Kinetic and Isotherm Studies for the Adsorption of *Phanerochaete Chrysosporium* on Lignites

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Abstract. Adsorption of microorganisms by coal is the first step for its microbial transformation. This study investigated the adhesion of Phanerochaete chrysosporium on Chinese lignite samples with different particle sizes. Langmuir and Freundlich models were applied to describe the biosorption isotherm of *P. chrysosporium* on lignites. Freundlich model fitted the equilibrium data better than Langmuir isotherm. The adhesion of *P. chrysosporium* on the coal surface was a spontaneous process. Pseudo-first-order and pseudo-second-order equations were applied to describe the biosorption dynamics of *P. chrysosporium* on lignites. Pseudo-second-order equation fitted the dynamics of adsorption better than the pseudo-first order. Adhesion experiments revealed that the adhesion rates of *P. chrysosporium* were 0.3539, 0.23827, and 0.20655L/(mol·min) on lignite with particle sizes of 0.25–0.5, 0.5–0.2, and 2–3mm, respectively under the contact time of 50min, 50g of lignite, 200mL of bacterial solution, and temperature of 25°C.

1. Introduction

Microbial transformation of coal is a promising, environmentally friendly, and efficient synthesis method for coal bed methane [1], hydrogen [2], methanol, ethanol, quinolone [3], and by-product humus acid [4]; this technique produces less carbon compared with the direct combustion of coal [5]. The flexibility and potential cost-effectiveness of the "biomining" make it attractive, but further understanding of the biosorption mechanisms of microorganisms and the development of transport methodology for optimizing microbial formulations and injection strategies are needed to realize its potential. And microbes bind to coal surfaces (via biosorption or bioaccumulation) is closely related to degradation degree and transportation. Coal is a natural porous medium, whereas microbial cells are composed of micron-level organism chitin, cellulose, and glucan that are 100 times larger than the atoms and molecules of coal. The recovery of target products is determined through microbial transmission in porous coals, and the first step involved in transmission is the microbial cell contact with the coal surface [6, 7].

Several studies have suggested that coal bioprocess must be preceded by cell attachment on coal to ensure efficacy [8]. The surface properties of coal vary based on its composition, porosity, and ranks [9], and its microbial characteristic codetermines its adsorption capacity [10]. Physical adsorption

through Van der Waals force occurs first, followed by chemical adsorption that lasts for a long time [11], and functional groups [12] (N–H and C–H) promote adhesion, so activated or inactivated microbial cells exhibit adsorption capacity. Coal attachment by microbial cells affects the surface tension of coal, and changes the pore diameter [13], and directly influences microbial conversion and transmission scope. Therefore, a theoretical basis for attachment and the adsorption rate dates must be provided for microbial transport.

Fungi [14, 15] are the main strains of coal degradation and transformation, and *P. chrysosporium* is considered as the representative fungi of coal degradation [16, 17]. This study used *P. chrysosporium* as the adsorbate and lignite as the adsorbent in equilibrium adsorption experiments to explore the type and characteristics of microbial adsorption on coal surface. The results of this paper aid in understanding transportation and retention, controlling and optimizing injection strategies, and predicting the degradation and retention rates.

2. Materials and methods

2.1. Coal samples
The coal samples used in the experiment were Inner Mongolia Baorixile lignite, and its industrial composition analysis is shown in Table 1. Lignite with different particle sizes of 0.25–0.5, 0.5–2, and 2–3mm were obtained using a ball mill pulverizer for 15min. The lignite samples were loaded in a kraft paper bag, placed in the autoclave sterilization, and dried at 45°C–50°C for 12h. The coal samples were weighed and stored in numbered test sample benchtop vials, and some experimental coal samples were reserved for inspection.

2.2. Preparation of P. chrysosporium
The microbial cells used in this experiment were *P. chrysosporium* purchased from ATCC (ATCC20696), and the *P. chrysosporium* specimen was activated to preserved slant and cultured media at 25°C for 48 h. The culture medium of *P. chrysosporium* contains 100g of potato, 5g of glucose, 5g of yeast extract, 2g of protein, 15g of agar, and 1 L of water and has a natural pH level.

The stable period of *P. chrysosporium* fermentation suspension was centrifuged for 10min with the rotation speed at 4,000 rpm. After centrifugation, the supernatant was washed twice. The wet weight of the cells was measured, and phosphate buffer was added to prepare a certain concentration of bacterial solution.The phosphate buffer contains 8.5g of KH_2PO_4, 21.75g of K_2HPO_4, 33.4g of Na_2HPO_4, 5g of NH_4Cl, and 1 L of water.

2.3. Concentration and absorbance (A–C) standard curve
Spectrophotometer was used to determine the concentration of microbial cells. A standard curve of A–C must be plotted first. The concentration of *P. chrysosporium* was measured by weighing, whereas the absorbance was measured at 600nm [18]. According to Lambert's law, the relationship between concentration and absorbance obeyed the following rule:

$$A = kLC \tag{1}$$

Where A is the absorbance, k (L/ (g·cm)) is the absorption coefficient related to the solution, L (cm) is the thickness of the cuvette (set as 1cm in this experiment), and C is the solution concentration (g/L). The A–C standard curve in this paper is shown in the Figure1.
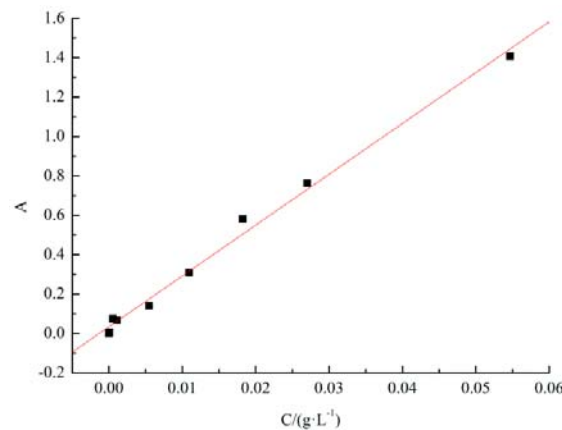
Figure 1. A–C standard curve

2.4. Adsorption Experiment

Static sequence batch experiment using different grain diameters was conducted. At the same temperature, 50g of sterilized coal samples with different particle diameters were placed in filter bottles labeled as 1, 2, and 3. Afterwards, 200mL of the same concentration of bacteria was quickly added to each of the filter bottles that were quickly sealed with lids. The experiment began after oscillation, and the supernatant was obtained every 5min to measure the absorbance. Three parallel experiments were conducted for each particle size. Sterilized water was used as a blank for deducting the adsorption error generated by coal particle suspension. All vessels were autoclaved for 20min, and the drugs used were of analytical grade.

2.5. Calculation of adsorption capacity

The concentration of the *P. chrysosporium* was determined by measuring the absorbance from the supernatant. The volume of bacterial solution was assumed constant before and after adsorption. The adsorption capacity was calculated by:

$$q = \frac{C_1 - C_2}{m} \times V \tag{2}$$

Where q is the adsorption capacity (g/g), C_1 (g/L) is the concentration of bacteria solution before adsorption, C_2 (g/L) is the concentration after adsorption, m (g) is the dry weight of coal sample, and V (L) is the volume of bacterial solution added to the coal sample.

2.6. Adsorption kinetic

Adsorption kinetic model is one of the important methods used to characterize the adsorption and predict the adsorption efficiency under different moments. The most widely used models are pseudo-first-order and pseudo-second-order kinetic models, which are shown in the following formulations [19]:

$$\frac{dq_t}{dt} = k_1 \left(q_{eq} - q_t \right) \tag{3}$$

$$\frac{dq_t}{dt} = k_2 \left(q_{eq} - q_t \right)^2 \tag{4}$$

Where q_t is the adsorption capacity (g/g) at the certain time t; q_{eq} is the adsorption capacity at adsorption equilibrium at g/g; and k_1 and k_2 are the rate constants of pseudo-first-order and pseudo-second-order equations. Eqs. (3) and (4) were integrated as follows:

$$\ln\left(q_t - q_{eq}\right) = -k_1 t \tag{5}$$

$$-\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{6}$$

The ln (q−q_{eq}) and t obtained by the experimental data were linearly fitted, and the resulting slope was given as k1. t/q and t were linearly fitted, and k2 and q_{eq} were obtained from the slope and intercept, respectively.

3. Results and discussion

3.1. Concentration and absorbance (A–C) standard curve

According to the A–C standard curve, the concentration of *P. chrysosporium* can be converted to absorbance to calculate the adsorption capacity. Figure 2 shows the relationship between concentration and adsorption capacity of *P. chrysosporium*. Langmuir and Freundlich models were used to fit the adsorption equilibrium data from the experimental data. Langmuir model and Freundlich models can be expressed as follows:

$$q = \frac{Q_0 bC}{1 + bC} \tag{7}$$

$$q = K_F C^{1/n} \tag{8}$$

where q (g/g) is the equilibrium adsorption capacity at concentration C, Q_0 (g/g) is the saturated adsorption capacity, b (L/g) is the adsorption constant, and KF and n are the adsorption constants that represent the adsorption ability and strength, respectively, in the Freundlich model.
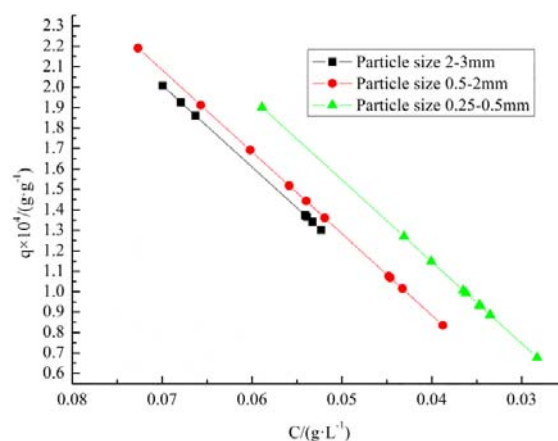

Figure 2. Adsorption isotherms of *P. chrysosporium* on lignite with different particle sizes

Figure 2 shows that the concentration of *P. chrysosporium* decreased, and the adsorption capacity on lignite increased throughout the adsorption. These results indicate that *P. chrysosporium* can be adsorbed on the coal surface. The increasing adsorption capacity could be attributed to the decreasing amount of *P. chrysosporium*.

Given that adsorption is an exothermic process, ΔH < 0. Bacteria move slowly as they are being adsorbed on the coal surface. According to the second law of thermodynamics, the adsorption entropy change is ΔS > 0. Thus, the Gibbs free energy obeys the following relationship:

$$\Delta G = \Delta H - T\Delta S < 0 \tag{9}$$

Therefore, the adsorption of *P. chrysosporium* on coal is a spontaneous process, which was in accordance with the experimental results.

Figure 2 shows that for the same concentration of *P. chrysosporium*, the adsorption capacity of *P. chrysosporium* decreased with increasing particle size. The adsorption capacity of *P. chrysosporium* on coal is closely related to its own particle size. The abrasiveness of coal results in a reduced particle size. On the one hand, the increased surface of the new sample and the specific surface area improved the adsorption of *P. chrysosporium*. On the other hand, the decrease in coal particle size results to the increase in external surface area of the coal, thereby effectively reducing the pore-blocking effect of the coal with relatively large molecular mass. Hence, the coal adsorption capacity increased significantly with small particle size [20].
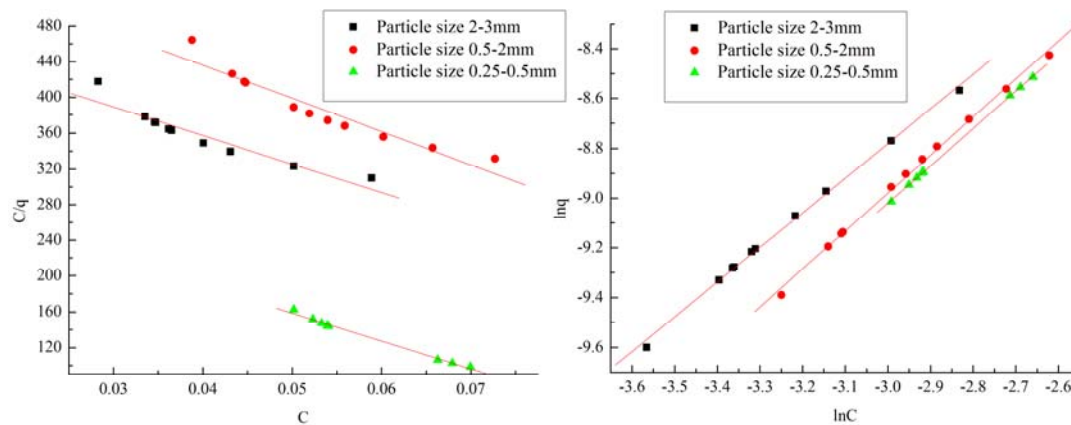

Figure 3. Fitting curve of Langmuir and Freundlich models of the adsorption capacity of *P. chrysosporium* on lignite with different particle sizes.

According to the fitting results of the two adsorption models in Figure 3 and Table 2, the slope of Langmuir model was negative regardless of the particle size. The fitting degree was smaller than that of Freundlich model, which indicated that Langmuir model was not suitable for the adsorption of *P. chrysosporium* on lignites. Langmuir model is based on the assumption that adsorption occurs on the monolayer, and the adsorption sites are evenly distributed. By contrast, the coal surface does not have uniform composition and adsorption sites, and the coal provides a carbon source, a nitrogen source, and a small amount of trace element for the *P. chrysosporium* growth. Hence, *P. chrysosporium* adsorption on the coal surface is selective and could not occur at any position, which are contrary to the hypothesis of the Langmuir model. Therefore, Langmuir model cannot be used to describe the coal adsorption of *P. chrysosporium.*
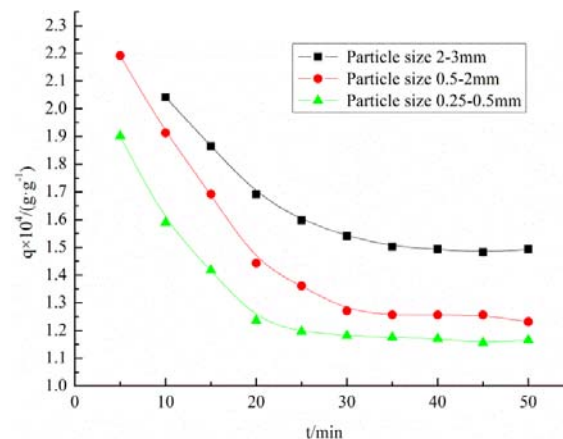
Table 1. Industrial Components of Coal Fitting results of Langmuir and Freundlich models of the adsorption capacity of *P. chrysosporium* on lignite with different particle sizes

Particle size/mm	Langmuir model	Correlatin coefficient R^2	Freundlich model	Correlation coefficient R^2
0.25–0.5	y = −3118.66191 x +314.370	0.9920	y = 1.49831 x − 4.52495	0.9998
0.5–2	y = −3709.97688x + 584.278	0.9186	y = 1.52691x − 4.39883	0.9975
2–3	y = −3180.23373 x + 484.441	0.8943	y = 1.39241 x − 4.60353	0.9977

The fitting correlation coefficients of Freundlich model with different coal particle sizes were all above 0.99, and the fitting degree of 0.25–0.5mm coal was the highest. These results were obtained because the small coal particles exposed the organic matter and the nutrients needed for the growth of *P. chrysosporium*. The presence of these materials indicates a large number of adsorption sites. Freundlich model is an empirical formula used to characterize multilayer adsorption and is suitable for uniform and non-uniform surfaces. The experimental results in this paper followed the Freundlich adsorption law, which indicates that the adsorption of *P. chrysosporium* is complex and multi-layered.

3.2. Analysis of adsorption kinetics
The adsorption was spontaneous and in line with the Freundlich model. The time to achieve adsorption equilibrium and the kinetics of *P. chrysosporium* adsorption on coal were also investigated.

Figure 4. Adsorption kinetic curve

The adsorption kinetic curves of coal samples with different particle sizes are shown in Figure 4. The kinetic curve showed that the smallest coal sample of 0.25–0.5mm had the highest adsorption capacity for *P. chrysosporium*, followed by 0.5–2mm coal. The largest 2–3mm coal had the lowest adsorption capacity. The coal samples with three particle sizes stabilized after 20, 25, and 30min, which can be considered as the adsorption equilibrium. In Figure4, the adsorption of *P. chrysosporium* on coal can be divided into two stages. From 0min to 30 min, the adsorption rate was extremely fast, and the concentration of *P. chrysosporium* decreased rapidly against time. After 30min, the adsorption capacity stabilized, and the adsorption rate was nearly zero.
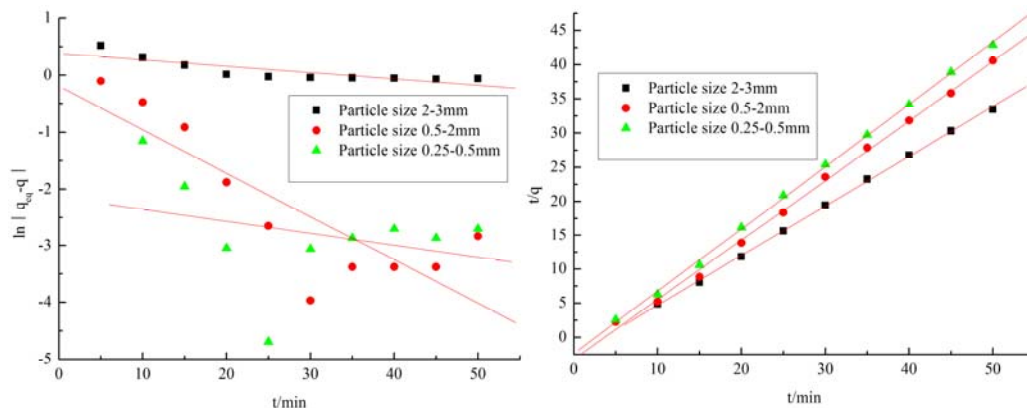
Figure 5. Fitting curves of pseudo-first-order and pseudo-second-order kinetic equations with different particle sizes of lignite.

Pseudo-first-order and pseudo-second-order kinetic equations were used to fit the adsorption experimental data and further reveal the effect of varying particle sizes on the adsorption of *P. chrysosporium*. The fitting parameters are shown in Figure 6 and Table 3. Pseudo-second-order kinetic equation more accurately described the adsorption than the pseudo-second-order kinetic equation, and the correlation coefficients of different coal samples were all above 0.99. When the particle size was 0.25–0.5mm, the correlation coefficient of pseudo-first-order kinetic equation was less than 0.1, indicating that this formula cannot be used to describe the adsorption of *P. chrysosporium* to coal with small diameter. When the particle size was increased to 0.5–2 and 2–3mm, the correlation coefficient of fitting was still low, although the pseudo-first-order kinetic equation fitting degree increased to ≥0.71. Therefore, the adsorption of coal to *P. chrysosporium* was accurately described by the pseudo-second-order kinetic equation.

Table 2. Fitting results of pseudo-first-order and pseudo-second-order kinetic equations with different particle sizes of lignite

Particle size/mm	Pseudo-first-order kinetic equation		Pseudo-second-order kinetic equation	
	k_1	R^2	k_2	R^2
0.25–0.5	0.02137	0.0957	0.3539	0.9907
0.5–2	0.07647	0.71118	0.23877	0.99787
2–3	0.01115	0.7123	0.20655	0.99935

The rate constant k directly reflects the speed. Table 2 shows that when the particle size is small, the rate constant increases, and the adsorption rate is faster. The change in particle size of the coal significantly affects its adsorption efficiency to *P. chrysosporium*. On the one hand, the increased adsorption rate was caused by the short distance required for *P. chrysosporium* in the liquid phase to reach the coal pores. Hence, the number of *P. chrysosporium* that reaches the coal surface adsorption site was increased per unit time. On the other hand, the newly exposed adsorption sites increased; hence, the coal can adsorb many *P. chrysosporium* cells per unit of time.

4. Conclusion
The main findings are as follows:

(1) Adsorption thermodynamics analysis shows that *P. chrysosporium* adsorption on coal was a spontaneous process. Langmuir and Freundlich models were used to fit the adsorption data of *P. chrysosporium* adsorbed in the coal samples with different particle sizes, and the results show that Freundlich model is suitable in describing the adsorption. The correlation coefficient was ≥ 0.99.

(2) Adsorption kinetic analysis shows that the kinetics of *P. chrysosporium* adsorption on coal was more accurately described by the pseudo-first-order kinetic equation than the pseudo-first-order dynamic equation. The adsorption constants k_2 0.3539, 0.23827, and 0.20655 L/(mol·min) corresponded to the lignite particle sizes of 0.25–0.5, 0.5–0.2, and 2–3 mm, respectively, that adsorbed *P. chrysosporium* cells. All correlation coefficients were above 0.99.

(3) Coal particle size had a significant effect on the adsorption efficiency of *P. chrysosporium*. Microbial cells were adsorbed on the coal adsorption sites that allow them to consume coal for growth. Coal with small particle size increased the exposure of organic matter and provided additional adsorption sites for *P. chrysosporium*. On the contrary, the decline of particle size increased the specific surface area and adsorption sites. The particle size of the coal minimally affects the adaptability of the Freundlich model. When the particle size decreased, the distance of *P. chrysosporium* in water phase from the coal pore was reduced, the number of *P. chrysosporium* on the coal surface adsorption site increased per unit time, and the adsorption rate increased.

A broad understanding of the basic adsorption mechanism of microbial cells, which confirms the adsorption types and determines the optimum parameters for multi-coupling biotransmission, will contribute in developing an environment-friendly method for sulfur removal. These reults may be useful to evaluate sites for field adsorption intended to microbes transport processes.

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