Immobilization of Dominant Bacteria by Polyvinyl Alcohol and Its Effect on COD Removal from Coking Wastewater

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Abstract. Coking wastewater is one of the most difficult wastewater to treat in industry, especially in COD removal. In this study, polyvinyl alcohol was used as carrier to immobilize the dominant bacteria which could efficiently remove COD from coking wastewater. Through single factor experiments and microscopic observation, the optimum immobilization conditions were determined to be 10% PVA concentration and 1% bacterial sludge concentration. Compared sludge containing dominant bacteria with polyvinyl alcohol carrier under optimal conditions, the results showed that polyvinyl alcohol carrier had better COD removal effect than sludge containing dominant bacteria. When the initial COD concentration was 1050 mg/L, the removal rate of COD by carrier arrived 84.23%, while that of sludge arrived 77.46%. In addition, the carrier exhibits better tolerance in treating coking wastewater with high concentration of COD than sludge, and its degradation capacity can continuously maintain more than 70% for a long time.

1. Introduction
Coking wastewater is a kind of industrial wastewater with large discharge and complex composition. It contains not only inorganic substances such as ammonium salt, sulfide and cyanide, but also a large number of refractory organic substances such as aromatic, fused and heterocyclic compounds, which directly threaten the environment as well as human health [1,2]. Compared with the traditional suspension biological method, the immobilized microorganism technology has the advantages of high efficiency, strong stability and good solid-liquid separation effect [5]. In this experiment, PVA material was used to immobilize dominant bacteria, and dilute hydrochloric acid and calcium carbonate reacted to produce bubbles to increase the porosity of the material. The degradation of COD in Coking Wastewater by carrier under different immobilization conditions was investigated. The advantages of this carrier were analyzed on the aspects of structure, degradation of organic matter and biological community.

2. Experiment and Methods

2.1. Experimental Content

2.1.1 Embedded mud culture. Beta-proteobacteria, a predominant bacterial strain which can degrade COD in coking wastewater, was isolated and screened in the laboratory and enriched in beef extract peptone medium. And centrifuge it. Rinse with PBS buffer solution and centrifuge again. Store the sludge in a refrigerator of 4 degrees. The sludge from the aerobic tank of the coking plant is domesticated, and the domesticated water is the effluent from the regulating tank of the coking plant (the water quality is shown in Table 1). The domesticated sludge was mixed with dominant bacteria at
a mass ratio of 1:5 to make mixed bacterial sludge. The mixed bacterial sludge was then stored in a refrigerator for use in preparing polyvinyl alcohol carrier.

Table 1. Coking wastewater quality.

| Item     | COD (mg/L) | NH₄-N (mg/L) | SS (mg/L) | pH       |
|----------|------------|--------------|-----------|----------|
| Index    | 2100       | 195          | 114.8     | 7.5-8.2  |

2.1.2 Preparation of polyvinyl alcohol carrier. The 1799 polyvinyl alcohol (PVA), 1g CaCO₃ and 95ml deionized water were mixed. The water was heated in a water bath at 85 degrees. When the PVA was completely dissolved, remove it from the water bath. After the PVA solution is slightly cooled, 5 ml hydrochloric acid solution with 5% mass fraction is added to the PVA solution and stirred rapidly to make CaCO₃ react with hydrochloric acid solution as fully as possible, resulting in a large number of bubbles, thus increasing the porosity of the carrier. When the PVA solution is cooled to about 30 degrees, the mixed bacterial is added to the PVA solution. After mixing, the solution was poured into a silica gel ice lattice of 1cm*1cm*1cm, frozen for 20h at -20 degrees, then removed, thawed for 4 h at room temperature, and the carrier was successfully prepared. The flow chart is shown in Figure 1.

![Figure 1. Preparation process of polyvinyl alcohol carrier.](image)

2.1.3 Optimization of immobilization conditions. By single factor experiment, PVA carriers with 5%, 8%, 10%, 12% and 15% concentration were prepared. Meanwhile, polyvinyl alcohol carriers containing 0.5g, 1g, 1.5g and 2g of mixed bacterial sludge were prepared. The optimum immobilization conditions were determined according to the degradability of the carrier for COD in coking wastewater and the analysis of the internal structure of the carrier.

2.1.4 Species and content distribution of microflora. The optimum immobilization conditions were used to prepare the carrier, which was added into the conical bottle according to the volume ratio of the carrier to the coking wastewater of 1:4. Two conical bottles were also taken, one containing the same amount of pure sludge as the encapsulated sludge, and the other containing the mixed bacterial. The amount of coking wastewater added in the above three conical bottles is the same. Then three conical bottles were put into a constant temperature shaker, and the supernatant of water samples was taken every 24 hours to measure its COD, and the supernatant was replaced.

In the later stage of the reaction, sludge was derived from the conical bottle with only carrier added. Some carriers, some sludge samples in the bottle and some mixed bacterial sludge were taken. Samples were sent to observe the species and content distribution of the flora.

2.2. Analysis Method

During the experiment, COD was detected by rapid digestion spectrophotometry, the internal structure of carrier was observed by SEM, and the species and content distribution of bacteria were observed by high throughput sequencing technology.
3. Results and Discussion

3.1. Effect of Different Immobilization Conditions on COD Degradation

Coking wastewater with initial COD of 700 mg/L was treated with PVA carriers under different optimum conditions. Water samples were taken every 24 hours and supernatant was replaced. After the quality of effluent is stable, COD values of water samples are measured and averaged for 3 consecutive days. The data obtained are shown in Figure 2.

The results showed that when the concentration of PVA was 5%, 8% and 10%, the removal rate of COD by carrier was higher than 77%, and the removal rate of COD by carrier with 10% PVA reached 81.23%. The removal rate of COD decreases with the increase of PVA concentration in the range of 10% to 15%. This is because the carrier formed by high concentration of PVA has high strength and density, which is not conducive to the mass transfer of nutrients and metabolites. The optimum embedding amount of mixed bacterial sludge showed that when the amount of bacterial sludge was 1 g, the removal rate of COD by carrier was the highest, reaching 77.34%. The amount of bacterial sludge is small, which leads to low bacterial content, and the rate of degradation of organic matter is slow; the amount of bacterial sludge is large, which leads to a large number of bacteria in the carrier, and their growth and reproduction lead to a high competitive pressure[6]. Therefore, the optimum dosage of mixed bacterial sludge is 1 g. Because the removal rate of COD is higher when the concentration of PVA is 5%, 8% and 10%, the optimum concentration of PVA will be screened out by SEM in the next experiment.

3.2. Effect of Different Immobilization Conditions on Carrier’s Structure

The fresh carriers with PVA concentration of 5%, 8% and 10% were sliced. After pretreatment and spraying, the sections were observed by SEM. The SEM images are shown in Figure 3 below.
SEM images show that pore exists in the carrier with different concentration, but the pore size is different. With the increase of PVA concentration, the pore diameter in the carrier decreases and the number of pore increases. The pore size is too large. In the reaction process, although the carrier's mass transfer performance is enhanced, the bacteria inside the carrier may also be lost. If the pore size is too small, the mass transfer performance of bacteria and wastewater in the carrier is not strong, which will also affect the treatment effect of COD\cite{7}. The dominant bacterium used in this experiment is Gram-negative bacilli. When the concentration of PVA is 10%, the bacilli in the pore can be clearly seen in the electron microscope picture. It can be seen that when the concentration of PVA is 10%, the dominant bacteria can be well fixed in the pore. Therefore, the optimal concentration of PVA was determined to be 10%.

### 3.3. Comparison of COD Degradation Ability of Carrier, Pure Mud and Mixed Bacteria Mud

The data obtained are shown in Figure 4.

![Comparison of COD degradation ability of carrier, pure mud and mixed bacteria mud.](image)

From Figure 4, it can be seen that the COD degradation ability of mixed sludge is higher than that of pure sludge solution, and the COD removal efficiency of mixed sludge is increased by about 20%. When the initial COD is 700 mg/L, the COD degradation ability of mixed bacterial sludge and carrier has little difference. When the initial COD is 1050 mg/L, the COD degradation ability of mixed bacterial sludge decreases, and carrier can still maintain high degradation ability. The COD removal rate can reach 84.23%. When the initial COD was 1400 mg/L, the carrier showed a certain tolerance: the removal rate of COD by mixed bacterial sludge decreased to 61.03%, while the removal rate of
COD by carrier remained above 70%. This is because, compared with the mixed sludge, the carrier can encapsulate bacteria in it. On the one hand, it can prevent the loss of sludge. On the other hand, it can prevent harmful substances in wastewater from poisoning bacteria and reduce bacterial activity[3,4].

3.4. Species and Content Distribution of Microflora
Fig. 5 shows the species and content of bacteria obtained by high throughput sequencing. A represents the sludge mixed with dominant bacteria, B represents the sludge derived from the carrier, and C represents the carrier.

Figure 5 shows that the dominant bacteria accounted for 62.93% of the total bacterial flora in the carrier, and its dominant bacteria content was 4.68 times that of the dominant bacteria in the mixed sludge. This is because the porous structure inside the carrier is conducive to the attachment of dominant bacteria, and dominant bacteria are in a favorable position for growth competition. Under sufficient nutrients conditions, dominant bacteria can get a large number of proliferation[4]. This is the main reason why the carrier’s ability to degrade COD is better than the other two groups. The dominant bacteria accounted for 0.01% of the total bacterial flora in the sludge derived from outside the carrier. It can be seen that with the reaction proceeding, the dominant bacteria embedded in the carrier would hardly be lost. The content of Thiobacillus in the derived sludge reaches 72.05%, because it is a common strain in wastewater. Without other competitive bacteria, Thiobacillus is in the growth advantage and has a large number of proliferation.

4. Conclusion
Polyvinyl alcohol carrier materials with perfect pore structure can be obtained by reaction of dilute hydrochloric acid and calcium carbonate. Polyvinyl alcohol immobilized dominant bacteria, through the performance of carrier to remove COD from coking wastewater and the observation of the carrier’s microstructure, the optimum immobilization conditions were determined: the concentration of PVA was 10%, and the amount of mixed bacterial sludge was 1 g.

Compared with pure sludge, mixed bacterial sludge containing dominant bacteria and carrier under optimal immobilization conditions, the removal efficiency of COD from coking wastewater was the highest, up to 84.23%, followed by mixed bacterial sludge, up to 77.38%, and the removal efficiency of pure sludge was the lowest, only 54.48%. In addition, compared with the mixed bacterial sludge, the embedding carrier showed a certain tolerance to coking wastewater with high concentration of COD: COD removal rate can still reach more than 70%.

5. Acknowledgments
This work was supported by the National Natural Science Foundations of China (No. 51174031) and Major Science and Technology Program for Water Pollution Control and Treatment (Grant No. 2017X07402001).
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