisms were augmented in a full-scale cokes wastewater treatment facility to enhance removal efficiency of cyanide compounds[3]. The studies[4-8] showed that adding efficient strains is good for biofilm starting up and the improvement of degradation rate. In this study, bioaugmentation was used biofilm starting up of BF-BFB system in coke plant wastewater, to build a sound the micro ecosystem, and to improve removal effect.

EXPERIMENTAL SECTION

2.1 Initial Wastewater and Bacteria Strains
The initial wastewater was taken from a coke plant in an Iron & Steel Company, Hebei Province, China. The concentration of COD and NH3-N are of 2610.50mg/L and 246.80mg/L respectively. The initial wastewater was diluted several times before entering the system.

The efficient bacterium, named *comamonas testosterone*, was separated and screened from coking wastewater. It was injected into BF-BFB reactors after regeneration and activation.

2.2 The Experimental System.
The experimental system is comprised by the anaerobic tower biofilter (BF), aerobic BFB, and sloping plate settling tank[9-10].

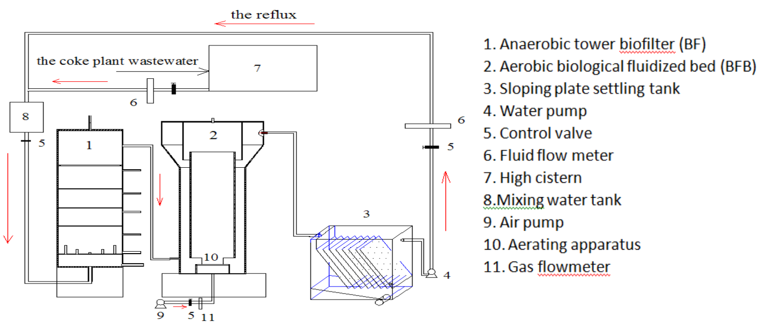


Fig. 1 The integrated biological fluidized bed reactor system diagram

In the experiments, the wastewater was pretreated by BF firstly, and then it flew into the bottom of aerobic three-phase BFB. At last, the effluent from the top of BFB flew into the sloping plate settling tank. The reflux flew back into the anaerobic tower biofilter to continue the denitrification process. Zeolites were filled in facultative anaerobic BF and anoxic BF. They played the role of filtration and isolation. The average particle size of zeolites is 2-5mm.

2.3 Analysis and Detection Methods
2.3.1 Chemical Analysis
The concentrations of COD and NH3-N were measured using Multi parameter water quality monitor instrument. A pH meter was used in pH analysis. The variation of dissolved oxygen was monitored with dissolved oxygen meter.

2.3.2 Biofilm Observation
The microbial communities in biofilm above suspended media were observed by optical microscope. The microscopic examination of microorganisms in the biofilm was followed by standard microscopic examination procedures. In stable operation phase, the microbe in biofilm was observed by SEM.

2.3.3 16SrRNA Clone Library Analysis
The major steps included DNA extraction, PCR amplification, product purification，connection and conversion, positive clones screening and digestion reaction. The DNA extraction method was cell disruption. The target DNA fragments were extracted through PCR amplification, product purification according to instruction of Fast DNA Spin Kit for soil (MP Biomedicals Corporation). The PCR products were sub-cloned using the pGEM-T easy vector system. Then the positive colonies were amplified and submitted for DNA sequencing. Sequence data from 16SrRNA gene fragments were subjected to phylogenetic analysis. Homology searches were conducted in using the GenBank server of the Nation Center for Biotechnology Information (NCBI) and the BLAST algorithm. Phylogenetic trees were designed using the software Mega5.0. The neighbor-joining method was used for tree construction , and Bootstrap value was 500.

2.4 System Startup and Operation
Intermittent and continuous operation mode[11] was adopted during the startup process to supplement coke plant wastewater into system. The COD and NH3-N concentration were increased gradually until one half of initial concentration. The *comamonas testosterone* and nutrition were added to reactors. The intermittent operation was conducted for 10 days, and stable operation was achieved. Then, the coke plant wastewater was supplied constantly without nutrition. Meanwhile, influent and effluent was sampled to monitor COD and NH3-N every day. The BF-BFB system was continuously operated with inflow 7L/d. Air was supplied enough in three-phase internal

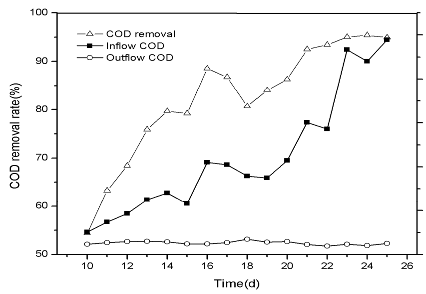
circulation aerobic BFB to make it in fluidized state, and aeration amount was 20L/h. Most effluent water was flowed back, except fresh wastewater injection.

RESULTS AND DISCUSSION

3.1 Removal of COD and NH3-N in Startup Process

During startup period, the efficient bacterium-Comamonas Testosterone was applied to coke plant wastewater with normal sludge in BFB of BF-BFB process, the biofilm was matured gradually after 23 days, and the both of COD and NH3-N removal rate were more than 90%. The experimental results were shown in Fig.2, Fig3.

Though the influent COD concentration was went up from 102.30 to 978.00 mg/L, the COD removal rate increased stably and the removal rate of COD was up to 95.39%. The minimum influent COD concentration was of 37.62mg/L.



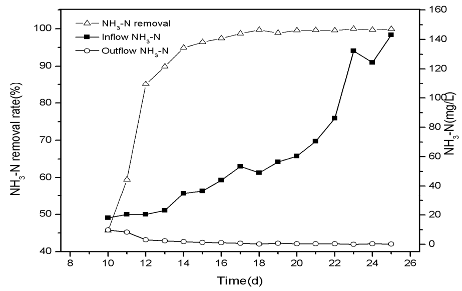


Fig.2 COD removal during startup period **Fig.3 NH3-N removal during startup period**

The performance of NH3-N removal was in steady state after 16 days domestication. The NH3-N removal rate was more than 98.00%, and the best degradation rate was up to 99.52%. Although influent NH3-H concentration was increased, the NH3-N removal efficiency also increased rapidly since 10th day and the minimum influent NH3-H concentration was 0.16mg/L. Therefore, the BF-BFB system had high efficiency on NH3-N removal.

The above results revealed that bioaugmentation technology by inoculating the BF-BFB system with specialized bacteria could improve effect of coke plant wastewater treatment. The system effluent COD and NH3-N concentrations reached the First-degree of National Discharge Standard.

3.2 The Variety of Dissolved Oxygen

Fig.4 shows us that the changes of DO concentration in BF and BFB reactors during the later period of start-up procedure. A decrease of DO concentration was observed in system. Under the basis of DO level (DO>2mg/L in aerobic reactor; DO<1mg/L in micro-aerobic reactor), BF and BFB are in micro-aerobic and aerobic state, respectively.

The oxygen was consumed because of the growth and reproduction of aerobic microbe. The BF reactor was in a closed anaerobic environment, therefore, the DO level was lower and dropped rapidly. The DO in BFB reactors also dropped rapidly, and was from 2.0 mg/L to 4.0 mg/L after start-up procedure.

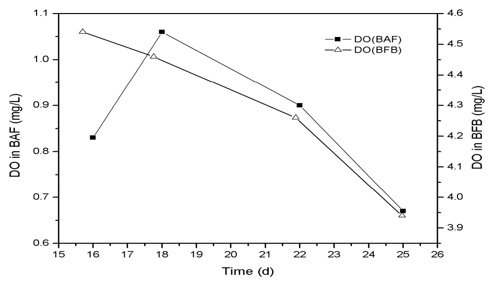


Fig.4 The variation of DO in startup process

3.3 The Dominant Strain and Micro-ecosystem

In stable operation period, abundance of bacterial community, algae and protozoa formed microbe ecological system.

By 16SrRNA and PCR technology, a total of 28 unique OTUs (operation taxonomy unit) were obtained from library. The clone sequence analysis had shown wide diversity of bacteria groups in aerobic sample. β-proteobacteria, α-proteobacteria and γ-proteobacteria were the dominant strains. The Proteobacteria was the largest bacteria group, accounting for 55.00% of the total library. All the OTUs clones were used to construct a phylogenetic tree (Fig.5).

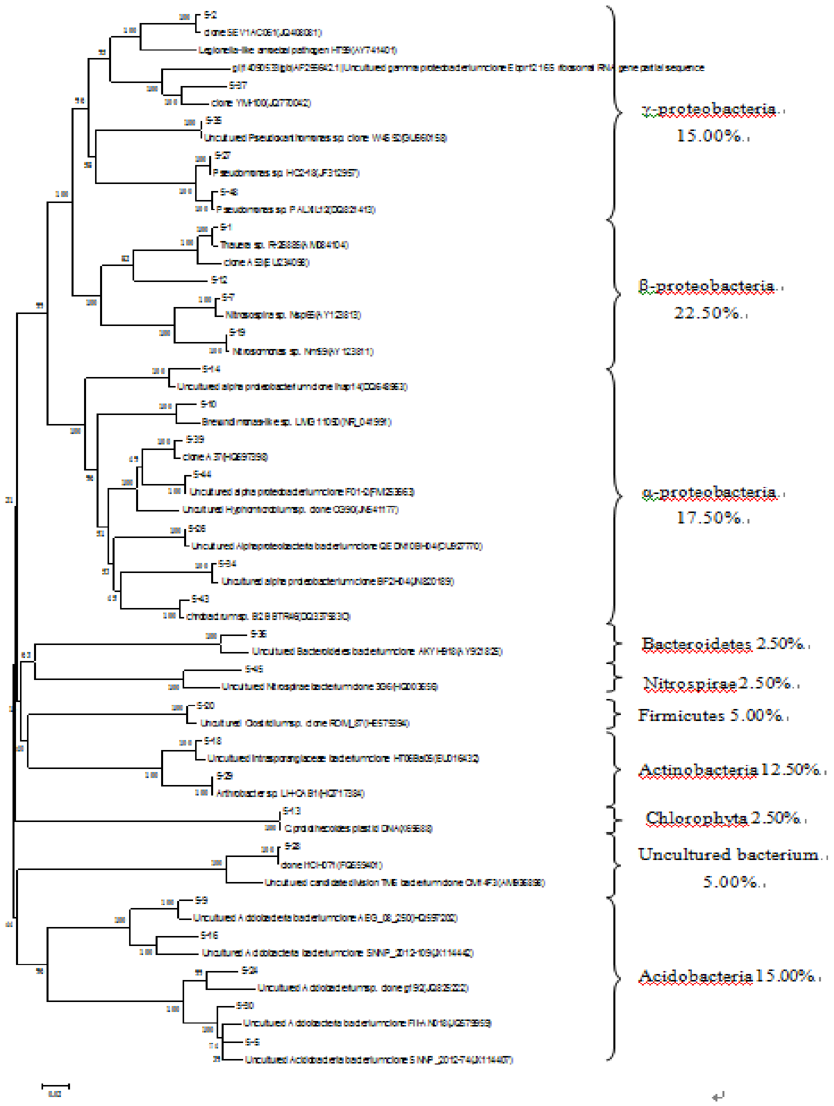


Fig.5 Phylogenetic trees for aerobic sample 16SrRNA sequence

The 28 OTUs belong to ten bacterial groups, β-proteobacteria was the largest population, accounting for 22.50% of total clone library, followed by α-proteobacteria (17.50%), γ-proteobacteria (15.00%), Acidobacteria (15.00%), Actinobacteria (12.50%), Uncultured bacterium (5.00%), Firmicutes (5.00%), Chlorophyta (2.50%), Nitrospirae (2.50%) and Bacteroidetes (2.50%). Several bacterial clones found had shown close affiliation with Thauera sp., and the other main relative species were Arthrobacter sp., Clostridium sp. and Nitrosospira sp.. Many of them involved in pollutant degradation and nitration reaction. Bacteria in Proteobacteria group could degrade high concentration organic pollutants in coke plant wastewater, and other bacteria also had important roles in biodegradation reaction.

In addition to bacteria，micro-organisms in the system were studied. Two types of dominant microorganisms--- algae and arcella in sarcodina were found. The dominant species observed in the biofilm changed as the influent concentration increased during the start up process. In the acclimatization period, filamentous colony mainly constituted the microorganisms in the biofilm (Fig.6 a). As the coke plant wastewater concentration increased, most filamentous organisms disappeared, and various algae were dominant in observation, including Navicula (Fig.6 b), Chlorella (Fig.6 c), Closterium (Fig.6 d).

The major dominant populations of non-bacteria microbe were observed by SEM in system (Fig.7). Navicula, Pinnularia, Closterium and Arcella were also found.

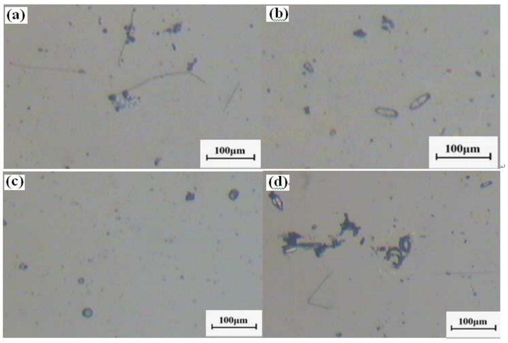


Fig.6 Microscope of microbe during startup process

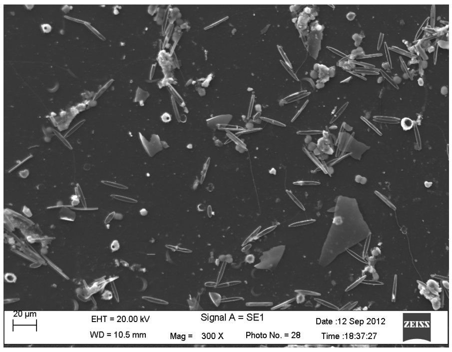


Fig.7 SEM of non-bacteria microbe in stable period

A variety of algae through photosynthesis release oxygen, which is available to bacteria for organic oxidation. Algae could also independently remove nitrogen and organic of coke plant wastewater. Algae played an important role during the biofilm formation process. Arcella belongs to amoebae and camps animal nutrition. The emergence of Arcella was a good sign for wastewater purification. Nitration reaction and organic degradation were promoted indirectly by Arcella, because of them feeding bacteria and protozoan.

The analyses above indicate that algae as producers, molds and protozoa as consumers, and some decomposition microbes were discovered in this system. Finally, a stable microecosystem had been formed by bacteria and non-bacteria microbe, a complete ecological island was formed, which made the system perfectly stable.

CONCLUSION

The experimental results revealed that bioaugmentation by adding comamonas testosterone in coke plant wastewater is very effective when it combined with BF-BFB system for removal of recalcitrant organic compounds.

The system`s startup was very quick and the biofilm was matured in 25 days. Under the optimum parameters, COD concentration could be decreased from 102.30-978.00mg/L mg/L to 37.62 mg/L and NH3-N concentration could be reduced from 18.05-143.05 mg/L to 0.16-2.86 mg/L.

By SEM and gene library technology, the proteobacteria were dominant strains and a stable micro ecosystem was formed ultimately. The results showed that algae as producers, fungi and protozoa as consumers, and decomposition bacteria as decomposers were existed in the system. They made the coking wastewater as its source and formed a more complete food ecological chain and a basic fully functional ecological island in the system, strengthened the digestion of pollutants with good stability.

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