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Study on in situ Bioremediation of Cr(VI) in Porous Media

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Abstract: Indigenous microorganism that show an ability for Cr (VI) reduction were isolated from a chromium-contaminated sediment. Sand column experiments were conducted using the isolated bacteria to investigate microorganism effects on Cr(VI) reduction in closed systems that simulated subsurface conditions. The results showed that when glucose and emulsified oil were used as carbon sources, the effective reduction of Cr(VI) can be achieved, and the reduction rate of Cr(VI) increases with the increase of carbon source. The reduction rate of Cr(VI) decreases with the increase of initial Cr(VI) concentration, which is consistent with the first-order reaction kinetics. The reaction rate constant decreases with the increase of initial Cr(VI) concentration. The reduction rate of Cr(VI) increased with the increase of the hydraulic residence time, that is, the emulsified oil has relatively high stability and low biodegradation rate, and can provide carbon source in the bioreduction process for a long time.

Keywords: Indigenous Microorganism; Cr(VI) Reduction; Column Experiment

Introduction

China is a major producer of chromium salt. According to statistics, it has produced 7 million tons of chromium slag, and more than 4 million tons of chromium slag have been discharged into the environment without reasonable treatment, causing serious environmental pollution and posing a serious threat to human health[1]. In the 45 environment, there are many valence states of 0 to +6, of which only the compounds of Cr(III) and Cr(VI) can exist stably in the environment, but the biological, geochemical and toxicological properties of the compounds of the two valence states are significantly different[2]. Cr(III) mainly exists in the form of Cr (OH) $_{2+}$,Cr $(OH)_{2+}$,$Cr(OH)_3$,$Cr(OH)_{4-}$,$Cr(OH)_{52-}$,etc. Its chemical compound has low bioavailability, mobility and solubility. When the pH in the water body is between 6-10, the Cr(III) is easy to hydrolyze into Cr $(OH)_3$ precipitate and separate. Therefore, its solubility, mobility and bioavailability are low, and its environmental risks are small.

The 50 exists in the form of $cro_{42-}$and Cr_2 $o_{72-}$and can accumulate in organisms, participate in the redox process in cells, and interfere with small amounts.

Normal life activities of cells. Cr(VI) compounds have carcinogenic, teratogenic and mutagenic effects and belong to a class of pollutants[3, 4]. Based on the difference of chemical properties between trivalent chromium and hexavalent chromium, the main idea of chromium pollution treatment is to change the presence of chromium in polluted environment form, transforming Cr(VI) with high toxicity and strong mobility into Cr(III) with low toxicity and poor mobility. At present, the remediation methods of heavy metal chromium pollution mainly include chemical remediation, physical remediation and bioremediation. Among them, microbial remediation method has the advantages of mild

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reaction, low operation cost, little disturbance to the environment, no secondary pollution, etc. It has received extensive attention and achieved rapid development[5].

Microbial remediation refers to a remediation method that uses indigenous microorganisms in the natural environment or adds specific exogenous micro-organisms to realize the transformation, degradation and removal of pollutants in the environment through their metabolic activities under artificially enhanced conditions [6]. At present, the microorganisms with Cr(VI) and reducing effect have been found to be Bacillus SP.[7,8], Arthrobacter SP.[9,10] and Ochrobactrum sp[11], Pseudomonas sp[12, 13], Shewanella sp.[14, 15], Desulfovibrio sp.[16], Arthrobacter sp[17, 18].

At present, the research on microbial remediation of chromium pollution in water environment is generally carried out under the conditions of sequencing batch experiments, but the experimental conditions of sequencing batch experiments are far from the actual environmental conditions, and the obtained experimental results cannot effectively reflect the reduction effect of microorganisms on Cr(VI) in actual fields. The migration and transformation of Cr(VI) in underground water is a highly dynamic process, and [19, 20] cannot be defined through simple sequencing batch experiments. Therefore, this experiment selects indigenous microorganisms that have been screened and domesticated in chromium contaminated sites. It uses one-dimensional flow field sand column experiment to simulate the migration and transformation process of Cr(VI) in groundwater environment, studies the influence of carbon source concentration, initial Cr(VI) concentration, hydraulic retention time and other factors on microbial reduction process, and further clarifies the feasibility of indigenous microbial reduction of Cr(VI) in actual field application.

1. Materials and methods

1.1 Experimental materials

The soil sample is taken from the surface soil (0-50cm) of a chromium slag yard in Changsha city, Hunan province. using the square method of random distribution, select 5 points near the chromium slag yard, and then take a mixed sample of about 1kg after mixing the soil samples evenly. Put it in a cowhide bag and bring it back to the laboratory. Take out the soil sample, remove the crushed stone, and then store it in a refrigerator at 4 C. It can be used as a micro-biological source for Cr(VI) reduction to screen chromium reducing bacteria.

1.2 Isolation, screening and enrichment of chromium reducing bacteria

Isolation and screening of chromium reducing bacteria: weighing 5g soil sample in a 100mL conical flask, placing 50mL of liquid culture medium which has been sterilized 85 bacteria at high temperature and cooled, and then culturing in a constant temperature oscillating incubator at 25 deg c and rotating speed of 150r/min for 24h to obtain bacteria suspension. Subsequently, 5mL of the bacterial suspension was taken and cultured in a liquid medium with Cr(VI) concentration of 43mg/L.

1.3 Sand column experiment

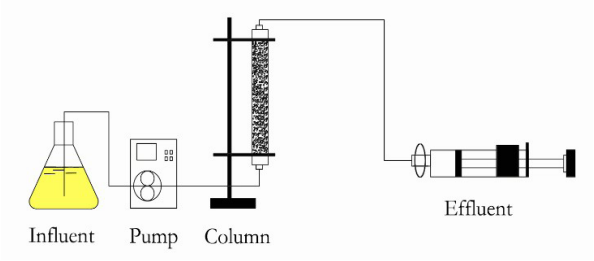

Figure 1. Schematic diagram of the column experimental setup.

The sand column experiment device is shown in figure 1. the column specification is φ4.0cm×15.0cm. distributors and stainless steel are installed at both ends of the column. Filter element to ensure water distribution uniformity. The filling medium in the column is natural river sand (40-50 mesh), and is compacted while adding river sand. After the column is filled, CO2 gas 30min is injected into the sand column from bottom to top to remove residual air in the column. Sand column experimental phase.

After the column loading is completed, a high performance liquid chromatography pump is connected, and AGW

solution after heating, degassing and cooling is introduced into the quartz sand column from bottom to top until the column keeps constant weight, and then the sand column reaches saturation. Subsequently, pentafluorobenzoic acid was introduced. PFBA is used as a conservative tracer and its penetration curve is drawn to verify the homogeneity of the sand column.

Subsequently, the bacteria suspension was circulated at a flow rate of 0.05mL/min for 14 days to enable microorganisms to grow and reproduce in a sand column uniform adhesion in the column forms biofilm. After the domestication in the microbial column is completed, under the condition of not adding carbon source, the method comprises the following steps of the Cr(VI) solution of 8.6mg/L was introduced at the flow rate of 0.05mL/min, and the Cr(VI) concentration of inflow and outflow was determined. It is true that determine whether there is adsorption of Cr(VI) by microorganisms and whether endogenous respiration of microorganisms can realize Cr(VI).

Influence of carbon source types on microbial reduction effect: the experiment was conducted in two groups. one group selected glucose as carbon source, and set initial Cr(VI) concentration 4.3mg/L and flow rate 0.05mL/min. the glucose concentration was 3, 8 and 13g/L in turn. the concentration of Cr(VI) in influent and effluent was measured. The other group chose emulsified oil as carbon source, and set the initial Cr(VI) concentration 4.3mg/L and flow rate 0.05mL/min. The emulsified oil concentration was 3, 8 and 13g/L in turn. The concentration of Cr(VI) in influent and effluent was measured.

Influence of initial Cr(VI) concentration on microbial reduction effect: set flow rate 0.05mL/min, emulsified oil concentration 13g/L, the initial Cr(VI) concentration was 4.3mg/L, 8.6mg/L and 21.5mg/L in sequence, and the Cr(VI) concentration in inflow and outflow was measured. Influence of hydraulic retention time on microbial reduction effect: set emulsified oil concentration 13g/L and initial Cr(VI) concentration as follows 8.6mg/L with flow rates set to 0.05mL/min, 0.02mL/min and 0.01mL/min in sequence. The concentration of Cr(VI) in influent and effluent was measured.

Each group of experiments were carried out according to the following steps: before each group of experiments was started, a AGW solution of 2PV was introduced to remove non-attached microorganisms in the column and pre-oxidation solution in the column. Then 4 PV of experimental solution are introduced at a flow rate of 0.05mL/min, and then 2~3 of simulated groundwater solution are introduced at the same flow rate. In the experiment, the introduction time of chromium-containing wastewater was taken as the 0 moment at the beginning of the experiment, and the hydraulic retention time was 24h at the flow rate of 0.05ml/L. Samples were taken every 24h and 5000r/min were separated from the center 10min, and then passed through a 0.22μm filter membrane to measure the Cr(VI) concentration of influent and effluent.

2. Results and discussions

2.1 Penetration curves of PFBA and Cr(VI)

Conservative tracer refers to the substance that is consistent with the migration rule of groundwater in the aquifer. It is only subjected to thrust flow and hydraulic dispersion and does not react in the groundwater environment. The influence of different factors on the migration of pollutants in groundwater is shown in the penetration curve in Figure 2. curve a indicates the ratio of initial concentration of pollutants to measured concentration in groundwater c/c 0 = 1.

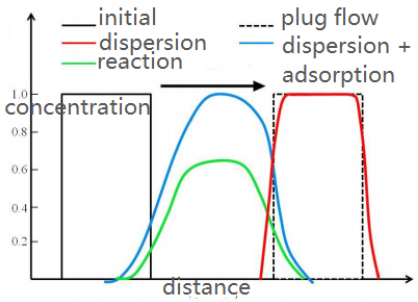

Figure 2. Schematic representation of contaminant in groundwater.

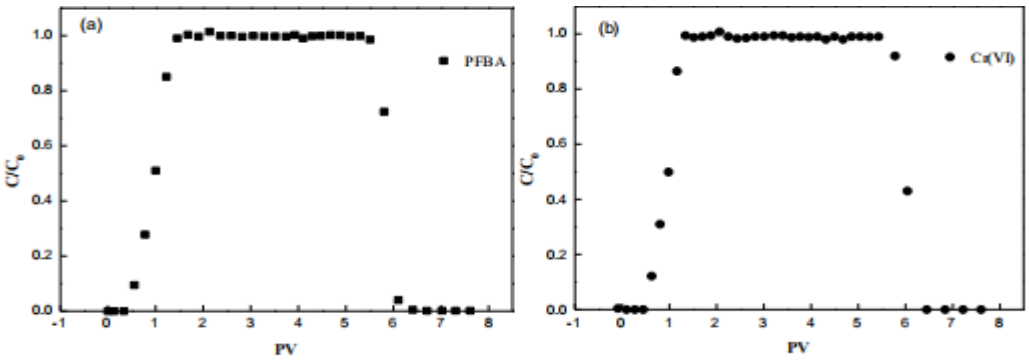

Figure 3. (a) Breakthrough curve of PFBA - (b) Breakthrough curve of Cr(VI) in the absence of carbon source.

The penetration curve of PFBA is shown in Figure 3(a). the relative concentration of PFBA solution in the effluent (the ratio of the concentration of PFBA in the effluent to the concentration of PFBA in the influent) rises rapidly. after about one PV, the relative concentration c/c 0 ≈ 0.5 of the effluent, and then the penetration curve continues to rise until the platform reaches 1 (c/c 0 = 1) and remains unchanged. After 4 PV's, the column was flushed with AGW solution, and the concentration of PFBA in the effluent was detected to rapidly decrease to zero. The penetration curve shows that there is no hysteresis and tailing phenomenon in the whole transmission process of the non-reactive tracer, i.e. the quartz sand in the column is evenly filled, and the sand column is used for experiment. In the absence of the source, the concentration of Cr(VI) in the effluent did not significantly decrease, and the platform of the breakthrough curve reached 0.99, indicating that microorganisms could not effectively reduce Cr(VI) in the absence of carbon source.

2.2 Effect of carbon source concentration on microbial reduction

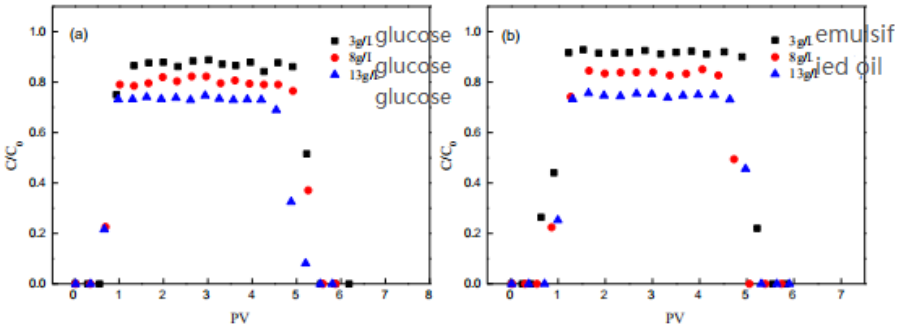

Figure 4. (a) Reduction of Cr(VI) by different concentrations of glucose; (b) Reduction of Cr(VI) by different concentrations of emulsified oil

An electron donor is required to provide electrons. Carbon source provides electron donor for Cr(VI) bioreduction, which is a very important influencing factor in the process of Cr(VI) bioreduction. The reduction effects of glucose and emulsified oil as carbon sources were compared, and the results are shown in Figure 4. When glucose is selected as carbon source, the reduction effect of microorganism increases with the increase of glucose concentration. when glucose concentration is 13g/L, the penetration curve platform of Cr(VI) reaches 0.73 after 24 hours of bioreduction. After 24h biological reduction, the reduction rate of Cr(VI) reached 27%.

The effect increases with the increase of emulsified oil concentration. When the concentration of emulsified oil is 13g/L, the platform of penetration curve is 0.74, i.e. the reduction rate of Cr(VI) reaches 26% after 24h biological reduction. The commonly used electron donor is due to the fact that glucose can be directly decomposed and metabolized into pyruvic acid through glycolysis and enters TCA Cycle. Other studies have shown that Bacillus sp has the best reduction effect[22] when glucose is used as an electron donor.

The oil is formed by mixing edible soybean oil, surfactant Tween-80 and water, wherein the main component triglyceride is converted into hydrogen and unsaturated fatty acid through anaerobic fermentation to further form glycerol and fatty acid dissolved in water, and then the glycerol is decomposed into 1, 3-propylene glycol and further degraded into acetic acid. Emulsified oil is superior to glucose due to its slow release function as a carbon source. The

potential is that not only microorganisms can provide carbon sources, but also electron donors can be continuously provided to realize the reduction of Cr(VI).

2.3 Effect of initial Cr(VI) concentration on microbial reduction

The experimental results of the effect of different initial Cr(VI) concentrations on microbial reduction are shown in Figure 5. The breakthrough curve shows that when the initial concentration of Cr(VI) is 4.3mg/L, the platform of the Cr(VI) breakthrough curve is stable at 0.74, that is, after 24h biorereduction reaction, the reduction rate of Cr(VI) is 26%, and the reduction amount is 1.118 mg ; When the initial concentration of Cr(VI) is 8.6mg/L, the platform of Cr(VI) penetration curve is stable at 0.82, i.e. after 24h bioreduction reaction, the reduction rate of Cr(VI) is 18%, and the reduction amount is 1.548 mg; When the initial concentration of Cr(VI) is 21.5mg/L, the platform of the Cr(VI) penetration curve is stable at 0.90, i.e. after 24h bioreduction reaction, the reduction rate of Cr(VI) is 10%, and the reduction amount is 2.15mg. The experimental results show that the microorganisms in the column can realize the reduction of Cr(VI) between the concentration of Cr(VI) 4.3~21.5mg/L, and the reduction rate of Cr(VI) decreases with the increase of the initial concentration of Cr(VI).

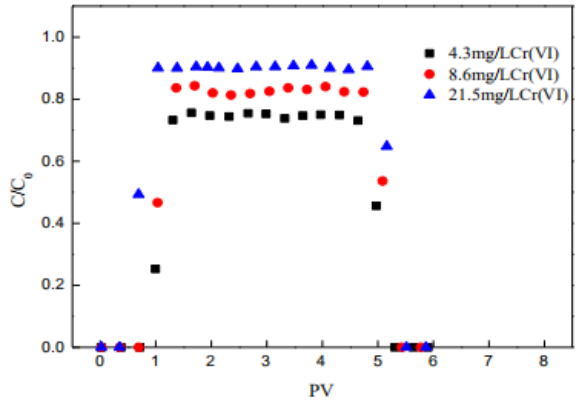

Figure 5. Effect of different initial Cr(VI) concentration on Cr(VI) bioreduction.

2.4 Effect of hydraulic retention time on microbial reduction

The time sequence is 24, 60 and 120h. According to the penetration curve in the figure, when the hydraulic retention time is 24h, after 24h bioreduction reaction, the platform of Cr(VI) is 0.82, i.e. the reduction rate is 18%; When the hydraulic retention time is 60h, the platform of Cr(VI) is 0.69, that is, the reduction rate is 31%; When the hydraulic retention time is 120h, the platform of Cr(VI) is 0.54, that is, the reduction rate is 46%. The results show that the reduction rate of Cr(VI) increases with the increase of hydraulic retention time.

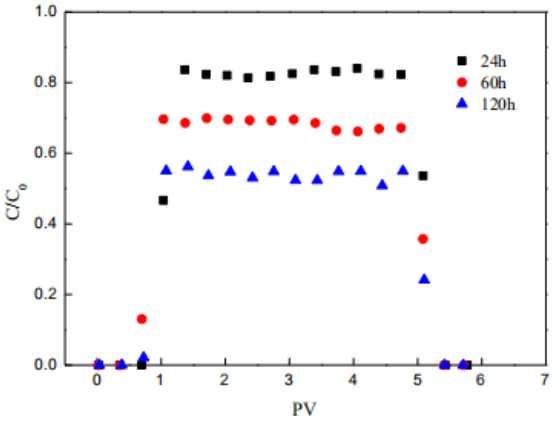

Figure 6. Effect of hydraulic residence time on Cr(VI) bioreduction

3. Conclusion

Glucose and emulsified oil as carbon sources can enhance the reduction of Cr(VI) by indigenous microorganisms, and the reduction rate increases with the increase of carbon source concentration. The reduction rate of Cr(VI)

decreases with the increase of initial concentration of Cr(VI), the reduction process conforms to the first-order reaction kinetics, and the reaction rate constant decreases with the increase of initial concentration of Cr(VI). Emulsified oil has slow release property, can continuously release electron donors, and can be completely degraded by indigenous microorganisms at the same time, thus having good application prospect in in-situ biological process of chromium pollution.

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