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Research Article

Experimental Study on Bioclogging in Porous Media during the Radioactive Effluent Percolation

Rong Gui,[1,2,3] **Yu-xiang Pan**,[2,3] **De-xin Ding**,[1,2,3] **Yong Liu**,[2,3] **and Zhi-jun Zhang**[2,3]

[1]*School of Resources and Safety Engineering, Central South University, Changsha, Hunan 420083, China*
[2]*Key Discipline Laboratory for National Defense for Biotechnology in Uranium Mining and Hydrometallurgy, University of South China, Hengyang, Hunan 421001, China*
[3]*Nuclear Resource Engineering College, University of South China, Hengyang, Hunan 421001, China*

Correspondence should be addressed to De-xin Ding; dingdxzzz@163.com and Zhi-jun Zhang; zzj181@163.com

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The sand columns inoculated with the indigenous microorganism (*Aspergillus niger*) were used to investigate the effect of bioclogging during the radioactive effluent percolation. The hydraulic gradient, volumetric flow rate, and uranyl ions concentration were monitored over time. The sand columns were operated with continuous radioactive effluent of uranium tailings reservoir. After 68 days, the hydraulic conductivity of the sand columns decreased more than 72%, and the adsorption rate of uranyl ions by *Aspergillus niger* reached more than 90%. Environmental scanning electron microscope imaging confirmed the biofilm covering the surface of sand particles and connecting sand particles together, which resulted in a reduction of hydraulic conductivity. The results indicated that the propagation of *Aspergillus niger* can clog the seepage channel and effectively adsorb the uranyl ions of radioactive effluent in the porous media, which provides a suitable measure for controlling the migration of radioactive effluent of uranium tailings reservoir into the subsurface environment.

1. Introduction

With the increasing demand of nuclear fuel, hundreds of uranium tailings reservoirs were built to store the radioactive nuclear wastes which were produced during the mineral extraction. Since the tailings reservoirs were built to be decommissioned for a long time, the tailings reservoirs could be leaked due to the failure of engineering barriers, geological changes, earthquakes, and so on. If the leakage was not treated promptly, radioactive effluent could contaminate the groundwater and damage the eco-environment, ever be accumulated along the food chain and cause damage to the human body [1–5].

Due to the particularity of the radioactive effluent, traditional technologies encounter some obstacles such as the high cost, difficult to find the sources of leakage, low efficiency, and so on [6]. The bioclogging technology has the advantages of detecting and sealing the sources of leakage automatically by inoculating the microorganism and nutrient into porous media, which can reduce the permeability of porous medium and adsorb pollutants by growth, reproduction, and death of microorganisms [7–12]. And it is suitable for complex environments.

Laboratory experiments and numerical simulations were carried out to research the phenomenon of bioclogging in recent years. Samso et al. analyzed the effects of bioclogging on the fluid dynamics of porous media in constructed wetlands through a standalone mathematical model [13]. Surasani et al. performed the water flooding numerical test based on a multicomponent reaction transport model to quantify the effect of bioclogging by injecting L. mesenteroides into a highly permeable formation [14]. Newcomer et al. established a one-dimensional numerical representation to study the effect of bioclogging process on seepage [15]. Hand et al. studied the effects of bioclogging process on hydraulic properties in porous media with different particle sizes under different organic carbon concentrations and oxygen content [16]. Pintelon et al. proposed a 3D biofilm

model considering the permeability of biofilm to study the effect of bioclogging on hydraulic conductivity in porous media [11]. Ben Rajeb et al. analyzed the generation of bioclogging and its effect on wastewater purification during the treatment of leachate wastewater by one-dimensional sand column experiment [17]. Vogt et al. used the magnetic resonance technology to analyze the effect of bioclogging process on seepage dynamics in porous media [18]. Caruso et al. investigated the blockade effect of hyporheic fluxes by bioclogging through modeling of aquatic biogeochemical models [19]. Ham et al. established a mathematical model of bioclogging in saturated porous media based on macroscopic methods to investigate the effects of biomass growth and attachment on permeability and porosity [20]. Berlin et al. studied the migration of nitrogen in the unsaturated porous media under the bioclogging process and found that the transport of *ammonium nitrogen* and *nitrate nitrogen* was significantly delayed [21]. Soleimani et al. used two-dimensional unsaturated water flow and transport models to analyze the effects of soil hydraulic properties and porosity on bioclogging mechanisms in unsaturated soils [22]. Thullner et al. found that bioclogging can reduce the permeability of porous media by 2-3 orders of magnitude by computational network simulations [23]. Ye et al. measured the bioclogging level of the anaerobic methanol-supply benchscale sand in a two-dimensional seepage field and used Taylor's model, Vandevivere's model, Seki's model, and Clement's model to simulate the process, respectively [24]. Thullner compared and analyzed the experimental results of bioclogging in one-dimensional and two-dimensional flow fields and simulated the results under different porosity [25]. The above researches indicated that the growth of microbial colonies and biofilm contributes to the porosity reduction and the reduction of hydraulic conductivity and leads to bioclogging.

The sand columns inoculated with *Aspergillus niger* were employed in this study to investigate the effects of bioclogging under constant percolation (radioactive effluent). This paper aims to explain the phenomena and mechanisms of bioclogging during the process of percolation and to provide experimental evidence for dealing with the leakage problem of uranium tailings reservoir with microbial technology.

2. Microbial Breeding

2.1. Strains. Five kinds of indigenous microorganisms (three bacteria and two fungi) for the experiment were from the surrounding of uranium tailings reservoir. The strains were saved in the ultralow temperature freezer and registered as *A*1, *A*2, *A*3, *A*4, and *A*5, respectively, before identification.

2.2. Experimental Method. The strains were inoculated into a test tube containing 10 mL liquid medium (the beef extract-peptone medium for bacteria, the potatoes medium for fungi) with a sterilized loop. Then the test tubes were put in a constant-temperature shaking incubator which was set at 30°C for 4 days. The culture supernatant was taken by the sterilized loop and performed streak inoculation on the solid medium plates, and then the inoculated plates were put in a constant-temperature incubator which was set to 30°C for 3 days until the spores were generated on the top of the mycelium.

The spores were scraped with a sterilized cotton swab and were eluted in a centrifuge tube of 2 mL liquid medium in a clean workbench. After shaking the centrifuge tube for 5 minutes, the mycelium was filtered to get the bacterial liquid, and the OD600 value of bacterial liquid was measured with the Protein Nucleic Acid Analyzer and was diluted to 1.0 with liquid medium.

In order to eliminate the interference of other microorganisms, the wrapped flask with 50 mL radioactive effluent was placed in an autoclave which was set to 121°C for 30 minutes, then 50 mL liquid medium was poured into the wrapped flask and was inoculated 1 ml bacterial liquid (OD600 = 1.0). The wrapped flask was put in a constant-temperature air-bath shaker incubator which was set to 30°C for 2 days, the rotation speed was set to 200 r/min, and the propagation of microorganisms was observed.

2.3. Selection of Microorganisms. The OD600 values of bacterial liquid of the five microorganisms are shown in Table 1. We can see that the OD600 value of *A*5 is higher than others, which means *A*5 could quickly form the obvious colonies during the propagation. Considering that the percolation velocity in sand columns is fast, the microorganisms can easily washed away, so it is difficult to achieve good clogging effect if they cannot propagate quickly. So, *A*5 was the most suitable microorganism for the bioclogging experiments and was identified as *Aspergillus niger*.

3. Bioclogging Experiments

3.1. Experimental Materials and Equipment

3.1.1. Sand. The sand used in the experiment was taken from 2 m underground around a uranium tailings reservoir. Screening test showed that the gradation parameters d_{10} of the samples is 0.093 mm, d_{30} is 0.28 mm, and d_{60} is 0.56 mm; the nonuniform coefficient C_u is 6.02 and the curvature coefficient C_c is 1.51; the relative density G_S is 2.56, the density ρ is 1.374 g/cm^3, and the void ratio e is 0.632. The cumulative curve of the particle composition of the sand samples is shown in Figure 1. The sands were sterilized in an autoclave before *Aspergillus niger* was inoculated.

3.1.2. Radioactive Effluent. The fluid was taken from the radioactive effluent collection well of the uranium tailings reservoir. The concentration of uranyl ions of the radioactive effluent is 1.75 mg/L by the spectrophotometer and pH = 7.85.

3.1.3. Cultivation of Aspergillus niger

(1) The Petri dishes were wrapped with the Kraft paper, and sterilized under high temperature. Then they are put in a clean room for use when the Petri dishes were cooled to room temperature.

(2) The prepared potato medium was placed in a flask for sterilization. When the temperature of the potato

Table 1: Summary table of OD600 value change.

Strains	The initial OD600 value	After 2d the OD600 value
*A*1	1.0495	1.3766
*A*2	1.0330	1.1803
*A*3	1.0196	0.8216
*A*4	1.0327	1.2225
*A*5	1.0274	2.2451

medium drops to 50–60°C, it was dispensed into the Petri dishes under the liquid state.

(3) After the potato medium was solidified, the Petri dishes were inverted to prevent the condensed water on the lid from contaminating the medium.

(4) After inoculating, the Petri dish was placed in a constant-temperature incubator which was set to 30°C for 2 days.

(5) The spores were scraped from the Petri dish and were inoculated into the liquid medium. Put the liquid medium in a constant-temperature shaking incubator which was set to 30°C for 2 days. The rotation speed was set to 200 r/min.

3.1.4. The Sand Columns Model. A schematic representation of the sand columns model was used in this experiment is shown in Figures 2 and 3. The physical model of the sand columns was constructed of polyvinyl chloride (PVC) 50 cm long with an inner diameter of 15 cm. Two piezometer tubes were installed on the top of the model at a distance of 20 cm to measure the water head difference. The water head difference could be controlled by adjusting the outlet flow controller. Nine holes with a diameter of 1.5 cm were reserved on the top of the model for providing oxygen, perfusing the nutrient solution, and taking the soil samples. In the model, two filter screens with a diameter of 1 mm are set up between the piezometer tubes. The sterilized sand was filled between two filter screens, and 4–5 mm gravel was filled between the filter screens to the inlet and outlet. Three sand columns (Column 1 and Column 2 inoculated with bacterial liquid, Column 3 as a blank reference) with the same weight were prepared for the experiment.

3.1.5. Inoculated Volume of Aspergillus niger and Nutrient Solution. According to the related literature [8, 9], the inoculated volume of *Aspergillus niger* spore suspension was 1% of water content of the saturated sand which was 30 mL per 10 cm^3. There are about 3,400 cm^3 sands in the sand columns, so the inoculated volume of *Aspergillus niger* spore suspension was about 100 mL. *Aspergillus niger* has strongest reproductive capacity when 500 mL of nutrient solution was uniformly poured into the sand columns per day though preliminary experiment.

3.1.6. Experimental Data Determination

(1) Hydraulic Conductivity Calculation. The hydraulic conductivity was measured as a function of time in each sand column with constant water head. Keep the water head difference of the piezometer tubes at 2 cm by adjusting the outlet flow controller (the hydraulic gradient is $i = 2\,\text{cm}/20\,\text{cm} = 0.1$, which is consistent with the hydraulic gradient of the actual environment), and the hydraulic conductivity K was calculated by Darcy's law as follows:

$$K = \frac{QL}{F} \times \Delta H, \tag{1}$$

where K: hydraulic conductivity (m/s), Q: volume flow of unit time (m^3/s), L: percolation length of specimen (m), ΔH: water head difference of the piezometer tube (m), and F: cross-sectional specimen area (m^2).

During the experiment, the average flow rate of each sand column was monitored over time, and the permeability coefficient K was calculated according to the Equation (1).

(2) Concentration of Uranyl Ion. During the experiment, 20 mL radioactive effluent sample of each sand column was collected over time, and the concentration of uranyl ions was measured with the spectrophotometer.

The concentration data of uranyl ions was processed:

$$R = \frac{C_0 - C_t}{C_0} \times 100\%, \tag{2}$$

where R: adsorption rate (%), C_0: initial concentration of uranyl ion (mg U/L), and C_t: concentration of uranyl ion at time T (mg U/L).

3.2. Bioclogging Tests. Open the inlet valve and let the radioactive effluent saturate the sand columns. The water head difference of the piezometer tubes was adjusted to obtain a time travel equal to 24 h, and then it was maintained constant until stabilization of the outflow. The initial permeability coefficient K_0 of the sand sample and the uranyl ion concentration were measured.

Close the inlet valve and inoculate 100 mL of *Aspergillus niger* spore suspension and pour 500 mL of nutrient solution into the sand columns evenly through the reserved hole. The permeability coefficient K and the uranyl ions concentration were monitored for 68 days under constant flow (every 2 days in the initial stage of the experiment, every 5 days after two weeks later). After the experiment, open the sand columns model and observe the sand sample by environmental scanning electron microscope.

3.3. Data Analysis. The change in hydraulic conductivity as a function of time is shown in Figure 4. From the figure, initial hydraulic conductivity of sand columns is about 6.3×10^{-5} m/s. After 68 days, the hydraulic conductivity of sand columns decreases to 1.68×10^{-5} m/s (Column 1) and 1.60×10^{-5} m/s (Column 2), which decreases by 74.4% and 74.6%, respectively. However, the hydraulic conductivity of blank column (Column 3) only decreases by 11.8%.

The changes in hydraulic conductivity as function of time can be divided into three stages. In stage 1 (days 1–15),

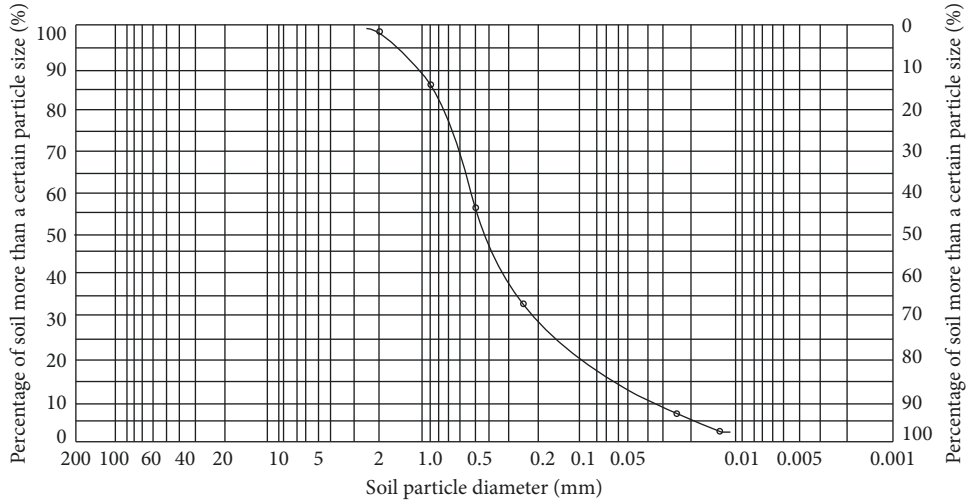

Figure 1: Sand sample particle cumulative curve.

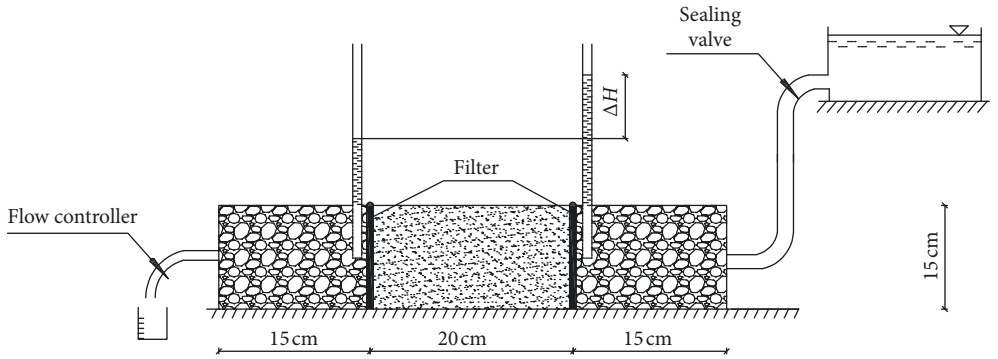

Figure 2: The facade schematic of experimental model.

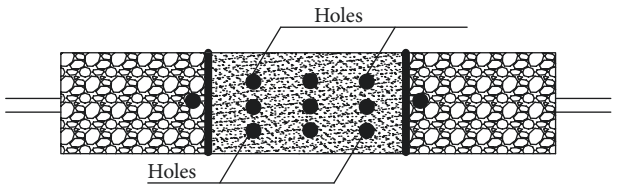

Figure 3: The plane sketch of experimental model.

there is the maximum decrease in hydraulic conductivity because of the growth of biomass. In stage 2 (days 16–40), the variation rate of hydraulic conductivity slows down. In stage 3 (days 41–65), the hydraulic conductivity of sand columns tends to be stable.

In the initial stage, *Aspergillus niger* propagated rapidly because of the loose growth environment of sand columns and adequate nutrient, and the pores between sands were blocked contribute to the decreases of hydraulic conductivity. Then, the decrease of the oxygen and the nutrient solution supply lead to the reproductive speed of *Aspergillus niger* slow down with the time and the permeability coefficient of sands keep decreasing. Finally, the propagation of *Aspergillus niger*

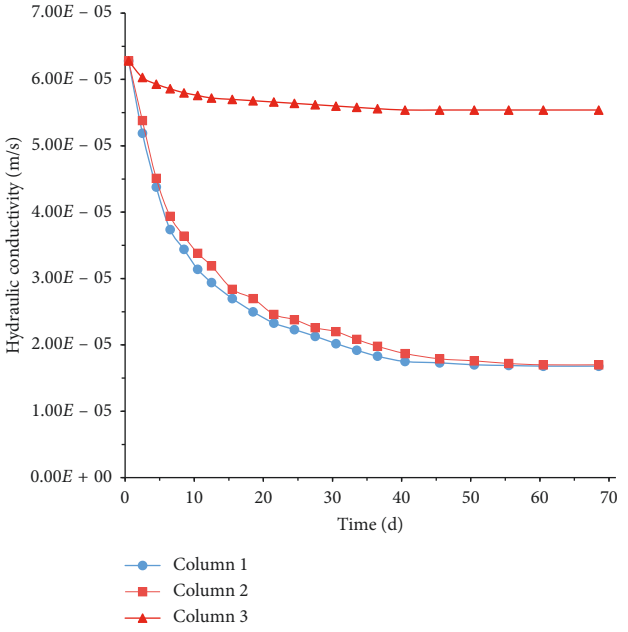

Figure 4: Changes in hydraulic conductivity as function of time.

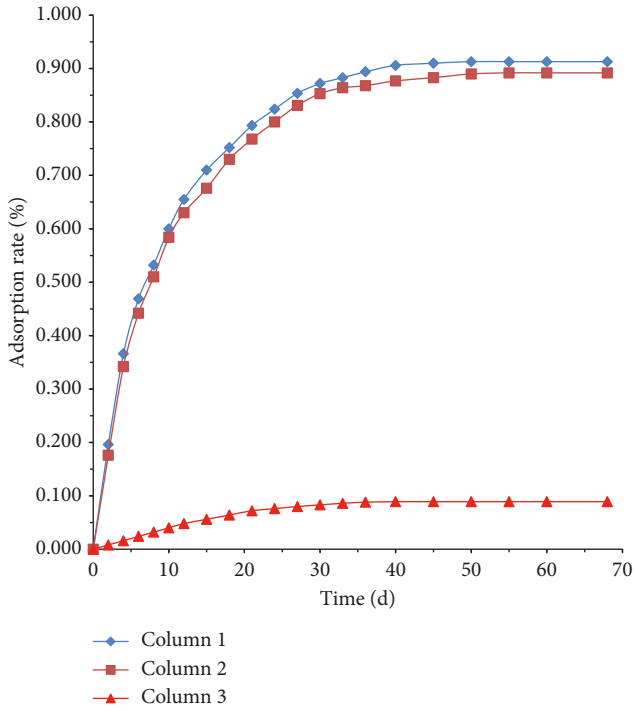

Figure 5: Changes in adsorption rate as function of time.

reached steady state, and the decline rate of hydraulic conductivity as function of time was not obvious until it stabilizes.

From Figure 5, the changes in adsorption rate of uranyl ion as a function of time could be divided into three stages. In the initial stage (1 d–15 d), the adsorption rate of uranyl ion by *Aspergillus niger* is fast and reaches to 47% after two weeks. In the second stage (16 d–40 d), the adsorption rate slows down with the decrease of effective adsorption sites. After 60 days, the adsorption rate of uranyl ions reaches to 91.3% and tends to be stable. The adsorption rate of uranyl ion of the blank column (Column 3) only decreases by 8.9%.

In the initial stage, the *Aspergillus niger* adsorbs the uranyl ions to its surface. This process was fast, reversible, passive adsorption, and no energy required. In the second stage, *Aspergillus niger* transfers the uranyl ions to the inside of cells for digestion and decomposition. This process was active adsorption and irreversible and energy required, related to cell metabolism.

3.4. Environmental Scanning Electron Micrographs. To research the bioclogging mechanism in sand columns, SEM was employed to observe the sand samples after the experiment. The scanning electron micrographs are presented in Figure 6, and it can be observed that the sand samples inoculated with *Aspergillus niger* have formed the biofilm which covered the surface of sand particles, connected the sand particles together, and resulted in the decrease of hydraulic conductivity.

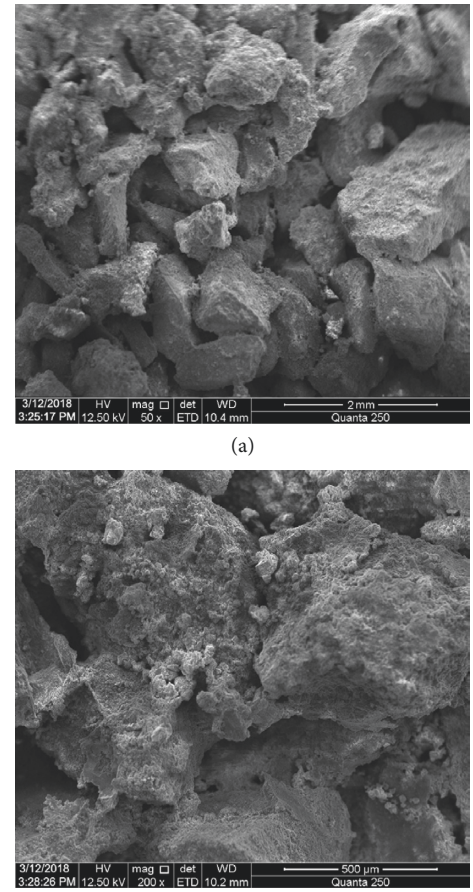

Figure 6: SEM images of the sand sample after experiment ((a) 50x; (b) 200x).

4. Conclusion

In this paper, *Aspergillus niger* was inoculated into the sand columns to investigate the effect of bioclogging and the adsorption rate of uranyl ion under the radioactive effluent percolation, and the following conclusions were obtained:

(1) In this experiment, five indigenous microorganisms were cultivated, and *Aspergillus niger* was chosen for the strongest reproductive capacity.

(2) Bioclogging experiments showed that the sand columns inoculated with *Aspergillus niger* can effectively clog the seepage channel of sands and adsorb uranyl ions. After 68 days, the hydraulic conductivity of sands decreased more than 72%, and the adsorption rate of uranyl ions reached more than 90%.

(3) The scanning electron micrographs showed that the biofilms produced by *Aspergillus niger* covered the surface of sand particles and blocked the seepage passage of porous media, which was the main reason for the decrease of hydraulic conductivity of sand columns.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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