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KINETIC STUDY OF BIOSORPTION OF ARSENIC FROM SOIL USING MICROORGANISMS

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ABSTRACT

This work studied the kinetics of biosorption of arsenic (As) from polluted soils using two bacteria [Proteus mirabilis (P. mirabilis) and Bacillus subtilis (B. subtilis)]. The bacteria obtained from the soils were cultured and utilized to treat the arsenic polluted soils through the process of batch biosorption experiment. The experimental data were fitted with four kinetic models (Pseudo-first order, pseudo-second order, Elovich and the intra-particle diffusion); and judgement on models performance was made using the values of the models' coefficient of determination (R^2).The four models tested were adequate for describing the biosorption kinetics but the leading performance of the intraparticle diffusion model with R^2 of 0.991 for sorption by P. mirabilis and 0.972 for sorption by B. subtilis proved the diffusion process to be the rate-limiting step. This indicated that the systems were transport controlled.

Keywords: *Arsenic, contaminated soils, biosorption, sorption kinetics, microorganisms.*

1. INTRODUCTION

Growth in industrial exploration of natural earth resources has triggered increased discharge and accumulation of heavy metals in environmental components; and these metals are injurious to these components [1, 2] as they bioaccumulate to become toxic to plants and animals' lives [3].

Though, some of these metals are useful to flora at adequate concentrations [2], arsenic being a principal environmental pollutant constitute environmental health problem for millions of humans on earth [4].

Almost every organ in human body is susceptible to arsenic exposure, with diverse health impacts including lung disease, skin lesions and cancer [5]. Chronic exposure to arsenic leads to inflammation of the conjunctiva membrane; vesicular rashes, skin pigmentation, hyperkeratosis, destruction of red-blood cells, bone marrow depression, renal damage, irrational speech, poor memory, paralysis and degeneration of inner ear [6].

To make the ecosystem fit for man and other live sustaining organisms, polluted soils with heavy metals need to be corrected through treatment [1, 2]. Some microorganisms are suitable for correcting soil concentration of heavy metals. This Process is known as bioremediation [1, 7]; and it is significantly

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considered for treating metals contaminated ecosystem [1].

Other convectional techniques of correcting metals contaminated soils exist, and are housed under physical and chemical techniques. They are effective for metals pollution control but are very expensive when heavy metals concentrations in soils are very low [1, 8].

This is why biological techniques have been under serious research. It is regarded as a sustainable remediation technology to correct and restore the natural state of soil [1, 2]. Blaylock and others in [9] reported the cleaning cost minimization in bioremediation as they were able to save fifty to sixty-five percent of cost on applying bioremediation as a treatment alternative in one acre of lead-contaminated soil compared with excavation and landfill [10].

Therefore, this work focuses on bioremediation of arsenic polluted soils using two organisms: *Proteus mirabilis* and *Bacillus subtilis* isolated from the same soils obtained from the forest of Amaonye in Ishiagu communities in Ebonyi State, Nigeria. It focuses specifically on the kinetics of biosorption of this metal from the soils by engaging four mathematical kinetic models (Pseudo-first order model, pseuodo-second order model, Elovich model and the intra-particle diffusion model) to fit experimental data.

2. MATERIALS AND METHODS

2.1 Materials

These include soil samples, pipettes, beakers, conical flasks, microscope, Petris dishes, inoculating needles, cotton wool, atomic absorption spectrophotometer, measuring cylinders, wire loops, incubator, MacCartney bottles, refrigerator, autoclave, what man filter papers, hot plate and magnetic stirrer.

2.2 Reagents and Nutrients

These include Lugo's iodine, nitric acid, potato dextrose agar, perchloric acid, crystal violent, Simon citrate ager, hydrogen peroxide, ethanol, peptone water, methylene blue, sulphuric acids, triple sugar iron ager, oxidase reagent, hydrochloric acid, nutrient agar, sodium hydroxide, Kovac's reagent, safranin and MacConkery ager.

2.3 Preparation of Nutrients

In line with the manufacturers' stipulations and the methods in [11], the solutions of triple sugar iron agar, peptone water powder, nutrient agar, Simon Citrate agar and MacConkey agar were prepared by measuring, soaking and swirling 65, 15, 28, 24 and 52grams of powder of the respective nutrients in 1000cm^3 of distilled water for ten minutes. The respective solutions were autoclaved for 15 minutes at 121^{o}C and 1.5 pounds per square inch (psi) and then cooled to 45^{0}C.

2.4 Recognition of Organisms:

Bacteriological study was conducted in microbiology laboratory on soil sample obtained from a forest in Amaonye – Ishiagu, Ebonyi State of Nigeria. The soil was serial diluted, and 0.1 ml each from $10^{-1}$of the serial dilution was inoculated into different sterile petri-dishes using pure plating approach [12, 13] and were incubated at temperature of 37^{o}C for 24 hours [11]. The grown Colonies were enumerated, sub cultured and characterized using the techniques in [11, 14, 15]

2.5 Optimum Factors Determination

Heavy metals contaminated land remediation is optimally efficient when it is conducted at the optimum values of factors enhancing remediation. Experimental screening of these factors is a prerequisite to their optimal selection. The factors considered are nutrient dosage (ml), organisms' weights (g), pH, temperature (^{o}C) and stirring frequency [per week (pw)]. The screening was conducted in batches and in triplicate [16, 17].

Nutrient dosage of 1, 2, 3, 4, 5, 6 and 7 ml were measured into twenty-one (21) identical 50 ml beakers, containing 4g of soil samples each. The soils were inoculated with respective 24 days old bacterium; and evaluated to determine the residual arsenic ion on the 21st day with Atomic Absorption Spectrophotometer (GBC Sens AA, Model no. A6358) after deleting the bacterial from samples through the use of centrifuge [17].

The process was adopted to screen the optimum values of factors from varied values of 1, 2, 3, 4, 5, 6, and 7g; 20, 25, 30, 35, 40 and 45^0 C; 4, 5, 6, 7, 8, 9 and 10; and 0, 1, 2, 3, 4, 5 and 6 per week (pw) of organisms' masses; weights; temperature; pH; and stirring frequency per week respectively.

2.6 Biosorption and Kinetics

Four (4) grams of soils each were inoculated respectively with the bacterium in thirty 50ml beakers (fifteen for each bacterium) and conditioned with the optimized values of pH, temperature (^{o}C), organisms' weights (g), stirring frequency (pw) and nutrient dosage (ml) of 7, 30°C, 1g, 6pw and 8 ml for Proteus mirabilis; and 8, 30°C, 5g, 6 pw and 8 ml for Bacillus subtilis.

The residual As ion in soil was determined with Atomic Absorption Spectrophotometer (GBC SensAA, Model no. A6358) in triplicate [16, 18] at times 7, 14, 21, 28 and 35 days after centrifuging the soil samples to eliminate the organisms [17, 18].

The ions removed with time and at equilibrium in mg/kg were determined from Equations (1) and (2) [19, 20].

$$q_t = \frac{(C_o - C_t)}{m}.V \qquad (1)$$

$$q_e = \frac{(C_o - C_e)}{m}.V \qquad (2)$$

C_0, C_t, C_e, q_t, q_e, V and m are the initial, residual, equilibrium ions in mg/kg present in soil; amount removed with time, amount removed at equilibrium in mg/kg; volume (m^3) of soil used and the mass (g) of organisms put in contact with the bacteria [17].

The experimental data was fitted with pseudo-first order, pseudo-second order, simple Elovich and intra-particle diffusion models to evaluate the kinetics of the metal ion biosorption from the soils.

3. RESULTS AND DISCUSSION

3.1 Microbiology Analysis

Microbial analysis conducted for characterizing the organisms produced the requisite bacteria from the developed colonies of 2.5 x 10^2cfu/ml. The organisms were recognized with the various biochemical tests. B. subtilis shown gram positive rods (GPR) in gram stain and positive catalase, positive oxidase, negative indole, negative citrate, positive glucose, positive lactose, negative H_2S and positive motility. P. mirabilis shown gram negative rods (GNR) in gram stain, and positive catalase, negative oxidase, negative indole, negative citrate, positive glucose, positive lactose, positive H_2S and positive motility.

3.2 Optimum Factors

The varied values of 1, 2, 3, 4, 5, 6 and 7ml; 1, 2, 3, 4, 5, 6 and 7g; 20, 25, 30, 35, 40 and 45°C; 3, 4, 5, 6, 7, 8, 9 and 10; and 0, 1, 2, 3, 4, 5 and 6 per week (pw) of Nutrient dosage, organisms' weights, temperature, pH and stirring frequency per week respectively yielded the optimum values of pH, temperature (°C), organisms' weights (g), stirring frequency (pw) and nutrient dosage (ml) of 7, 30°C, 1g, 6pw and 8 ml for Proteus mirabilis; and 8, 30°C, 5g, 6 pw and 8 ml for Bacillus subtilis.

3.3 Pseudo-First Order Model

Pseudo first order equation generally expressed as given in equation (3) was linearized. The model parameter q_e is the sorption capacity at equilibrium ($mg.kg^{-1}$); q_t is the sorption capacity at time t ($mg.kg^{-1}$); and k is the rate constant of pseudo first order adsorption (d^{-1}).

$$\frac{dq_t}{dt} = k(q_e - q_t) \qquad (3)$$

The linear fits between ln (q_e-q_t) and t for the ion removal was obtained as shown in Figure 1 showing the regression equations and the coefficients of determination, R^2. The models' parameters, K and q_e deduced from the fit are -0.085$d^{-1}$and 0.0385 for Bacillus subtilis; -0.097$d^{-1}$and 0.1084 for Proteus mirabilis. The very high R^2 values of the models indicated that batched experimental data can be described by pseudo-first-order kinetic models.

The model showed a better fit for removal by Bacillus subtilis than Proteus mirabilis. This was ascertained from their R^2 of 0.965 for Bacillus subtilis and 0.933 for Proteus mirabilis. The order of ion removal by the organisms as depicted by the K values of -0.085$d^{-1}$and -0.097d^{-1} is B. Subtilis before P. mirabilis.

3.4 Pseudo-Second Order Model

The pseudo second order kinetic equation expressed by [21] and presented in equation (4) was linearized. The parameter, q_e is the sorption capacity at equilibrium ($mg.kg^{-1}$); q_t is the sorption capacity at time t in $mg.kg^{-1}$; and k_1 is the rate constant of pseudo second order sorption in kg. $mg^{-1}d^{-1}$.

$$\frac{dq_t}{dt} = k_1(q_e - q_t)^2 \qquad (4)$$

The linear fits between t/q_t versus t for the ion was obtained and shown in Figure 2 bearing the regression equations and the coefficients of determination, R^2 while models' parameters, K_1, q_e and h_o were calculated.

The very high R^2 values of 0.955 and 0.894 of the model for arsenic ion removal by Proteus mirabilis and bacillus subtilis respectively showed very good correlation, and indicated that the batched experimental data can be described by pseudo-second-order kinetic model.

The order of removal of the ion as described by K_1 values is Bacillus subtilis before Proteus mirabilis.

These K_1 values were derived to be 0.7437 kg/mg.d and 0.2002 kg/mg.d respectively. This is an indication that the removal capacity of Bacillus subtilis exceeded that of Proteus mirabilis. The h_o values were calculated to be 0.0016 $mg.kg^{-1}.d^{-1}$ and 0.0032 $mg.kg^{-1}.d^{-1}$ for B. subtilis and P. mirabilis respectively.

3.5 Elovich Model

The Elovich equation expressed in [22] and presented in equation (5) was linearized and employed to fit experimented data. The parameter α is the initial sorption rate in $mg.kg^{-1}$; and β is desorption rate constant $mg.kg^{-1}d^{-1}$.

$$\frac{dq_t}{dt} = \alpha \exp\left(-\beta q_t\right) \tag{5}$$

The linear relationship fits between q_t versus ln(t) for arsenic ions biosorption using the selected microorganisms are shown in Figures 3. This displayed the regression equations and the coefficients of determination R^2, while the values of models parameters α and β were derived to be 0.0037 $mg.kg^{-1}$ and 100.00 $mg.kg^{-1}d^{-1}$ for B. subtilis; 0.0072 $mg.kg^{-1}$ and 40.000 $mg.kg^{-1}d^{-1}$ for P. mirabilis respectively.

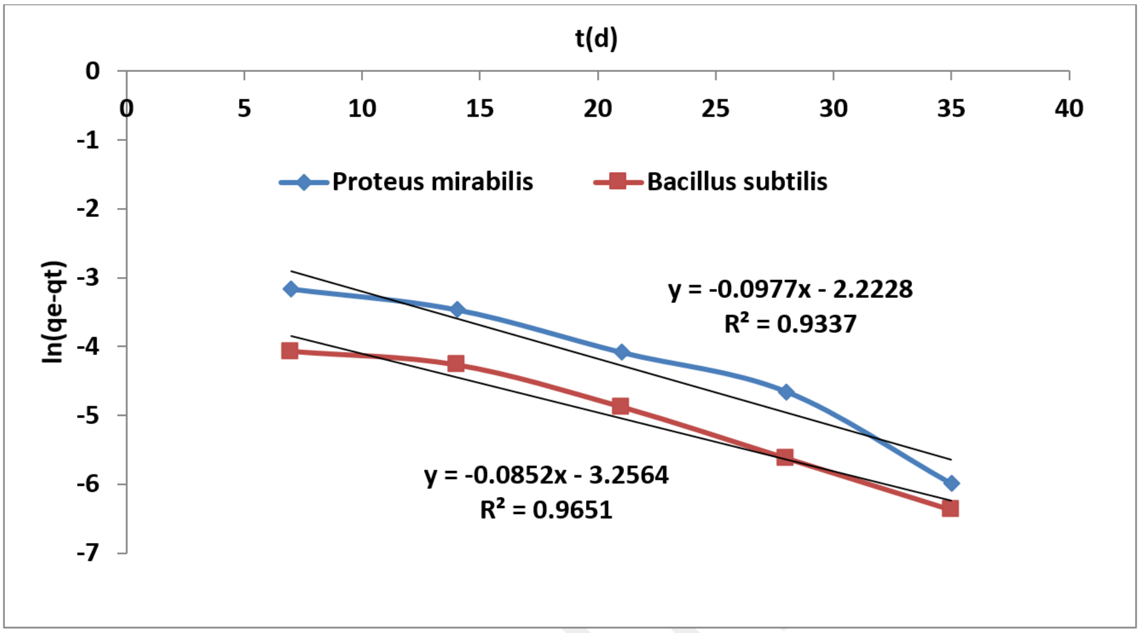

Figure 1: Fitting of Pseudo-First Order Model

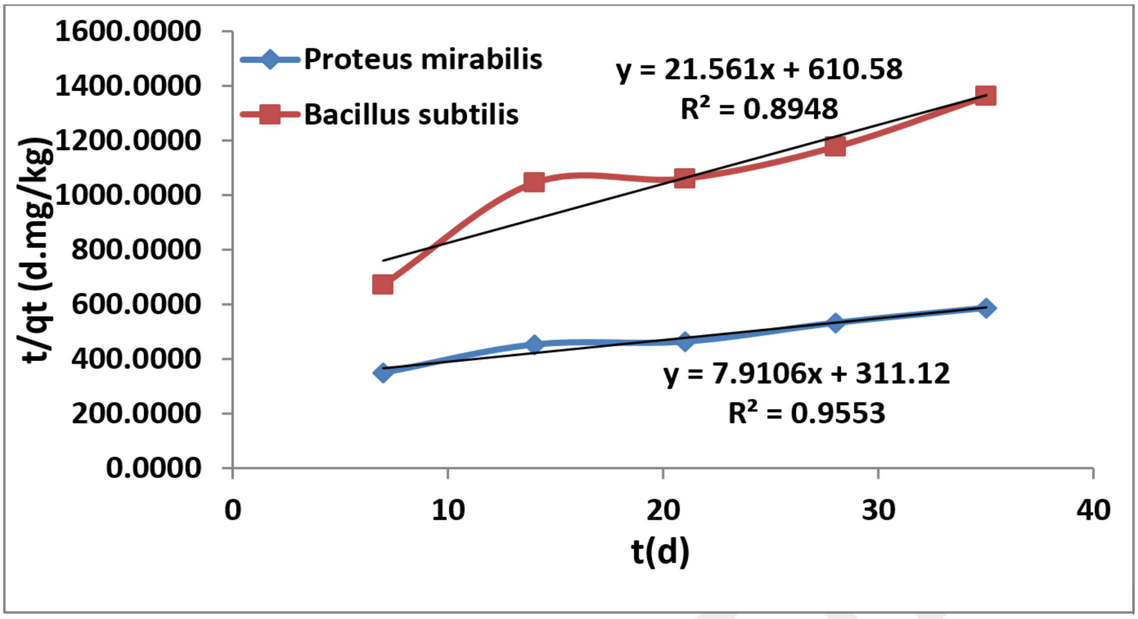

Figure 2: Fitting of Pseudo-Second Order Model

The very high R^2 values of 0.975 and 0.944 of the models for arsenic removal by Proteus mirabilis and Bacillus substilis respectively showed very good correlation, and indicated that the experimental data can be described with Elovich model.

3.6 Intra-particle Diffusion Model

According to [23], intra-particle diffusion model is expressed as presented in equation (6). The model was linearized and used in fitting the experimental data on arsenic removal by the organisms employed. The parameter X is the boundary layer diffusion effects; K_2 is the rate constant for intra-particle diffusion.

$$q_t = K_2 t^{\frac{1}{2}} + X \qquad (6)$$

The linear relationship fits between q_t and $t^{1/2}$ for arsenic ions biosorption using the selected organisms are shown in Figures 4 displaying regression equations and the coefficients of determination, R^2. The sorption capacity K_2 was corresponded to the slope. The very high R^2 values of the models showed very good correlation, indicating that batched experimental data can be described by intra-particle diffusion kinetic model.

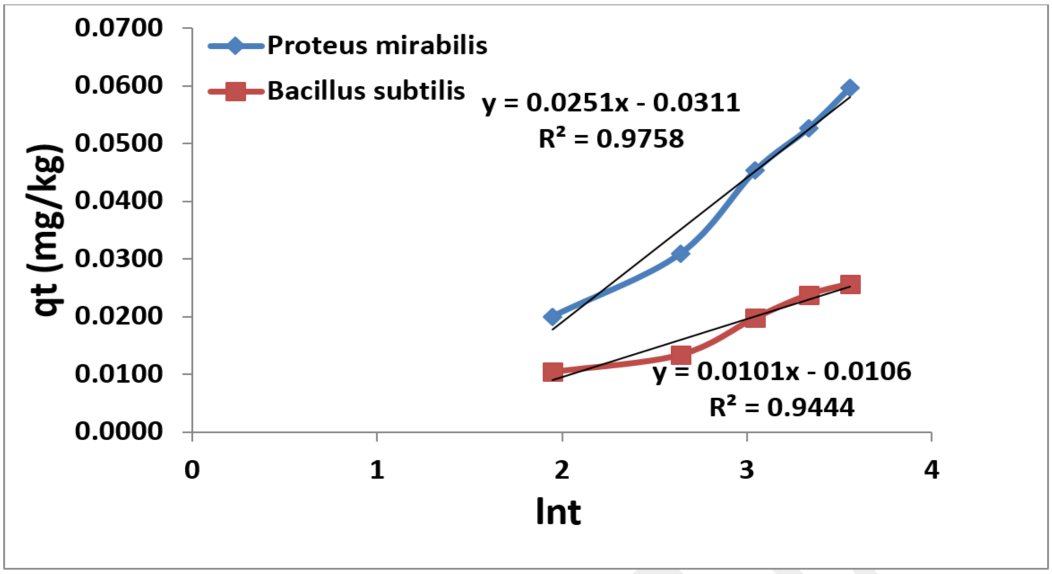

Figure 3: Fitting of Elovich Model

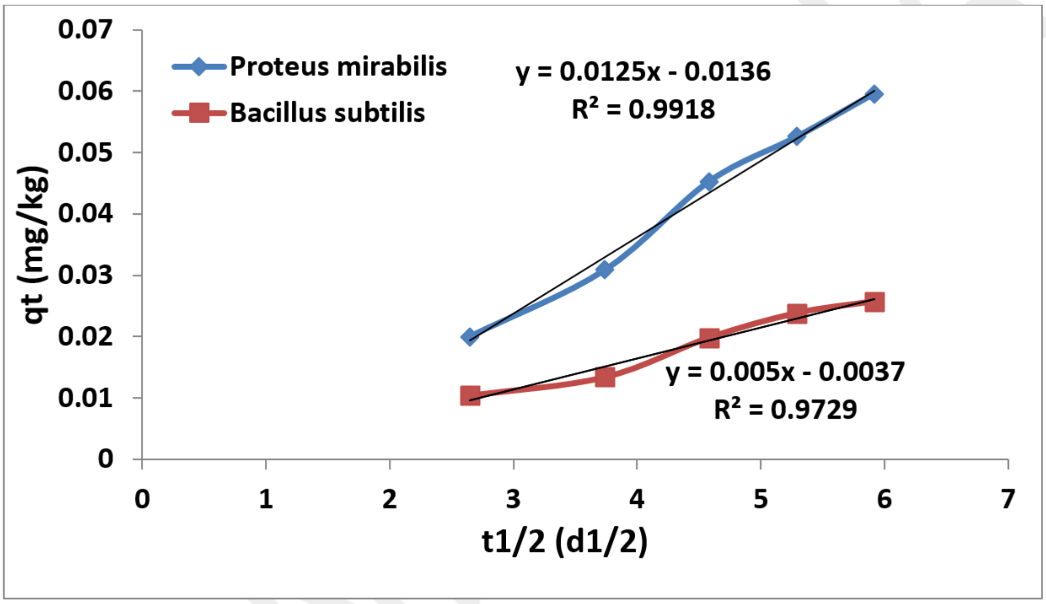

Figure 4: Fitting of Intra-particle Diffusion Model

Table 1: Comparison of Coefficient of Determination

Microorganisms	Coefficient of Determination (R^2)			
	Pseudo-Second Order Model	Pseudo-First Order Model	Elovich Model	Intra-particle Diffusion Model
Proteus mirabilis	0.933	0.955	0.975	0.991
Bacillus subtilis	0.965	0.894	0.944	0.972

The order of fit as deduced from R^2 values is 0.991 for Proteus mirabilis and 0.972 for Bacillus subtilis. The order of sorption capacity as deduced from K_2 values was also Proteus mirabilis with a value of 0.009 mg/kg.$d^{1/2}$ and Bacillus substilis with a value of 0.005 mg/kg.$d^{1/2}$.

Based on the respective models' R^2 values, the four models gave very good fit for arsenic sorption by the organisms. But from the comparison in Table 1, it was discovered that the intra-particle diffusion model gave the best fit with R^2 of 0.991 for sorption by P. mirabilis and 0.972 for sorption by B. subtilis. This indicated that the diffusion process was the rate-limiting step and the systems were transport controlled.

4. CONCLUSION

This work has studied the kinetics of arsenic biosorption by Proteus mirabilis and Bacillus subtilis. It focused on testing the suitability of existing kinetic models in describing the control process of As ion biosorption from contaminated soils.

The four models tested were adequate in describing the sorption kinetics but the extra performance of the intra-particle diffusion model proved the diffusion process to be the rate-limiting step. This indicated that the systems were transport controlled.

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