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Application of Bioaugmentation Technology in Cold-Rolling Emulsion Wastewater Treatment and Analysis of Microbial Community

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Abstract. Cold-rolling emulsion wastewater (CREW) is characterized by high oil content, complex organic components and certain toxicity. By traditional physical and chemical methods, it could not be completely degraded at low cost. In order to optimize treatment process of CREW, a highly effective strain (Pseudomonas fluorescens) was isolated and combined with bioaugmentation technology, biofilm and fluidized bed technology to treat CREW. After 78 days of the system operation, the removal rate of COD and oil reached 94% and 97%. COD and oil content of the effluent were stably below 70mg/L and 4.7mg/L, which met the first order of the national discharge standard (GB8978-1996). The result of high throughput 16SrRNA sequencing showed there were 23 genera of bacteria in the biofilm community of the reactor. This microbial community structure could effectively degrade COD and oil in CREW. At phylum level, γ-proteobacteria, Firmicutes and β-proteobacteria widely existed in carrier and were the main members of pollutant degradation.

1. Introduction

With the continuous development of automation technology, more and more mechanical equipment has been used in modern industrial production in China. At the same time, the amount of industrial oil such as lube oil and antiseptic oil has increased greatly, which brought a lot of emulsion wastewater in the process of industrial production[1]. In the steel industry, more than one hundred thousand tons of emulsion wastewater generated during cold rolling each year. As one of the most refractory steel industry wastewater, it will cause potential harm to natural water and human health without proper treatment[2]. A large number of literatures showed that the existing cold rolling emulsion wastewater (CREW) treatment methods are mostly the combination of physical, chemical and biological technologies, such as air-floatation (AF), gas-energy-flocculation (GEF), electrochemical catalytic oxidation (ECO)[3], membrane bioreactor (MBR), ultra-filtration(UF)[4], biochemical treatment[5], etc. In these technologies, biological treatment has become an important research object because of its high efficiency and minimum secondary pollution[6]. Facing the increasingly difficult situation of CREW treatment, this study combined biofilm technology, biological fluidized bed and bioaugmentation technology to treat CREW. During the long time (85 days) operation of the reactor, the treatment effect was monitored, and the microbial community in the reactor was identified and analyzed in order to find the relationship between the biological community and the treatment performance, which provided the theoretical basis for the engineering application.

2. Material and methods

2.1. Wastewater, chemicals and bacterial strain

In this study, cold rolling emulsion wastewater (CREW) and activated sludge came from a cold rolling mill in Tianjin. The quality of pretreated wastewater and emission standard is shown in Table 1. In addition, LB medium (A liter contains 10 g tryptone, 10 g beef extract, and 5 g NaCl) was used for bacterial enrichment. LB medium was mixed with CREW with different ratios as the separation medium (SM) for isolation of high effective bacteria. The initial pH of all the medium was set at 7, and all the media were sterilized at 121 °C for 20 minutes. During the start-up process, KH_2PO_4 were used as phosphorus sources to promote biofilm growth, and the isolated strain was inoculated into activated sludge to enhance the microbial community structure. All chemicals used in the experiment are analytical grade.

Table 1. Wastewater quality and discharge standard.

	COD_{cr} (mg/L)	Oil content (mg/L)	SS (mg/L)	pH
Wastewater	1263	227	536.4	6.49
Emission limit	80	5	50	6 - 9
Detection method	Potassium dichromate method	Infrared spectrophotometry	Gravimetric method	Glass electrode method
National standard	GB/T 11914-1989	GB/T 16488-1996	GB/T 11901-1989	GB/T 6920-1986

2.2. *Experimental set-up*

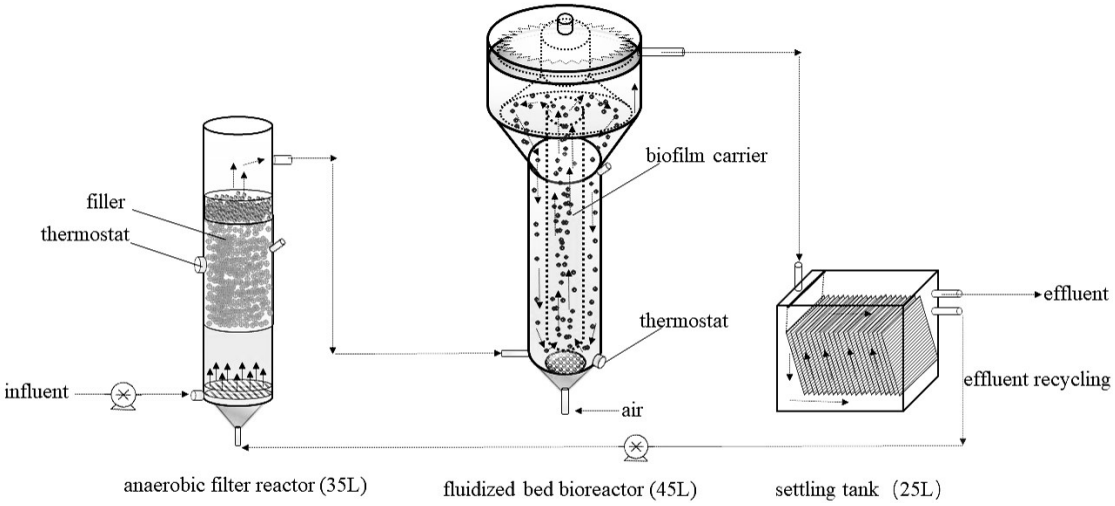

Figure 1. The experiment device structure

The experiment device consists of an up-flow anaerobic filter (AF, 35L), a three-phase fluidized bed reactor (FBR, 45L) and a settling tank (25L). The AF used polystyrene solid particles as microorganism fillers. The three-phase FBR involves the gas-liquid-solid process, where the density of the biofilm carrier was slightly less than the water. The wastewater discharged from the FBR partly returned to the AF for denitrification through settling tank, and each reactor was equipped with a thermostat to adjust the temperature, as shown in Figure 1.

2.3. *Analytical methods*

During the whole experiment, dissolved oxygen (DO), temperature and pH were detected by DO and pH meters, respectively. The determination of COD and oil content was based on the national standard method of China, as shown in Table 1. High efficiency strain was identified by 16SrDNA sequencing technology. At the 600nm standard wavelength, the optical density(OD_{600}) of the bacterial solution was measured by visible spectrophotometer to reflect the growth state of the bacteria. In addition, microbial community analysis of the reactor carrier biofilm was carried out through high-throughput 16SrRNA sequencing technology.

3. Results and discussion

3.1. Strain isolation, identification and characterization

10 ml activated sludge was enriched two times at 30℃ and 130 r/min in LB medium (90 mL). Then, the bacteria suspension (5 ml) was inoculated into the SM medium for five rounds of screening. After that, a consortium with high resistance and excellent biodegradability was selected. By spread plate method, four typical strains (YK1~YK4) were isolated from this consortium. For these strains, we conducted an independent degradation test. The results showed that YK2 strain had high degradation ability (COD:53%, Oil:23%), as shown in Figure 2(a). Compared with GenBank sequence, it has the highest similarity with Pseudomonas fluorescens (99%). In addition, Fig. 2(b) showed the optimum growth pH and temperature of the isolate were 7 and 30℃ respectively. Every experiment was carried out three times.

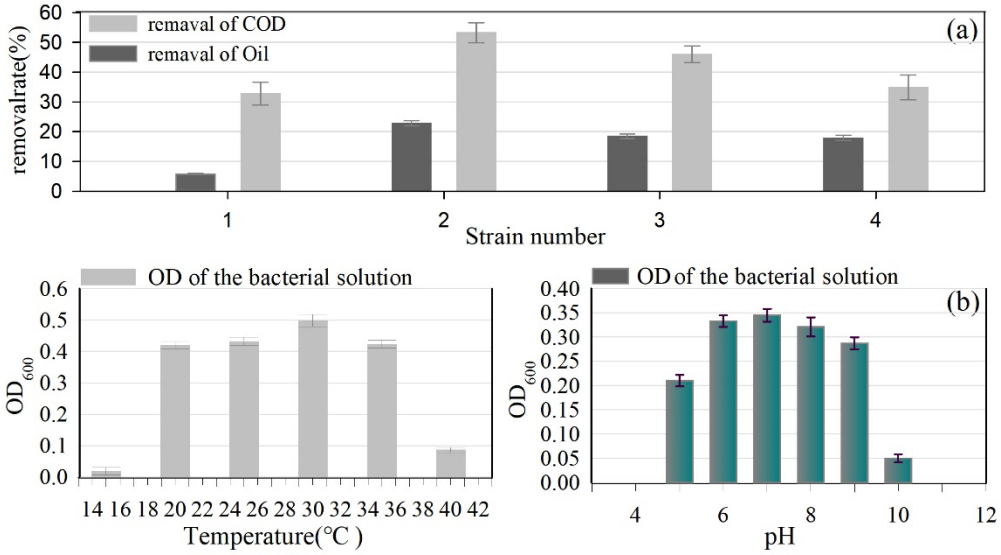

Figure 2. Degradation test of four strains and characterization of the isolate strain
COD and oil removal rate of four strains **(a)**
The optimum pH and temperature of the isolate growth **(b)**

3.2. Treatment effect of COD and oil by AF-FBR reactor

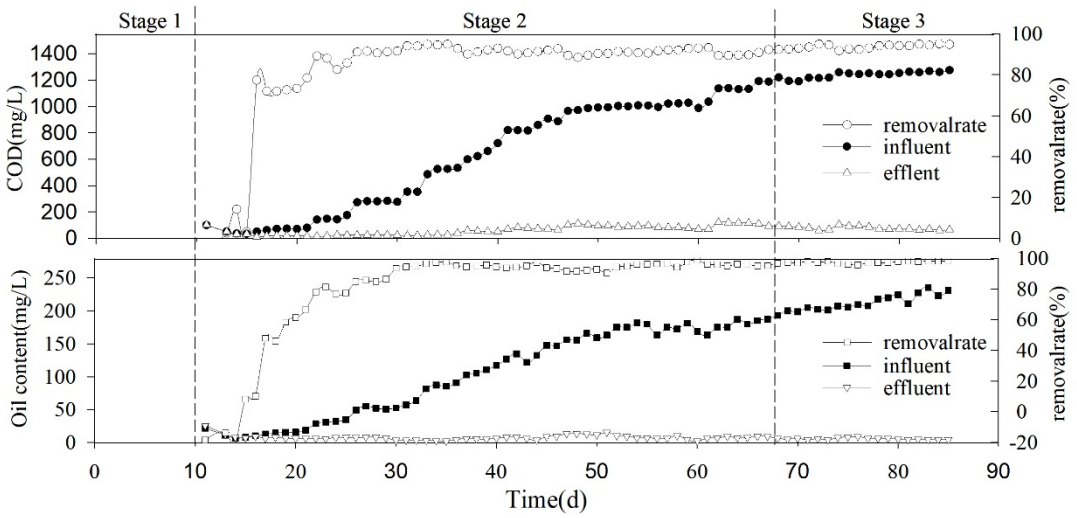

Figure 3. Removal rate of COD and oil

The experiment was divided into three stages, as shown in Figure 3. The first stage (1-10 days) was an independent start-up process of AF and FBR, through which inoculated sludge with high effective strain was adapted to the CREW. The second stage (11-67 days) was a load growth stage, during which the AF-FBR system was connected in series and the influent load gradually increased when the removal rate of COD and oil were separately stable over 80%. 67 days later, the system entered the third stage and started pumping into raw water. The dissolved oxygen (DO) of the AF reactor was kept below 0.3mg/L, and DO in FBR was maintained between 4~6mg/L. Meanwhile, the temperature was kept at 37℃, and the hydraulic retention time (HRT) was adjusted to 24h. After optimizing the operating parameters, the removal rate of COD and oil were stable at 92% and 95%, respectively. After 78 days, the removal rate of COD and oil reached 94% and 97%. COD and oil content of the effluent were stably below 70mg/L and 4.7mg/L, which met the first order of the national discharge standard (GB8978-1996). On 85th day, a small amount of biofilm was taken from AF and FBR carriers for analysis of microbial community.

3.3. Microbial community analysis

The result of high throughput 16SrRNA sequencing showed there were 23 genera of bacteria in the biofilm community of the reactor carrier, among which the AF group mainly included Nitrosospira, Comamonas, Mycobacterium, Sarcina, Micrococcus Cohn, Enterobacter and Methanolinea. The FBR included Pseudomonas pseudoalcaligenes, Pseudomonas, Pseudomonas fluorescens, Alcaligenes and Clostridium. This microbial community structure can degrade many kinds of aromatic compounds and PAHs pollutants[7], so which could effectively degrade COD and oil in CREW. At phylum level, γ -proteobacteria, Firmicutes and β -proteobacteria widely existed in carrier and were the main members of pollutant degradation.

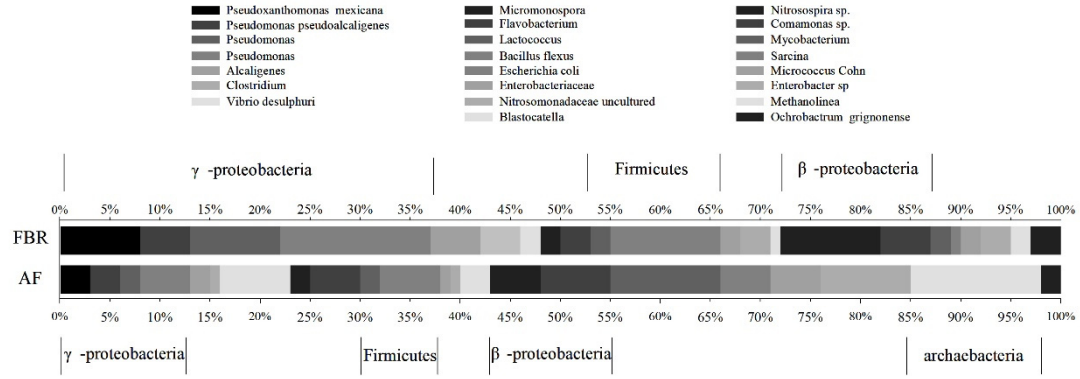

Figure 4. Microbial community of the AF and FBR biofilm

4. Conclusions

A highly effective strain (Pseudomonas fluorescens) was isolated and combined with biological augmentation technology, biofilm and biological fluidized bed technology to treat CREW. After 78 days of the system operation, the COD and oil content of the effluent were stably below 70mg/L and 4.7mg/L, which met the first order of the national discharge standard (GB8978-1996). There were 23 genera of bacteria in the biofilm community of the reactor. This microbial community structure could effectively degrade COD and oil in CREW. At phylum level, γ -proteobacteria, Firmicutes and β -proteobacteria widely existed in carrier and were the main members of pollutant degradation.

Acknowledgments

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