The Sorption of Cesium on Fungi Cell: Kinetic and Isotherm Study

Prapamon Seeprasert, Minoru Yoneda, Yoko Shimada, and Yasuto Matsui
Department of Environmental Engineering, Kyoto University, Kyoto, Japan
Email: noon@risk.env.kyoto-u.ac.jp, yoneda@risk.env.kyoto-u.ac.jp, shimada@risk.env.kyoto-u.ac.jp, ymatsui@risk.env.kyoto-u.ac.jp

***Abstract*—The increase in soil contamination by cesium (Cs) resulting from nuclear accidents has led to a resurgence of interest in microbe-radioactive interaction. The fate of Cs in the environment is mostly influenced by sorption processes. The aims of this study were as follow: 1) to apply a kinetic model of nonlinear regression and 2) to quantify the ability of soil fungi to adsorb Cs and determine the sorption isotherm. The results show that the r^2 values for the pseudo-first-order kinetic model are higher than those for the pseudo-second-order kinetic model for all fungi genera (*Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp.). Then, the sorption equilibrium data were fitted to describe the sorption characteristics and quantify the sorption capacity with Langmuir and Freundlich isotherm. The results contribute to a better understanding of biosorption phenomena, under single-element conditions; the monolayer sorption capacities for Cs ions were 64, 38, and 30 μg/g cell for *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp., respectively which was best described by the Langmuir isotherm, indicating a monolayer arrangement of Cs on the external cell surface.[1]**

***Index Terms* — cesium, kinetic, soil fungi**

I. Introduction

Cesium (Cs) is the rarest alkaline metal and is strongly sorbed onto clay minerals within the soil system [1], [2]. Nowadays, soil contamination by Cs from the nuclear accidents has led to a resurgence of interest in microbe-radioactive interactions. The fate of Cs in the natural has a low rate of vertical movement and low bioavailability for plant uptake [3], [4]. In case study of Chernobyl fallout in 1986, Cs was found to be localized mainly in the organic soil layer more than a decade after its deposition. Same behavior show in case of atomic bomb in Nagasaki, Japan, which is show that after 40 years the Cs monitoring showed 95% of the fallout was still present in the uppermost 10 cm of local soil [5]. Then, it can be assumed that the large quantities of Cs which released from the Fukushima accident in 2011 are still present in terrestrial ecosystems with the strong retention in the organic layer.

The effects of fungal and microbiological activity in organic matter have likely contributed substantially to the long-term retention of Cs in the organic layer. Previous studies indicated that 70% of spiked Cs into the organic materials was strongly bound in the presence of soil microorganisms, compared to only 10% occurred in abiotic systems [6]. It can be confirm that the microbial biomass and their activities in biotic systems play an important role in accumulating not only the nutrient elements but also the radionuclides. In general, the fate of Cs in the environment is primarily influenced by sorption process [7].

The nature of a given sorption process depends on the physical and chemical characteristics of the adsorbent system and the system conditions. Thus, the prediction of batch sorption kinetics is necessary to describe the sorption process, and kinetic expressions are commonly used to explain how fast the rate of sorption occurs. It is important to determine the time needed to reach equilibrium and the examination of the rates of adsorption that can be used to develop models and to understand the solutes on the adsorbent surface. The pseudo-first order rate has long been widely applied for these types of systems. A pseudo first-order rate best fit a lead sorption system [8]. The expression is shown in equation (1)

$$\frac{\mathrm{d}q_t}{\mathrm{d}t} = k_1(q_1 - q_t) \quad (1)$$

This expression can be rearranged for linearized data plotting by integrating for the boundary conditions $t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$, as shown by equation (2)

$$\log(q_1 - q_t) = \log(q_1) - \frac{k_1}{2.303}t \quad (2)$$

Where k_1 is the rate constant for first order sorption, q_1 is the amount of solute adsorbed at equilibrium, and q_t is amount of solute adsorbed on the surface of the fungi cell at time = t

The pseudo first-order kinetic rate equation often provides an excellent fit with experimental kinetic data for various sorption processes. However, in some previous studies, it has failed to theoretically predict the q_1 values, indicating that these sorption processes deviate from theory [9]. The pseudo second-order rate equation has also been found to fit most experimental data for sorption systems [10], and in particular, well explain the kinetics of the sorption of heavy metals [11] and other inorganic matter [12]. For adsorption systems following pseudo second-order kinetics, the Cs or Sr ions are assumed to be adsorbed onto two surface sites. Thus, the

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sorption kinetics following pseudo second-order kinetics can be expressed by rewriting equation (1) as equation (3)

$$\frac{\mathrm{d}q}{(q_1 - q_t)^2} = K_2 \mathrm{d}t \quad (3)$$

Integrating equation (3) for the boundary conditions $t = 0$ and $t = t$ and $q = 0$ and $q = q$ affords equation (4)

$$\frac{1}{(q_1 - q_t)} = \frac{1}{q_e} + K_2 t \quad (4)$$

The nonlinear pseudo second-order and pseudo first-order kinetic models and their linearized expressions are summarized in Table I.

TABLE I. LINEAR AND NONLINEAR FORMS OF THE PSEUDO FIRST-ORDER AND PSEUDO SECOND-ORDER KINETIC MODELS

Type	Nonlinear	Linear
1st order	$q = q_1(1 - \mathrm{e}^{-K_1 t})$	$\log(q_1 - q_t) = \log(q_1) - \frac{k_1}{2.303} t$
2nd order	$q = \frac{K_2 q_1^2 t}{1 + K_2 q_1 t}$	$\frac{t}{q} = \frac{1}{K_2 q_1^2} + \frac{1}{q_1} t$

In the present study, nonlinear regressions of the pseudo-first-order and pseudo-second-order models were first performed. The sorption characteristics were then plotted and fitted with the Langmuir and Freundlich isotherms to quantify the ability of soil fungi to accumulate Cs based on the sorption isotherms. The data enhance our understanding of how soil fungi accumulating cesium (Cs^+) via sorption process.

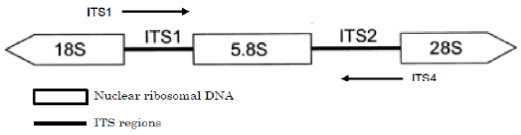

Figure 1. Schematic representation of fungal ribosomal genes and PCR primers.

II. MATERIALS AND METHOD

A. Cell Preparation

Soil fungi, which are found in the rhizosphere, were used in this study. They were isolated by making a soil solution dilution. The soil sampling sites were located in a deciduous and coniferous forest at Takizawa research forest of Iwate University, Japan (39°46′40N, 141°9′26E), in which Japanese oak is the main plant species. Each serial dilution (1 ml) was taken and spread onto a potato dextrose agar (PDA) plate. The dilutions were then incubated at 25 ℃-30 ℃ for seven days. Colonies were selected and re-cultured on fresh PDA plates, and DNA extraction and PCR (polymerase chain reaction) amplification were conducted. For the entire internal transcribed spacer (ITS), the ITS regions (Fig. 1), which are potentially useful in the rapid and accurate diagnosis of fungal isolation, were sequenced [13], [14]. ITS1 (5'TCC GTA GGT GAA CCTTGC GG 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') were used as the forward and reverse primers, respectively. The basic local alignment search tool was applied to identify soil fungi.

B. Preparation of the Resting Cell and Batch Sorption Experiment

The identified soil fungi that were determined to be representative were cultured in potato dextrose broth. The active cells were then cultivated for seven days. To prepare the resting cells, the cells were washed three times with sterile distilled water (SDW) to restrict cell growth.

For kinetic study, the resting cells were re-suspended in 25 mL of SDW that was amended with Cs^+. The total concentration of Cs in the final solution was 5 ppb. Suspension aliquots (3 mL) were sampled at various time intervals (0, 0.5, 1, 3, 6, and 24 h).

For isotherm study, the resting cells were re-suspended in 25 mL of SDW that was amended with different concentrations of Cs^+. The total concentrations of Cs in the final solution were 1, 10, 20, 25, and 50 ppb. Suspension aliquots (3 ml) were sampled after cultivation for 3 h.

C. Analysis

Samples collected from batch sorption studies were filtrated through dried filter paper (Whatman no. 1). The dry weight of cell pellet was measured. The remaining Cs^+ in the aliquot was then measured using Inductively Coupled Plasma Mass Spectrometry (ICP–MS) following the standard sample preparation methods (ISO17294-2 and EPA 6020a) [15].

III. RESULTS AND DISCUSSION

A. Soil Fungi Isolation and Identification

Soil solution was used to isolate single strains with the hyphal tip method. The hyphal tip method is employed to obtain pure cultures of fungi by taking a single hypha from the edge of the culture and cutting the hyphal tip immediately before the last branching point using a scalpel and a minutien pin [16]. Next, the tip is transferred to a new culture plate and incubated at a temperature of 25°C - 30°C. This method is economic because antibiotics are not needed in the culture plate as the precise manipulation means that the isolated spores are free of bacterial contamination. Unlike other isolation approaches, this method can reduce the excessive use of sterile pipettes, agar plates, and other sterile items, as well as spore dilutions. This method is useful because it can be modified to isolate various fungi from different taxonomic groups [17]. However, this method is also complex when selecting a single hypha if the fungal growth is dense and compact. This method cannot be used to distinguish all of the species of microorganisms found in different forest soils. However, some of the fungi with the most abundant growth were selected as representatives.

DNA extraction and PCR amplification were employed to identify representative members of the soil fungal community. Molecular techniques facilitate this type of research. The representative fungi selected using the hyphal tip isolation technique in this study were assigned to three genera, *Fusarium*, *Trichoderma*, and *Aspergillus*, which are 90% - 98% similar (data not shown). *Fusarium*, which is a large genus of filamentous fungi with widespread distributions in soil, and they are also associated with plants. *Trichoderma* is a genus of saprotrophic fungi that produces various types of secondary metabolites, and *Aspergillus* is a genus that was originally isolated from soil. These can all be assumed to be saprotrophic fungi that are free living in the soil, where they have the ability to decompose dead organisms and organic residues.

B. Cs Sorption Kinetics

The experiment was performed to determine the sorption kinetics of the stable isotopes of Cs: individual studies, wherein the sorption rate for each element was determined separately in batch experiment. The quantities of the elements adsorbed onto the fungi cells were fitted with the nonlinear regression results for the pseudo first-order and pseudo second-order models (Fig. 2).

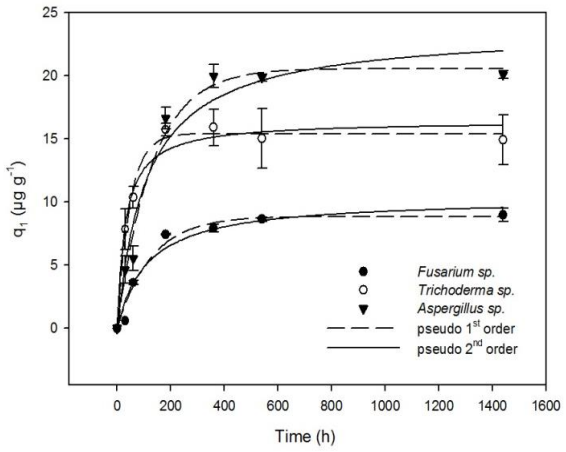

Figure 2. Concentration vs. time profiles for the adsorption of Cs on resting cells ([Cs^+] = 5 ppm).

The parameters and correlation coefficients (r^2) of the pseudo-first-order and pseudo-second-order nonlinear regression equations are shown in Table II. For all of the studied systems, the values of r^2 for the pseudo-first-order fits are higher than those of the pseudo-second-order fits. Conversely, the q_1 values of the pseudo-second-order equation are higher than those of the pseudo-first-order one; q_1 (2) > q_1 (1).

The result from experimental indicate that increase of the contact time above the equilibrium time did not result in any significant increase in the sorption of Cs by the fungi cells. In addition, the sorption rates were rapid when the concentrations of the element ions were high, such as during the initial period. However, as the contact time increased, the rates, and consequently the sorption efficiencies for the element ions by the fungi cells, decreased and eventually became nearly constant, likely due to saturation of the ions on the cell surfaces.

TABLE II. KINETIC PARAMETERS AND CORRELATION COEFFICIENTS FOR PSEUDO-FIRST-ORDER AND PSEUDO-SECOND-ORDER NONLINEAR REGRESSION

Cell	pseudo-first order			pseudo-second order		
	k_1 (min^{-1})	q_1 (1) ($\mu g.g^{-1}$)	r^2	k_2 ($\mu g.g^{-1}$ min^{-1})	q_1 (2) ($\mu g.g^{-1}$)	r^2
Tri.	0.47	8.91	0.97	0.05	10.22	0.95
Fus.	1.36	1.63	0.99	1.18	1.75	0.99
Asp.	0.46	17.52	0.97	0.03	19.44	0.97

To optimize the experimental conditions, batch studies, the concentration of the element 5 ppb were load to determine the time reached to equilibrium. The results show that for Cs, the r^2 values for the pseudo-first-order equation are higher than those of the pseudo-second-order equation for *Fusarium* sp. *and Trichoderma* sp. A previous study showed that the sorption of ions follows a two-step mechanism: (1) ions are taken up onto the surface of the fungi cells via a reversible physical/chemical process (namely, passive transport) with a relatively short contact time and (2) the ions are taken up into the cells via a biological mechanism (namely, active transport) [18], which requires a long contact time to reach equilibrium [19]. In this study, the fungi cells were washed with the sterile purified water to stop cell growth and cell activity. Thus, the biological function was then no longer active, and sorption mainly took place on the cell surface. Therefore, sorption equilibrium was achieved quickly within 60 min, and no further sorption was observed thereafter.

C. Cs Sorption Isotherm

The sorption process considered involves a solid phase, which contains fungal cells and a liquid phase, which contains the Cs solution to be sorbed. The sorption isotherms are plots between the sorption uptake and the final equilibrium concentration of the residual Cs in the solution. Correspondingly, the amount of Cs bound to the cell that disappears from the solution can be calculated on the basis of the mass balance in the system. The relationship between Cs was sorbed onto the fungi cell (q) and the residue of Cs in the solution (C_e) can also be mathematically expressed. Firstly, using the Langmuir isotherm, which is the relationship of the hyperbolic form represented by Equation 5:

$$q_e = \frac{q_m K_L C_e}{1 + C_e} \quad (5)$$

Where q_e is the amount of Cs sorbed, C_e is the amount of Cs residue in the solution, q_m is the maximum amount of Cs sorbed, and K_L is an equilibrium constant representing the affinity between the fungi cell and Cs solution The Langmuir isotherm considers sorption as a chemical phenomenon. The Langmuir constant K_L, which is related to the energy of adsorption through the Arrhenius equation, can also be interpreted as the total

number of binding sites that are in fact occupied by Cs at the concentration (C_e). The Langmuir model provides information on uptake capabilities and assumes that the forces that are exerted are chemically unsaturated, which do not extend further than the diameter of one sorbed molecule; therefore, sorption is restricted to the monolayer.

The Freundlich isotherm relationship is exponential and is represented by Equation 6

$$q_e = K_F C_e^{1/n} \quad (6)$$

where q_e is the amount of Cs or Sr sorbed, C_e is the amount of Cs or Sr residue in the solution, K_f is a constant that is related to sorption capacity and n is an empirical parameter that varies with the degree of heterogeneity.

The Freundlich relationship is an empirical equation that does not indicate a finite uptake capacity of Cs or Sr and, thus, can only be reasonably applied in low to intermediate concentration ranges. However, the Freundlich relationship is easier to represent mathematically in more complex calculations where it may quite frequently appear. The Freundlich model can be easily linearized by plotting the data in a log format and it has become the most commonly used empirical model because it contains two useful and easily conceptualized parameters, which are more easily understandable because they reflect the two important characteristics of the sorption system [20], [21].

To determine the mechanistic parameters associated with Cs sorption, data were gathered using two sorption isotherms. The best fitting model was indicated with high values of the correlation coefficient of determination. The results obtained by the Langmuir and Freundlich models are depicted in Fig. 3. The results mostly indicated that Cs was sorbed in agreement with the Langmuir isotherm (Table III), which was theoretically derived assuming that no interaction between the elements and the cell surface took place, and that adsorption occurred on fixed homogenous adsorption sites forming a monolayer surface coverage.

TABLE III. ISOTHERM PARAMETERS FOR CESIUM AND STRONTIUM SORPTION

Cell	Langmuir constants			Freundlich constants		
	q_m	K_L	R^2	K_f	$1/n$	R^2
Fus.	63.74	16.84	**0.9915**	5.12	0.60	0.9741
Tri.	37.72	17.87	**0.9604**	3.32	0.58	0.9174
Asp.	29.85	4.47	**0.9304**	8.20	0.35	0.7516

Table III shows the maximum amount of Cs that can be adsorbed derived from the Langmuir model. More Cs can be sorbed onto *Fusarium* sp. than onto *Trichoderma* sp. and *Aspergillus* sp. Conversely, the Freundlich isotherm does not indicate saturation of the adsorbent surface. The amount of Cs adsorbed increases indefinitely and proportionally to the Cs concentration of the solution.

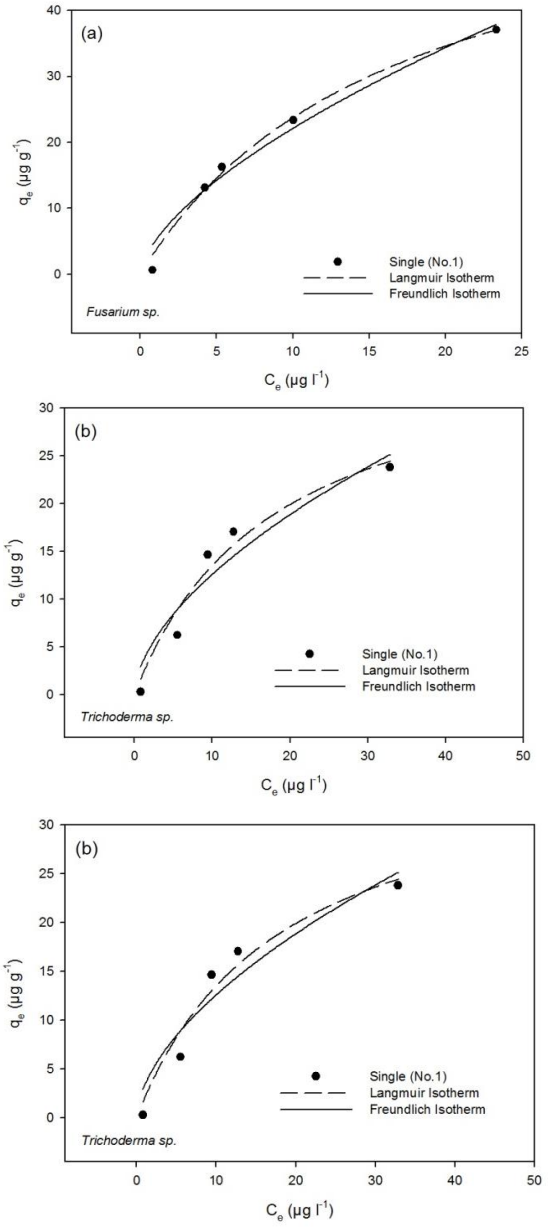

Figure 3. Data fit with the Langmuir and Freundlich isotherms: (a) *Trichoderma* sp.; (b) *Fusarium* sp.; and (c) *Aspergillus* sp..

The ability to accumulate Cs was dependent on the species of microorganisms, as reported in previous studies. For example *Pseudomonas fluorescens* and *Rhodococcus* sp. isolated from the soil were grown in the presence of Cs, and no detectable Cs accumulation was observed for *P. fluorescens* [22]. In this study, *Fusarium* sp. is greater adsorb follow by *Trichoderma* sp. and *Aspergillus* sp., respectively.

The sorption capacity of stable isotopes of Cs increased when the initial concentration of the elements were increased and showed a specific trend pertaining to approaching a plateau. A similar was show in Lan *et al.* (2014) which is the result assumed the biosorption of Cs onto *Rhodosporidium fluviale* (Cs concentration varied from 0.01 to 2.0 mg/L) was a monolayer adsorption through electrostatic attraction [23]. However, some of the treatments under the competitive with others ion such

as Sr^{2+} were in strong agreement with the Freundlich isotherm [24].

IV. Conclusion

In this study, the kinetics and isotherms of cesium adsorption on resting cells of soil fungi (*Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp.) were determined. For this purpose, nonlinear methods were used to estimate the kinetic parameters. The sorption characteristics were examined for different contact times to generate kinetic data, which were fit with two kinetic equations, namely pseudo-first-order and pseudo-second-order, to represent the experimental data. The results show that the correlation coefficients for the pseudo-first-order fit are higher than those of the pseudo-second-order fit for all soil fungi genera.

The adsorption equilibrium data were fitted with Langmuir and Freundlich isotherms. The fitting of Cs adsorption onto fungi cells with the Langmuir model indicates that the maximum amount of Cs adsorbed on *Fusarium* sp. is greater than that on *Trichoderma* sp. when the initial concentration of Cs was increased. Additionally, Cs adsorption was best described by the Langmuir model, indicating a monolayer arrangement of Cs on the cell external surface. The monolayer sorption capacities for Cs ions were 64, 38, and 30 μg/g cell for *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp., respectively. The experimental results indicated that fungi cells contributed substantially to the long-term retention of Cs in the organic layer. This retention is essential to reduce metal migration in soil layers by directly binding fungal cells extracellularly or actively taking up ions intracellularly. The intracellular uptake of ions from soil should be studied in the future.

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Prapamon Seeprasert was born in 1986 in Samutprakarn, Thailand. In 2009, she received her B.Sc. in environmental science from Kasetsart University, Thailand. In 2012, she received her M.Sc in environmental technology and management from the same university with a thesis focused on evaluating cadmium uptake in rice using neutron activating analysis. In the same year, she also received a B.P.H. in occupational health and safety form Sukhothai Thammathirat Open University, Thailand.