Kinetics of Arsenic Contaminated Soils Remediation with *Arthrobacter nicotiniae* and *Klebsiella pneumoniae*

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**Abstract**- This work is on bioremediation of soils contaminated with arsenic (As) using *Arthrobacter nicotiniae* (*A. nicotiniae*) and *Klebsiella pneumoniae* (*K. pneumoniae*) with a special focus on the kinetics of the bioremediation. The organisms were indigenous to the treated soils. They were respectively inoculated into 5 g each of soils samples dressed to attain a condition preset with optimum influencers earlier screened from first phase of remediation study. The samples were studied on 7, 14, 21, 28 and 35 days for residual As, and the laboratory results were assessed with four kinetics models. The tested models well described the bioremediation process. However, bioremediation with *A. nicotiniae* was overwhelmed by chemical process and the bioremediation with *K. pneumoniae* was taken charge of by physical process as determined from the rate-limiting steps of chemisorption and diffusion for the influences of *A. nicotiniae* and *K. pneumoniae* respectively. This is vital for the design and operation of an effective treatment system.

**Keywords**- Kinetics, soils, bioremediation, systems

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**1 INTRODUCTION**

The environment is continuously been burdened with diverse injurious metals from manufacturing, mining and agriculture (Kure *et al.*, 2018). The excess load of these injurious elements in soils has limited crop production (Valdman *et al.*, 2001; Vijaya *et al.*, 2010) and portable water availability (Valdman *et al.*, 2001; Vijaya *et al.*, 2010; Adigun and Kayode, 2019). While some metals at desired concentrations are useful for wellbeing of living organisms, some are termed non-essential as they are not in any way of biological significance but are potentially toxic (Ray and Ray, 2009; Musa *et al.*, 2017).

Because of the detrimental impacts of heavy metals, their availability in soil and other environmental components are prevented, monitored and eliminated to protect public health and ecosystems (Salawu *et al.*, 2014). The elimination of the metals from environmental components especially soils and water is through treatment of the contaminated components. There are various methods of treatments. These are grouped under physical, chemical and biological (Kure *et al.*, 2018). Attention is shifting from the physical and chemical methods to the biological methods because of the peculiar disadvantages of the previous. They are expensive, not friendly to ecosystems, not effective at low concentrations; and they cause greater problem of toxicity from the post-treatment products they leave in treated media (Kure *et al.*, 2018). Because of the disadvantages of physical and chemical treatment alternatives, biological treatment method has gained more research attention. It has edge over the physical and chemical methods since it is not accompanied with demerits of economic, environmental and toxicological problems and pains (Kure *et al.*, 2018).

Biological methods involve the use of organisms for treatment of contaminated soils and water (Singh and Gupta, 2016). It encompasses the use of plant (phytoremediation) and the use of microorganisms-living or dead cells (bioremediation) (Kure *et al.*, 2018). Numerous studies have shown that metals contaminated soils can be cleaned using microorganisms. Bioremediation technology depends on enhancing the growth of capable indigenous microbial consortia in contaminated media ([Girma, 2015; Kang *et al.*, 2016]. These organisms could be bacterial, fungi, yeast (Kure *et al.*, 2018) and their consortia can be setup by growth enhancement through nutrient addition, temperature and moisture control or by terminal electron acceptor addition (Boyle *et al.*, 1999; Kulshreshtha *et al.*, 2014).

The kinetics of these organisms’ actions of decontaminating soils is very vital in bioremediation study. The knowledge of kinetics parameters is very important for the design, operation and maintenance of bioremediation systems. In line with the requirement for bioremediation kinetics information, this study is on the kinetics of bioremediation of soils contaminated with arsenic using two microorganisms namely *Arthrobacter nicotiniae* and *Klebsiella pneumoniae*.

**2 MATERIALS AND METHODS**

This study was conducted in Nigeria at Delta State University using facilities in the microbiology laboratory. Nutrient and MacConkey agars prepared in line with Cheesebrough(2000) guidelines were purred on 0.1 ml soil aliquot each in different petri dishes in line with methods of Baron *et al.* (1994). This aliquot was obtained from soil dilutions 10⁻¹, 10⁻² and 10⁻³. The soil was obtained from a contaminated forest soil at Amaonye-Ishiagun in Ebonyi State, Nigeria. The petri dishes contents were maintained at 37°C for 24 hours and the noticed colonies were harnessed and sub-cultured in line with guidelines in Cheesebrough (2000) and Holt (1994).

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3 RESULTS AND DISCUSSION

Microbiology and biochemical study in line with Cheesebrough (2000) and Holt (1994) methods generated the results of K. pneumoniae and A. nicotiniae from the analyzed colony of 4.2 \times 10^2. The organisms were recognized with negative oxidase response; positive citrate response; negative response to hydrogen sulphide; positive response to catalase; positive to glucose; positive motility; positive response to indole; gram negative rods from gram staining for both organisms; positive lactose for A. nicotiniae; and positive lactose for K. pneumoniae. Analysis of factors for optimum actions of organisms portrayed that bioremediation of the soils would be best favored by a combination of 6 ml nutrient, 6pw stirring frequency, 35°C temperature, pH 7, and 1g organism’s weight for A. nicotiniae; and 10 ml nutrient, 6pw stirring frequency, 35°C temperature, pH 7, and 5g organism’s weight for K. pneumoniae. This is in accordance with the report of Deepali (2011) that bioremediation requires a good blend of parameters to promote optimal activities of organisms.

The kinetics evaluated by linearizing the intra-particle diffusion, pseudo-first order, elovich, and pseudo-second order models and plotting their respective \( q_t \) versus \( t^{1/2} \); \( \ln (q_t-q_e) \) versus \( t \); \( q_t \) versus \( \ln (t) \); and \( t/q_t \) versus \( t \) components was informative. Kinetics results are relevant for comprehending remediation rate (Owamah, 2014) and parameters selection for remediation design purpose (Owamah, 2014; Boparai et al., 2011). The pseudo-first-order was studied by fitting \( \ln (q_t-q_e) \) and \( t \) for the action of the organisms and presented in Figure 1 with the \( R^2 \) and the regression equations displayed. The parameters \( k \) and \( q_0 \) of model as deduced are -0.1053 \( d^1 \) and 0.0640 mg/kg for K. pneumoniae and -0.082 \( d^1 \) and 0.0667 mg/kg for A. nicotiniae. The \( R^2 \) values reflected good correlation and depicts that the removals by both organisms followed the model profile and can be describe by the model. The good fits are not with the exclusion of preference as shown by the values of the \( R^2 \). The preference was in the order of A. nicotiniae before K. pneumoniae with \( R^2 \) of 0.958 and 0.933 respectively. The \( k \) values showed that action of A. nicotiniae has a greater removal rate than K. pneumoniae.

The Pseudo-second-order was tested with the fits of \( t/q_t \) against \( t \) in Figure 2. The regression equations generated from the fits and the \( R^2 \) are displayed on Figure 2. The deduced parameters \( k_1 \) and \( q_e \) are 0.5752 kg.mg\(^{-1}d^1\) and 0.06184 mg/kg for K. pneumoniae and 1.2260 kg.mg\(^{-1}d^1\) and 0.0880 mg/kg for A. nicotiniae. The fit results showed a good description of laboratory data by the tested model of \( R^2 \) values of 0.995 and 0.927 for the actions of A. nicotiniae and K. pneumoniae respectively. The A. nicotiniae had a higher rate of removal than K. pneumoniae.

The laboratory data was tested with elovich model by plotting \( q_t \) versus \( \ln (t) \) as shown in Figure 3. The \( R^2 \) values are 0.982 for A. nicotiniae and 0.957 for K. pneumoniae. These values showed that bioremediation results followed the elovich model profile. K. pneumoniae was with a higher initial sorption rate, \( \alpha \) than A. nicotiniae. The \( \alpha \) values were 0.3407 for K. pneumoniae and 0.0190 for A.
The intraparticle diffusion output followed the laboratory data as indicated by the $R^2$ values of 0.985 for *K. pneumoniae*, 0.954 for *A. nicotiniae* in the plot of $q_t$ against $t^{1/2}$ presented in Figure 4. The model showed a better fit for removal by *A. nicotiniae* than *K. pneumoniae*. The order of sorption capacity revealed by the values of $k_2$ is *A. nicotiniae* with $k_2$ value of 0.009 mg/kg.d$^{1/2}$ before *K. pneumoniae* with $k_2$ value of 0.006 mg/kg.d$^{1/2}$.
The evaluation showed that the laboratory data followed the profiles of the four fitted models very well. Atikpo et al. (2019) reported similar fits of the four models with laboratory data of As sorption from soils using Proteus mirabilis and Bacillus subtilis. Ihimekpen et al. (2020) also reported similar fits of the four models with laboratory data of cadmium removal from soils using the mentioned organisms in Atikpo et al. (2019) and Escherichia coli.

However, in this study, further scrutiny evidenced that kinetics of treatment with A. nicotiniae followed best the pseudo second order profile while that of treatment with K. pneumoniae followed best the intraparticle diffusion profile. These revealed that the method of this metal removal by A. nicotiniae was influenced more by chemical process; and that of removal with K. pneumoniae was more influenced by physical process.

4 CONCLUSION
This is a study of the kinetics of bioremediation of soils contaminated with arsenic using Arthrobacter nicotiniae and Klebsiella pneumoniae. The tested models well described the bioremediation process. However, bioremediation with A. nicotiniae was taken-over by chemical process and the bioremediation with K. pneumoniae was taken-over by physical process. These were obvious from the rate-controlling steps of chemisorption and diffusion for the bioremediation with A. nicotiniae and K. pneumoniae respectively. This will be useful for the design and operation of a suitable treatment system.

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