

## Application of Langmuir and Freundlich Isotherm Models on Biosorption of Pb<sup>2+</sup> by Freeze-dried Biomass of *Pseudomonas aeruginosa*

Abdel-Hamied M. Rasmey<sup>#</sup>, Akram A. Aboseidah and Alshimaa K. Youssef

Botany and Microbiology Department, Faculty of Science, Suez University, Suez, Egypt.

**T**ODAY, the pollution of soil and aquatic environment by lead metal ion results from the discharged industrial waste water represents serious environmental problem. Biosorption is an ecofriendly technology that uses microbial biomasses to bind heavy metals on their surfaces by physicochemical pathways from waste water. Seventy-two bacterial isolates resistant to lead metal ion were recovered from 30 sewage water samples collected from different streets of Faisal district in Suez Governorate, Egypt. Interestingly, the isolate number 2103 was selected among them as the most highly resistant to high concentrations of Pb<sup>2+</sup>. This isolate was characterized morphologically, biochemically and identified by 16S rRNA gene sequencing as *Pseudomonas aeruginosa* and deposited in the GenBank database under the accession number KY712434. Studying the effects of contact time and pH on Pb<sup>2+</sup> biosorption by the freeze-dried biomass of *Pseudomonas aeruginosa* 2103 revealed that the maximum biosorption was achieved within 30min at pH 6. The maximum adsorption capacity ( $q_{max}$ ) of Pb<sup>2+</sup> removal by the freeze-dried biomass of *Pseudomonas aeruginosa* 2103 was 114.94mg/g. The regression coefficients (R<sup>2</sup>) were 0.8847 and 0.9751 from the Langmuir and Freundlich isotherm models, respectively, so the biosorption of Pb<sup>2+</sup> onto the biomass of *Pseudomonas aeruginosa* 2103 has been found to fits Freundlich isotherm model better than Langmuir isotherm model.

**Keywords:** Lead, *Pseudomonas*, Langmuir, Freundlich, Biosorption, GenBank.

### Introduction

Over the last years, the increase of urbanization, populations, industrialization and human activities have been increased the environmental pollution in different parts of the world (Abdi & Kazemi, 2015). One of the most serious environmental problems in the industrial areas of our country (Suez Governorate) is the discharge of untreated waste water in soil and aquatic ecosystem. Of all pollutants in the discharged waste water, heavy metals are of concern due to the fact that they have lethal effects and toxicity on all forms of life and are non-biodegradable and hence persist for a long time in the aquatic ecosystems (Jackson et al., 2001; Argun & Dursun, 2006; Attahiru et al., 2012 and Coronado et al., 2017). Lead (Pb) is considered the major metal in the discharged waste water that comes from several industrial processes, such as petroleum refining, lead arsenate insecticides, lead water pipes, chemical manufacturing, electroplating, mining and battery manufacturing, metal finishing, tanning, automobile, pipes, mechanics and dyes industry

(Jarosławiecka & Piotrowska-Seget, 2014 and Kariuki et al., 2017).

It was declared that unlike zinc, copper and manganese metal ions, lead is not known to be of any useful biological activity and it is toxic for human, animal and plants at very low concentrations (Bruins et al., 2000). In humans lead accumulation in blood leads to disturbance in liver and kidneys function, damage the central nervous system and reproductive organs (Rodriguez-Tirado et al., 2012 and Mohy El-Din, 2017). Other symptoms of lead severe toxicity include irritability, muscle tremor, dullness, poor attention span, restlessness, hallucinations, loss of memory and headaches (ATSDR, 1990). Altered membrane integrity, function, permeability resulted from the interactions of lead with oxidation of membrane lipid (Jan et al., 2015). Also, the exposure to lead compounds results in the increase in the level of total cholesterol, triglycerides, and elevated lipoprotein content, which associated with cardiovascular (Poreba et al., 2011). Substitution of calcium in bone by lead

<sup>#</sup>Corresponding author email: am\_rasmey@yahoo.com

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is another cause of lead toxicity (Abdi & Kazemi, 2015).

In plants, lead affects physiological processes, for example: A plant with high lead concentration can secure the generation of reactive oxygen species (ROS), which causes lipid membrane damage that eventually leads to hazard damage of chlorophyll and photosynthetic processes and in the end suppresses the overall growth of the plant (Najeeb et al., 2014). While at low concentrations, lead treatment was found to cause high instability in ion uptake by plants and this in turn leads to important metabolic changes in photosynthetic limit and at last in a strong inhibition of plant development (Jaishankar et al., 2014).

Due to the above mentioned negative impacts of lead metal ions on the life forms and environment, removal of this metal from wastewaters is required before discharge. Removal of heavy metals by the used conventional methods such as chemical precipitation, electro-deposition, ion exchange, membrane separation, coagulation and reverse osmosis is suitable at high concentrations but these methods are not applicable at the low concentrations and also result in secondary pollutants like sludge (Zouboulis et al., 2004; Igwe & Abia, 2006; Onyanha et al., 2008 and Abdel-Aty et al., 2013). Therefore, it is essential to find more effective alternative method for removing the toxic heavy metals from the industrial effluents (Wang & Chen, 2009 and Pahlavanzadeh et al., 2010).

There are various microbial species including bacteria, fungi and algae can grow in wastewaters and resist the toxic metals are known to be able of accumulating heavy metals onto their biomass surfaces (Vijayaraghavan & Yun, 2008). The detoxifying capability of these resistant microbes can be employed for bioremediation of these heavy metals through the processes as bioaccumulation, biosorption and bioprecipitation in the environment (Haroun et al., 2017). The biological removal of heavy metals from industrial effluents offers high efficiency when metals are in low concentrations. Bacterial species are most commonly used as biosorbents due to their small size, ubiquity and capability to grow under controlled conditions and pliability to a wide range of environmental conditions (Ramya & Thatheyus, 2018).

Biosorption is metal uptake from the environment by microbial biomass due to the presence of different functional groups such as amino, hydroxyl and carboxyl which bind the metal by adsorption (Nilanjana et al., 2007 and Wang & Chen, 2009). Biosorption has certain inherent advantages over conventional processes such as using a waste biomass, non-consuming time, high efficiency, cost effectiveness and no sludge formation during treatment (Volesky, 2001). Moreover, metal can be readily recovered from the biomass by desorption if the value and amount of recovered metals are significant and if the biomass is plentiful, metal-bound biomass can be incinerated, thereby extracting further treatment. The biosorption process depends on the type of metal ions, the cell wall structure of microbial biomass, cells shape, as well as physicochemical factors such as pH, temperature, contact time, ionic strength, and metal concentration (Gabr et al., 2008 and Wierzba, 2015).

The main objective of this study was to isolate and characterize lead resistant bacterial isolate as well as to determine the maximum absorption capacity of its dried biomass for removal of lead metal ions from aqueous solutions.

## **Materials and Methods**

### *Isolation of lead metal resistant bacteria*

Thirty samples of sewage water were collected in sterilized plastic bottles from different locations in Suez Governorate and transferred immediately to the laboratory for examination. The lead metal resistant bacterial species were isolated from the collected samples by pour plate method on tryptic soya agar (TSA) medium of pH 6.5 supplemented with 25ppm of lead metal ( $Pb(NO_3)_2$ ). The inoculated plates with 1ml of  $10^{-5}$  diluted sample were incubated after solidification at 37°C for 4 days. The grown separated bacterial colonies on agar plates were picked up and purified then stored on TSA slants at 4°C for further experiments. The concentration of 1000ppm Pb (II) solution was prepared by dissolving 1.598g of  $Pb(NO_3)_2$  in 1000ml deionized and the other concentrations were prepared by a serial dilution of this stock. The isolated bacteria were cultivated again on tryptic soya broth (TSB) of pH 6.5 with different concentrations of  $Pb^{2+}$  (25–200ppm with 25 intervals) to select the most highly resistant bacterial isolate applicable for biosorption of lead metal.

*Phenotypic and genotypic identification of the selected bacterial isolate 2103*

*Morphological and biochemical characterizations*

The colony characteristics of the bacterial isolate 2103 such as shape, color, surface and margin on nutrient agar medium were examined by the naked eye. The bacterial cells after 24h incubation were stained by Gram stain and the cells shape and arrangement were observed by the oil lens of the light microscope. Also, this isolate was characterized biochemically according to Bergey's manual of systematic bacteriology (Holt et al., 1986). The examined biochemical tests were oxidase, citrate, catalase, MR-VP (Methyl Red and Voges-Proskauer), urease, indole, H<sub>2</sub>S production, gelatin liquefaction, starch hydrolysis, and glucose and lactose fermentation.

*Molecular characterization*

DNA was extracted from the bacterial cell pellets by an SDS/CTAB lysis using the phenol/chloroform extraction method (Ausubel, 2002). The 16S rRNA gene was PCR-amplified using the two primers 16SF: 5'-GAGTTTGATCCTGGCTTAG-3' and 16SR: 5'-GGTTACCTTGTTACGACTT-3'. The PCR amplification was done using Qiagen Proof-start Tag Polymerase kit (Qiagen, Hilden, Germany). The total reaction volume of 25µl including 2µl of template DNA (20ng/µl), 12.5µl PCR Master Mix, 20 pmol (2µl) each of forward and reverse primers and 8.5µl of DNAase free water. The reaction conditions were: An initial denaturation at 94°C for 5min, 37 cycles of denaturation at 94°C for 30sec, annealing at 51°C for 30sec and extension at 72°C for 30sec. A final extension was directed at 72°C for 5min. PCR products were detected by electrophoresis on 1.5% (w/v) agarose in 1X TAE buffer. PCR product of 1500bp was purified from gel with QIA quick gel extraction kit (Qiagen, Hilden, Germany). PCR product was sequenced by cycle sequencing with dideoxy mediated chain-termination (Sanger et al., 1977). The obtained nucleotides sequence of 16S rRNA gene was subjected to the BLAST software at the NCBI server: <http://www.ncbi.nlm.gov/BLAST/> to examine the similarity and dissimilarity percent. The alignment of sequence was searched by CLUSTALW program (<http://clustalw.ddbj.nig.ac.jp/top-ehtml>). Phylogenetic tree derived from 16S rRNA sequences of the most related species in GenBank database was performed by the TREE VIEW program.

*Growth pattern of Pseudomonas aeruginosa 2103*

Bacterial cells were inoculated in TSB (Tryptic soya broth) of pH 6.5 supplemented with different concentrations of Pb<sup>2+</sup> (5, 50, 90 and 110ppm) in comparison with control (without Pb<sup>2+</sup>) and incubated for 55h at 37°C. The growth was estimated at different time intervals by determining the optical density (OD) of broth turbidity at 630nm by the spectrophotometer.

*Biosorption of Pb<sup>2+</sup> by Pseudomonas aeruginosa 2103*

The bacterial biomass was prepared by inoculation of fresh (24h) bacterial cells on TSB medium and incubated for 40h at 37°C and then the grown biomass was collected by centrifugation at 5000g for 15min. The collected biomass was freeze-dried and stored at room temperature for biosorption experiments. The biosorption of lead metal from aqueous solution by the freeze-dried biomass of the bacterium was performed by agitation (200rpm) of constant weight (20mg) of biomass in constant volume (20ml) of the lead metal solution for one hour at room temperature. The reaction medium was centrifuged at 5000g for 15min and the remaining concentration of Pb<sup>2+</sup> in the supernatant was estimated by atomic absorption spectrophotometer (CPU analyzers, Faculty of Agriculture, Suez Canal University).

The equilibrium adsorption amount ( $q_e$ ) of lead metal ions by the freeze-dried biomass of bacterium was calculated from the general condition:

$$q_e \text{ (mg/g)} = [(C_i - C_e) * V] / W \quad (1) \text{ (Tunali et al., 2006).}$$

where  $q_e$  is the amount (mg/g) of metal ions adsorbed on the biomass,  $C_i$  is the initial metal ion concentration (mg/L),  $C_e$  is the final metal ion concentration (mg/L),  $V$  is the volume (ml) of the solution and  $W$  is the weight (g) of the used biomass.

*Optimization of pH, contact time and Pb<sup>2+</sup> initial concentration*

The impact of pH was tested with the agitation of 20mg freeze-dried biomass of *Pseudomonas aeruginosa* 2103 in 20ml of 100ppm Pb(II) solution of different pH values (2, 4, 6 and 7) at 200rpm for 60min at room temperature (25±2). The impact of contact time was also tested by varying the incubation period (0–60min) of

the reaction medium at the ideal pH and room temperature. Also effect of initial concentrations of lead solution on the biosorption efficiency were studied by adding 20mg of the freeze-dried biomass in 20ml of different initial concentrations (0–200ppm) of Pb(II) and agitated at 200rpm at the ideal contact time and pH incubated at room temperature.

## Results and Discussion

### *Phenotypic and genotypic identification of the bacterial isolate 2103*

Isolation of highly resistant bacterial species is required to fulfill the bioremediation of toxic heavy metals from waste water and inhibit their accumulation in the environment. Seventy-two bacterial isolates resistant to 25ppm of Pb<sup>2+</sup> were recovered from 30 sewage water samples. All these isolates were screened for their capacity to grow on broth medium with different concentrations (25–200ppm) of Pb<sup>2+</sup> and the data obtained indicated that 16 bacterial isolates were able to grow on 130ppm. The only bacterial isolate no. 2103 was able to survive until 170ppm. Several studies by various authors isolated lead metal resistant bacteria and used them in the removal of lead metal from soil and water (Pardo et al., 2003; Selatnia et al., 2004; Murthy et al., 2012 and Wierzba, 2015). Velusamy et al. (2011) reported that the isolate *Bacillus* X4 which was recovered from contaminated soil was resistant to 50ppm of Pb(II).

The isolate number 2103 was selected as the most highly lead metal resistant bacteria for further investigation and subjected to the phenotypic and genotypic characterizations. Different morphological and biochemical characteristics of the isolate no. 2103 were examined according to Bergey's manual of systematic bacteriology (Kreig & Holt, 1984 and Murthy et al., 2012). Data shown in Table 1 indicated that the isolate characterized by irregular, oval, mucoid colonies with diffusible green pigment on nutrient agar medium. The cells are Gram negative rod shaped. The isolate was negative for each of H<sub>2</sub>S production, urease production, starch hydrolysis, indole, glucose and lactose fermentation tests and was positive for the other biochemical tests such as gelatin liquefaction, oxidase production, catalase production, methyl red and Voges-Proskauer. According to these phenotypic characteristics, the bacterial isolate number 2103 was identified

as *Pseudomonas* sp. For confirmation of this identification and identify the isolate on species level, the 16S rRNA gene was PCR-amplified and the nucleotides sequence was studied. The obtained sequence was aligned and contrasted with other recorded gene sequences in the database of National Center for Biotechnology Information (NCBI). The results demonstrated that the isolate no. 2103 is 100% identical to *Pseudomonas aeruginosa* NR118644.1. So, the isolate no. 2103 was identified as *Pseudomonas aeruginosa* and assigned the accession number KY712434 in the Gene Bank database. The sequence of the isolate was contrasted with different 16S rRNA gene sequences and the phylogenic tree was shown in Fig. 1. *Pseudomonas aeruginosa* efficiency for metal uptake has been reported by several authors (Strandberg et al., 1981 and Texier et al., 1999). König-Péter et al. (2014) selected the genus *Pseudomonas* as a biosorbent for lead (II), copper (II) and nickel (II), among 12 bacteria isolated from activated sludge.

**TABLE 1. Phenotypic characteristics of the bacterial isolate no. 2103.**

Characteristics	Observation
Colony colour	blue- green
Colony shape	oval
Colony margin	irregular
Colony surface	mucoid
Gram reaction	-
Cells shape	rod
Motility	+
Lactose fermentation	-
Glucose fermentation	-
Catalase production	+
Oxidase production	+
Indole production	-
Voges-Proskauer	+
Methyl red	+
Citrate utilization	+
Urease production	-
H <sub>2</sub> S production	-
Gelatin hydrolysis	+
Starch hydrolysis	-

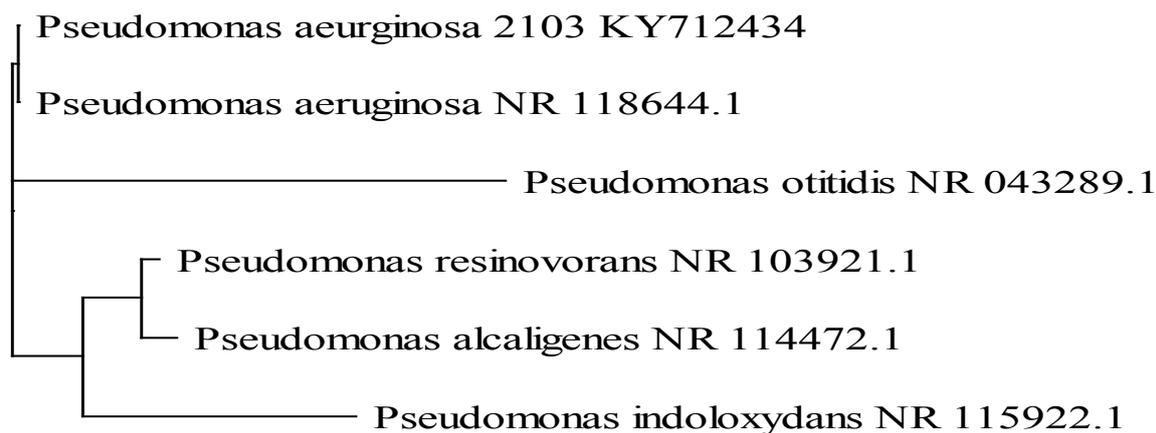


Fig. 1. The neighbor-joining tree of the isolate 2103 and the related strains based on 16S rRNA gene sequences.

#### Resistance of *P. aeruginosa* 2103 to different concentrations of Pb (II)

*Pseudomonas aeruginosa* 2103 was grown on TSB medium amended with different concentrations (5, 50, 90 and 110ppm) of Pb<sup>2+</sup> in comparison with control and the growth pattern was determined as shown in Fig. 2. The isolate showed a high resistance to the tested concentrations of Pb<sup>2+</sup>. The growth of the isolate increased gradually with time in all Pb<sup>2+</sup> tested concentrations along with the control at the first hours of incubation. The reduction rate of growth was obtained at 50, 90 and 110 of Pb<sup>2+</sup> after 28h of incubation. The higher reduction rate of growth was recorded as 11.79% for 110ppm at 55h incubation. These results indicated the potentiality of isolate *Pseudomonas aeruginosa* 2103 to resist high concentrations of Pb<sup>2+</sup>. Kafilzadeh et al. (2012) studied the growth of *Corynebacterium* and *Pseudomonas* sp. on different concentrations of lead metal from 0.4 to 0.7g/l with consideration of incubation time and found that the growth increase (lag phase) until reach stationary phase at 360min. Growth pattern of *P. aeruginosa* strain BC15 on different concentrations of Cr, Ni, Pb and Cd metals was studied by Raja et al. (2006). It was reported by different authors that the growth of the bacterial isolates is reduced as affected by the metals in higher concentrations comparing to the control (Suresh et al., 1998; Suresh et al., 2001 and Pal et al., 2004).

#### Optimization of pH on biosorption

The impact of hydrogen ion concentrations on the biosorption of heavy metals has been concerned in many studies, which revealed the significance of this parameter on the solubility of the metal ions and also on the ionization

of the binding sites (Sassi et al., 2010; Joo et al., 2010 and Ji et al., 2011). The effect of pH values on biosorption of Pb<sup>2+</sup> by the freeze-dried *Pseudomonas aeruginosa* 2103 was studied and presented in Fig. 3. It is obvious that equilibrium adsorption amount (Q<sub>e</sub>) of Pb<sup>2+</sup> is low at pH 2 and increased with increasing pH and achieved the maximum (83.77mg/g) at pH 6. König-Péter et al. (2014) reported in their study that the pH 5 was the optimum for Pb (II) biosorption by *P. aeruginosa* PAO1. Also, pH 6 was the optimum for lead biosorption by *P. aeruginosa* ASU 6a (Gabr et al., 2008). Wierzba (2015) reported that the optimum pH was 5 for Pb (II) biosorption by *Stenotrophomonas maltophilia* with maximum capacity (q<sub>e</sub>) of 71.4mg/g. Also, Veglió et al. (1997) found that the optimum pH for lead by *Arthrobacter* sp. was 5. At low pH values, cell wall ligands were nearly associated with hydronium ions (H<sub>3</sub>O<sup>+</sup>) thus limit the biosorption of metal ions as a result of the competition between H<sub>3</sub>O<sup>+</sup> and the heavy metals with the bacterial cell wall ligands (Liu et al., 2009). The bacterial cell wall contains negatively charged functional groups, for example carboxyl, phosphate, imidazole and amino groups. They are fundamentally in charge of the anionic character and metal binding capacity of the cell wall by Gram-negative bacteria. With increasing pH, the negative charge on the cell surface increases, which supports the adsorption of the heavy metal cations (König-Péter et al., 2014). The metal biosorption relies upon on the protonation or unprotonation of the functional groups on the cell wall (i.e., carboxylic, hydroxyl and amino groups) (Selatnia et al., 2004; Sautel et al., 1991; Fourest & Volesky, 1997 and Fourest & Roux, 1992).

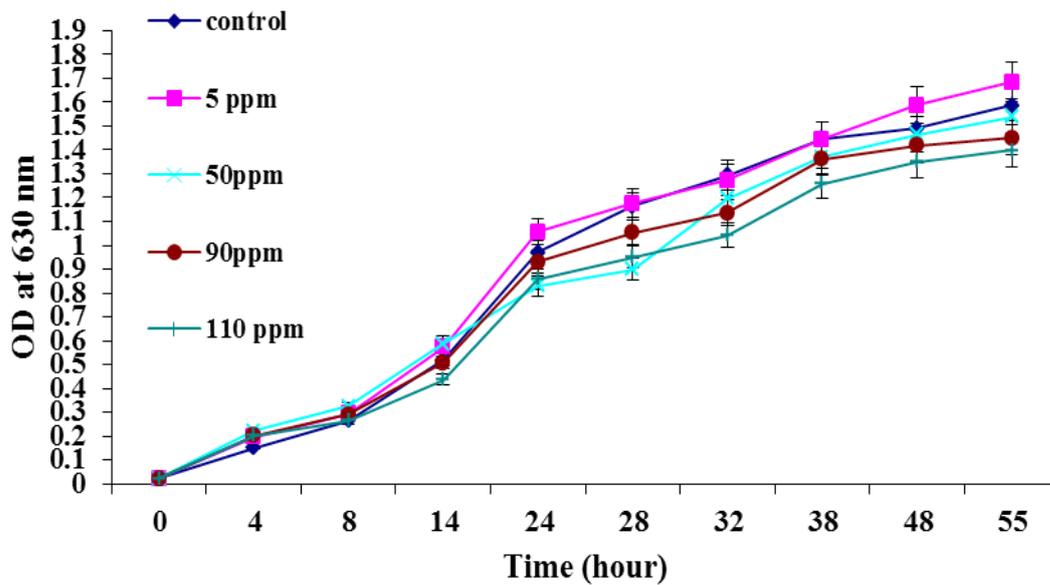


Fig. 2. Growth curve of *Pseudomonas aeruginosa* 2103 on different concentrations of lead metal ions.

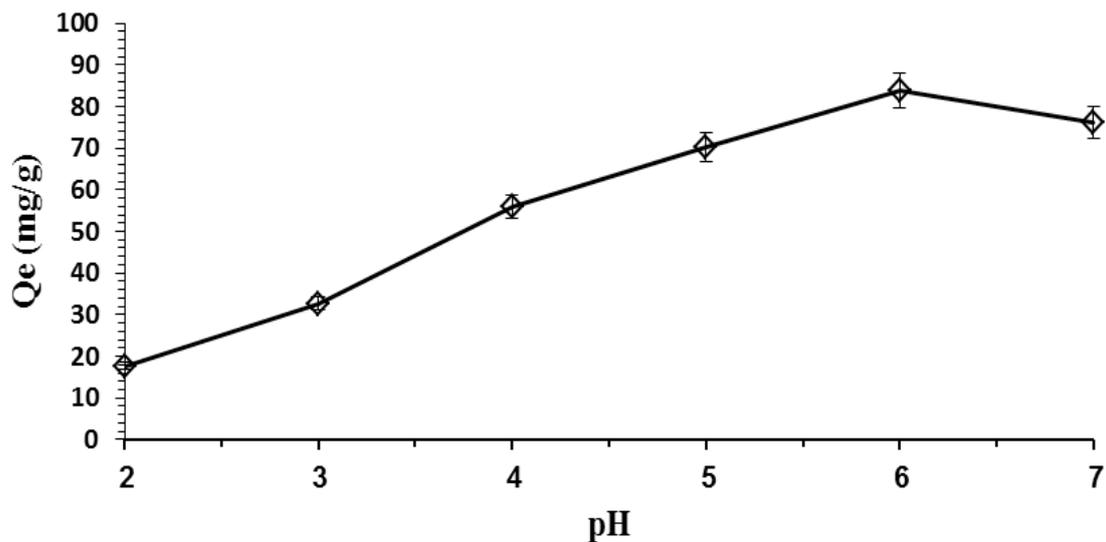


Fig. 3. Effect of pH on biosorption of  $Pb^{2+}$  by freeze-dried biomass of *Pseudomonas aeruginosa* 2103.

#### Optimization of contact time on biosorption

The kinetics of metal ion sorption is a critical parameter for designing sorption frameworks and is required for choosing the optimum operating conditions for full scale bacterial process of metal removal (Liu et al., 2009). The uptake of heavy metals by biomass increased with increasing contact time (König-Péter et al., 2014). The assurance of the ideal contact time for recovery of  $Pb^{2+}$  from the solution is required to raise the efficiency of biosorption process.

The data shown in Fig. 4 revealed the impact of contact time on the lead metal biosorption by freeze-dried biomass of *Pseudomonas aeruginosa* 2103. The finding showed that there is increase in lead uptake quickly in the initial 20min then the rate of removal moderates leading the balance time for metal biosorption at 30min. This short time required for biosorption is in accordance with the results given by other studies (Wierzba, 2015; Joo et al., 2010; Gabr et al. 2008; Pahlavanzadeh et al., 2010; Chen et al., 2005; Ji et al., 2011 and Li et al., 2010).

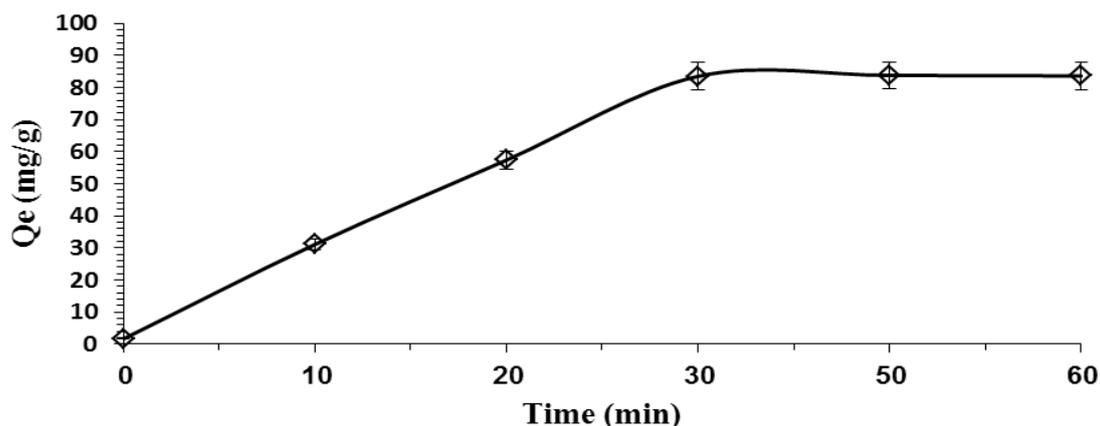


Fig. 4. Effect of contact time on biosorption of  $Pb^{2+}$  by freeze-dried biomass of *Pseudomonas aeruginosa* 2103.

#### Optimization of initial concentration on biosorption

Many investigations have demonstrated that at low  $Pb^{2+}$  concentrations, the amount of adsorbed  $Pb^{2+}$  per unit mass of biosorbent was directly proportional to the ionic concentration in solution (Selatnia et al., 2004). Lead biosorption viability by freeze-dried *Pseudomonas aeruginosa* 2103 at various starting concentrations (0-200ppm) of  $Pb^{2+}$  was assessed at the optimum pH and contact time as appeared in Fig. 5. It is announced that the biosorption limit of the bacterial biomass expanded continuously with the expansion in beginning grouping of  $Pb^{2+}$  and afterward gradually to be consistent at high concentrations. The maximum quantity of adsorbed  $Pb^{2+}$  by the freeze-dried biomass was approximately 98mg/g. This value is higher than that obtained by Tunali et al. (2006), who reported that maximum biosorption capacity of *Bacillus* sp. ATS-1 biomass for Pb (II) was  $92.27 \pm 1.17 \text{ mg g}^{-1}$ .

#### Biosorption isotherms

The biosorption isotherm curve represents the equilibrium distribution of metal ions between the aqueous and solid phases (Wierzbna, 2015 and Kariuki et al., 2017). Several kinetic models exist for the adsorption of heavy metals (Vijayaraghavan & Yun, 2008). Langmuir and Freundlich isotherm models are widely applied in equilibrium analysis to understand sorption mechanisms (Joo et al., 2010; Gabr et al., 2008; Chen et al., 2005; Ji et al., 2011 and Shroff & Vaidya, 2011). The Freundlich equation is:

$$q_e = K_f C_e^{1/n} \quad (2) \text{ (Freundlich, 1906).}$$

The linear form of this model is:

$$\ln q_e = \ln K_f + 1/n \ln C_e \quad (3)$$

where  $K_f$  and  $n$  are the adsorption capacity and the intensity of adsorption, respectively. The Langmuir equation is shown as:

$$q_{eq} = q_{max} \frac{b C_{eq}}{1 + b C_{eq}} \quad (4) \text{ (Langmuir, 1916).}$$

And the linear form of this model is:

$$C_{eq}/q_{eq} = 1/q_{max} b + C_{eq}/q_{max} \quad (5)$$

where  $q_{max}$  and  $b$  are the Langmuir constants.

The Langmuir model considers sorption by monolayer type and supposes that all the active sites on the sorbent surface have the same affinity for heavy metal ions (Hawari & Mulligan, 2006). The Freundlich isotherm is an empirical equation which assumes a heterogeneous biosorption system with different active sites (Li et al., 2010). Freundlich isotherm model is used to estimate the adsorption intensity ( $n$ ) and the adsorption capacity ( $K_f$ ) while Langmuir isotherm model is used to estimate maximum adsorption capacity ( $q_{max}$ ) of  $Pb^{2+}$  biosorption by the freeze-dried *Pseudomonas aeruginosa* 2103 along with values of constant  $b$ . The Langmuir and Freundlich constants have been calculated through the corresponding plots of the metal biosorption as described in Fig. 6 and 7. The calculated values were presented in Table 2. The regression coefficient ( $R^2$ ) obtained for  $Pb^{2+}$  from Langmuir and Freundlich isotherm models were 0.8847 and 0.9751, respectively. In this manner Freundlich isotherm fits better

with the equilibrium of biosorption of lead metal than Langmuir isotherm model. The maximum adsorption capacity of the freeze-dried biomass of *Pseudomonas aeruginosa* 2103 for  $Pb^{2+}$  was 114.94mg/g according to the Langmuir isotherm model. This value is higher than that obtained by Tunali et al. (2006), which was 96.15mg/g of  $Pb^{2+}$  by *Bacillus* sp. ATS-1. Gabr et al. (2008)

demonstrated that for Ni (II) and Pb (II) biosorption by living and lyophilized *P. aeruginosa* ASU 6a cells, the adsorption equilibrium data fitted well with the Langmuir and Freundlich models for metal ions. The findings of this investigation indicate the efficiency of the freeze-dried biomass of the bacterial isolate *Pseudomonas aeruginosa* 2103 for  $Pb^{2+}$  uptake from aqueous solutions.

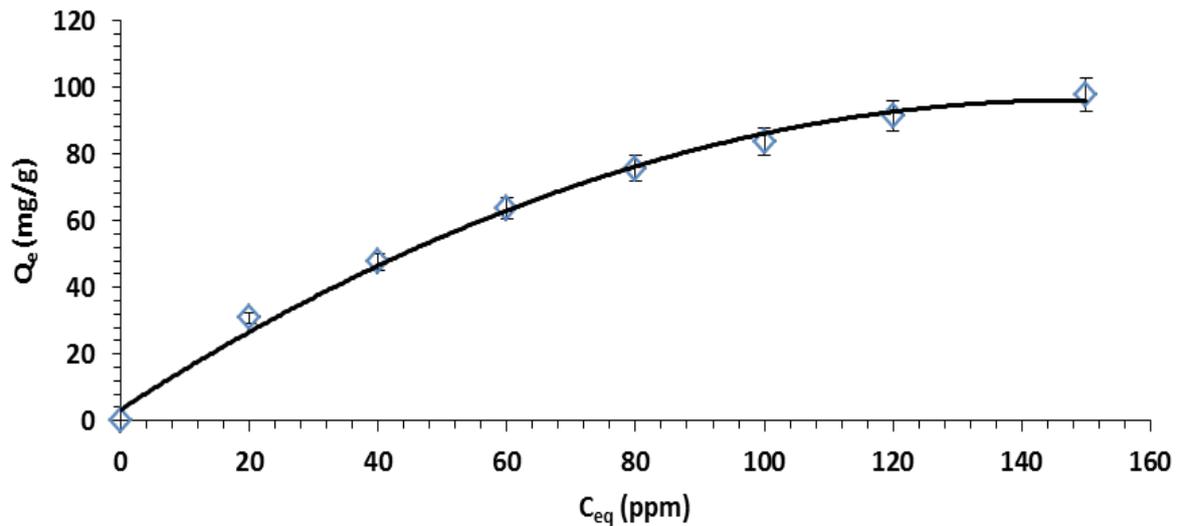


Fig. 5. Adsorption isotherm of  $Pb^{2+}$  by freeze-dried biomass of *Pseudomonas aeruginosa* 2103 at different initial concentrations.

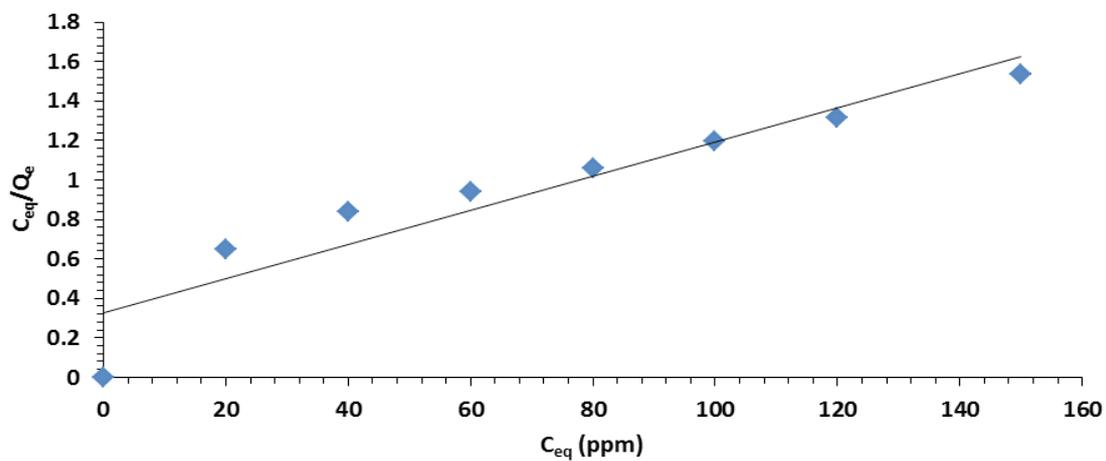


Fig. 6. The linear form of the Langmuir adsorption isotherm of  $Pb^{2+}$  by freeze-dried biomass of *Pseudomonas aeruginosa* 2103.

Table 2. The Freundlich and Langmuir adsorption isotherm constants for  $Pb^{2+}$  biosorption by *Pseudomonas aeruginosa* 2103.

Freundlich			Langmuir		
$k_f$	$n$	$R^2$	$q_{max}$	$b$	$R^2$
1.263	1.077	0.9751	114.94	0.0268	0.8847

## Conclusion

The bacterial isolate *Pseudomonas aeruginosa* 2103 recovered from sewage water is highly resistant to Pb<sup>2+</sup> ions. The freeze-dried biomass of this isolate has proved to be a successful biosorbent for lead removal from aqueous solutions. The equilibrium pH and contact time for lead removal was 6 and 30min, respectively at room temperature. The maximum adsorption capacity ( $q_{max}$ ) from Langmuir isotherm model was 114.94mg/g. These findings indicate the possibility of using *Pseudomonas aeruginosa* 2103 biomass for Pb<sup>2+</sup> biosorption. More investigations are required to study the effect of different parameters and other adsorption isotherms on biosorption process and to clear the mechanisms of action to ensure complete bioremediation of lead metal.

*Conflict of Interest:* The authors have declared no conflict of interest

## References

- Abdel-Aty, A., Ammar, N., Hany, H., Abdel, G. and Rizka, K. (2013) Biosorption of cadmium and lead from aqueous solution by fresh water algae *Anabaena sphaerica* biomass. *J. Adv. Res.* **4**, 367-374.
- Abdi, O. and Kazemi, M. (2015) A review study of biosorption of heavy metals and comparison between different biosorbents. *J. Mater. Environ. Sci.* **6**(5), 1386-1399.
- Argun, M.E. and Dursun, S. (2006) Removal of heavy metal ions using chemically modified adsorbents. *J. Int. Environ. Appl. Sci.* **1**(1-2), 27-40.
- ATSDR [Agency for Toxic Substances and Disease] and Public Health Service/U.S] (1990) Environmental Protection Agency Syracuse Research Corporation Registry. Toxicological profile for lead.
- Attahiru, S., Shiundu, M. and Wambu, W. (2012) Removal of Cr (III) from aqueous solution using a micaceous poly-mineral from Kenya. *Int. J. Phys. Sci.* **7**(8), 1198-1204.
- Ausubel, F.M. (2002) "*Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*", Wiley, New York.
- Bruins, M., Kapil, S. and Oehme, F. (2000) Microbial resistance to metals in the environment. *Ecotoxicol. Environ. Saf.* **45**(3), 198-207.
- Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X. and Chen, Y.X. (2005) Biosorption of copper (II) and zinc (II) from aqueous solution by *Pseudomonas putida* CZ1. *Colloid Surface B*, **46**(2), 101-107.
- Coronado, F., Gacho, C., Tansengco, M. and Barcelo, J. (2017) Isolation and characterization of heavy metals-resistant bacteria from contaminated wastewater in mine tailings of Itogon, Benguet Philippines. *Adv. Biol. BioMed.* **4**(1), 1-13
- Fourest, E. and Roux, J.C. (1992) Heavy metal biosorption by fungal mycelial by-products. Mechanisms and influence of pH. *Appl. Microb. Biotechnol.* **37**(3), 399-402.
- Fourest, E. and Volesky, B. (1997) Alginate properties and heavy metal biosorption by marine algae. *Biochem. Biotechnol.* **67**(3), 215-226.
- Freundlich, H.M. (1906) Over the adsorption in solution. *J. Phys. Chem.* **57**, 1100-1107.
- Gabr, R.M., Hassan, S.H. and Shoreit, A.A. (2008) Biosorption of lead and nickel by living and non-living cells of *Pseudomonas aeruginosa* ASU 6a. *Int. Biodeter. Biodegr.* **62**(2), 195-203.
- Haroun, A., Kamaluddeen, K., Alhaji, I., Magaji, Y. and Oaikhena, E. (2017) Evaluation of heavy metal tolerance level (MIC) and bioremediation potentials of *Pseudomonas aeruginosa* isolated from Makera-Kakuri industrial drain in Kaduna, Nigeria. *Eur. Exp. Biol.* **7**(5), 28.
- Hawari, A. and Mulligan, C. (2006) Biosorption of lead (II), cadmium (II), copper (II) and nickel (II) by anaerobic granular biomass. *Bioresource Technol.* **97**(4), 692-700.
- Holt, J.G., Krieg, N.R., Sneath, P.H. and Staley, J.T. (1986) "*Bergey's Manual of Systematic Bacteriology*" Vol. 2. Williams and Wilkins, Baltimore, USA.
- Igwe, J. and Abia, A. (2006) A bioseparation process for removing heavy metal from wastewater using biosorbents. *Afr. J. Biotechnol.* **5**(120), 1167-1179.
- Jackson, R., Carpenter, S., Dehm, C., McKnight, D.,

- Naiman, R., Postel, S. and Running, S. (2001) Water in a changing world. *Ecol. Appl.* **11**(4), 1027-1045.
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N. (2014) Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.* **7**(2), 60-72.
- Jan, A.T., Azam, M., Siddiqui, K., Ali, A., Choi, I. and Haq, Q.M. (2015) Heavy metals and human health: Mechanistic insight into toxicity and counter defense system of antioxidants: A review. *Int. J. Mol. Sci.* **16**(12), 29592-29630.
- Jaroslawska, A. and Piotrowska-Seget, Z. (2014) Lead resistance in micro-organisms. *Microbiology*, **160**(1), 12-25.
- Ji, Y., Gao, H., Sun, J. and Cai, F. (2011) Experimental probation on the binding kinetics and thermodynamics of Au (III) onto *Bacillus subtilis*. *Chem. Eng. J.* **172**(1), 122-128.
- Joo, J.H., Hassan, S.H.A. and Oh, S.E. (2010) Comparative study of biosorption of Zn<sup>2+</sup> by *Pseudomonas aeruginosa* and *Bacillus cereus*. *Int. Biodeter. Biodegr.* **64**(8), 734-741.
- Kafilzadeh, F., Afrough, R., Johari, H. and Tahery, Y. (2012) Range determination for resistance/tolerance and growth kinetic of indigenous bacteria isolated from lead contaminated soils near gas stations (Iran). *Euro. J. Exp. Bio.* **2**(1), 62-69.
- Kariuki, Z., Kiptoo, J. and Onyancha, D. (2017) Biosorption studies of lead and copper using rogers mushroom biomass '*Lepiota hystrix*'. *South Afr. J. Chem. Eng.* **23**, 62-70.
- König-Péter, A., Kocsis, B., Kilár, F. and Pernyeszi, T. (2014) Bioadsorption characteristics of *Pseudomonas aeruginosa* PAOI. *J. Serb. Chem. Soc.* **79**(4), 495-508.
- Kreig, N.R. and Holt, J.G. (1984) "*Bergey's Manual of Systematic Bacteriology*".
- Langmuir, I. (1916) The constitution and fundamental properties of solids and liquids. Part I. Solids. *J. Amer. Chem. Soc.* **38**(11), 2221-2295.
- Li, H., Lin, Y., Guan, W., Chang, J., Xu, L., Guo, J. and Wei, G. (2010) Biosorption of Zn (II) by live and dead cells of *Streptomyces ciscaucasicus* strain CCNWHX 72-14. *J. Hazard Mater.* **179**(1), 151-159.
- Liu, Y., Cao, Q., Luo, F. and Chen, J. (2009) Biosorption of Cd<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, and Zn<sup>2+</sup> ions from aqueous solutions by pretreated biomass of brown algae. *J. Hazard Mater.* **163**(2), 931-938.
- Mohy El-Din, S.M. (2017) Effect of copper and lead on growth and some metabolic activities of cyanobacterium *Spirulina platensis* (Nordstedt). *Egypt. J. Bot.* **57**(3), 445-456.
- Murthy, S., Bali, G. and Sarangi, S.K. (2012) Lead biosorption by a bacterium isolated from industrial effluents. *Int. J. Microbiol.* **4**(3), 192-196.
- Najeeb, U., Ahmad, W., Zia, M.H., Zaffar, M. and Zhou, W. (2014) Enhancing the lead phytostabilization in wet land plant *Juncus effusus* L through somaclonal manipulation and EDTA enrichment. *Arab J. Chem.* **10**, 3310-3317.
- Nilanjana, D., Vinala, R. and Karthika, P. (2007) Biosorption of heavy metal (an overview). *Indian J. Biotechnol.* **7**, 159-169.
- Onyancha, D., Mavura, W., Ngali, C., Ongoma, P. and Chacha, P. (2008) Studies of chromium removal from tannery waste water by algae biosorbents, *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*. *J. Hazard Mater.* **158**, 605-624.
- Pahlavanzadeh, H., Keshtkar, A.R., Sfadari, J. and Abadi, Z. (2010) Biosorption of nikel (II) from aqueous solution by Brown algae: Equilibrium, dynamic and thermodynamic studies. *J. Hazard Mater.* **175**(1), 304-310.
- Pal, A., Choudhuri, P., Dutta, S., Mukherjee, P.K. and Paul, A.K. (2004) Isolation and characterization of nickel-resistant microflora from serpentine soils of Andaman. *World Journal of Microbiol. Biotechnol.* **20**(9), 881-886.
- Pardo, R., Herguedas, M., Barrado, E. and Vega, M. (2003) Biosorption of cadmium, copper, lead and zinc by inactive biomass of *Pseudomonas Putida*. *Anal. Bioanal. Chem.* **376**(1), 26-32.
- Poreba, R., Gac, P., Poreba, M. and Andrzejak, R. (2011) Environmental and occupational exposure to lead as a potential risk factor for cardiovascular

- diseases. *Environ. Toxicol. Pharm.* **31**(2), 267-277.
- Raja, C.E., Anbazhagan, K. and Selvam, G.S. (2006) Isolation and characterization of a metal-resistant *Pseudomonas aeruginosa* strain. *World J. of Microbiol. Biotechnol.* **22**(6), 577-585
- Ramya, D. and Thatheyus, A. (2018) Microscopic investigations on the biosorption of heavy metals by bacterial cells: A review. *Sci. Int.* **6**, 11-17.
- Rodriguez-Tirado, V., Green-Ruiz, C. and Gómez-Gil, B. (2012) Cu and Pb biosorption on *Bacillus thio-parans* strain U3 in aqueous solution: Kinetic and equilibrium studies. *Chem. Eng. J.* **181**, 352-359.
- Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, **74**(12), 5463-5467.
- Sassi, M., Bestani, B., Said, A., Benderdouche, N. and Guibal, E. (2010) Removal of heavy metal ions from aqueous solutions by a local dairy sludge as a biosorbant. *Desalination*, **262**(1), 243-250.
- Sautel, G., Roulph, C. and Leclerc, P. (1991) Cadmium biofixation by *Pseudomonas putida* bacteria. *Récents Progrès en Génie des Procédés*, **5**, 203-208.
- Selatnia, A., Boukazola, A., Kechid, N., Bakhti, M.Z., Chergui, A. and Kerchich, Y. (2004) Biosorption of lead (II) from aqueous solution by a bacterial dead *Streptomyces rimosus* biomass. *Biochem. Eng. J.* **19**(2), 127-135.
- Shroff, K. and Vaidya, V. (2011) Kinetic and equilibrium studies on biosorption of nickel from aqueous solution by dead fungal biomass of *Mucor hiemalis*. *Chem. Eng. J.* **171**(3), 1234-1245.
- Strandberg, G.M., Shumate, S.E. and Parott, J.R. (1981) Microbial cells as biosorbents for heavy metals: Accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* **41**(1), 237-245.
- Suresh, K.M.K., Selvam, G.S. and Jasmine, P.S. (1998) Cadmium tolerance and metal accumulation by bacterial strain from industrial effluents. *J. Sci. Ind. Res.* **57**(10-11), 817-820.
- Suresh, K.M.K., Krishnan, H.H. and Selvam, G.S. (2001) Cadmium resistant bacteria *Pseudomonas aeruginosa* species isolated from Cochin environment. In: "*Emerging Trends in Environmental Science*" (1<sup>st</sup> ed), pp. 298-304. Asiatech Publishers Inc., New Delhi.
- Texier, A.C., Andrès, Y. and Le Cloirec, P. (1999) Selective biosorption of lanthanide (La, Eu, Yb) ions by *Pseudomonas aeruginosa*. *Environ. Sci. Technol.* **33**(3), 489-495.
- Tunali, S., Cabuk, A. and Akar, T. (2006) Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. *Chem. Eng. J.* **115**(3), 203-211.
- Veglió, F., Beolchini, F. and Gasbarro, A. (1997) Biosorption of toxic metals: An equilibrium study using free cells of *Arthrobacter* sp. *Process Biochem.* **32**(2), 99-105.
- Velusamy, P., Awad, Y.M., Abd El-Azeem, S.A.M. and Ok, Y.S. (2011) Screening of heavy metal resistant bacteria isolated from hydrocarbon contaminated soil in Korea. *J. Agr. Life Env. Sci.* **23**(1), 40-43.
- Vijayaraghavan, K. and Yun, Y.S. (2008) Bacterial biosorbents and biosorption. *Biotechnol. Adv.* **26** (3), 266-291.
- Volesky, B. (2001) Detoxification of metal-bearing effluents: Biosorption for the next century. *Hydrometallurgy*, **59**(2), 203-216.
- Wang, J. and Chen, C. (2009) Biosorbents for heavy metal removal and their future. *Biotechnol. Adv.* **27**, 195-226.
- Wierzba, S. (2015) Biosorption of lead (II), zinc (II) and nickel (II) from industrial wastewater by *Stenotrophomonas maltophilia* and *Bacillus subtilis*. *Polish J. Chem. Tech.* **17**(1), 79-87.
- Zouboulis, A., Loukidou, M. and Matis, K. (2004) Biosorption of toxic metal from aqueous solutions by bacterium strains isolated from metal polluted soils. *Process Biochem* **39**, 909-916.

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## تطبيق نموذجي الأيسوسيرم لانجيومير وفرينديش على الإمتصاص الحيوي لأيونات الرصاص بواسطة الكتلة الحيوية المجففة بالتجميد لبكتيريا *Pseudomonas aeruginosa*

عبد الحميد محمد رسمي، أكرم عبدالرحمن ابوسعدة و الشيماء خالد يوسف  
قسم النبات والميكروبيولوجي – كلية العلوم – جامعة السويس – السويس – مصر.

يمثل الآن تلوث التربة والبيئة المائية بأيون الرصاص الناتج من صرف مياه الصرف الصناعي، مشكلة بيئية خطيرة. وتعتبر تقنية الأمتصاص الحيوي للعناصر الثقيلة من مياه الصرف بواسطة الكتلة الحيوية الميكروبية عن طريق ادمصاصها المعادن الثقيلة على أسطحها بطرق فيزيائية كيميائية هي تقنية صديقة للبيئة. في هذه الدراسة تم عزل عدد 72 عزلة بكتيرية مقاومة لأيون الرصاص من 30 عينة مياه صرف صحي تم تجميعها من شوارع حي فيصل بمحافظة السويس، مصر. ومن المثير للإهتمام، أنه تم اختبار العزلة البكتيرية رقم 2103 من بينهم الأكثر مقاومة للتركيزات العالية من أيون الرصاص. وقد تم وصف هذه العزلة مورفولوجيا وبالإختبارات الكيميائية الحيوية، وكما تم تعريفها وراثيا بواسطة تسلسل الجين 16 س حمض الريبونوكليك الريبوسومي على أنها *بيسيدوموناس اريوجونوزا* وتم تسجيلها في بنك الجينات برقم KY712434. وأظهرت دراسة تأثير زمن التفاعل ودرجة الحموضة على امتصاص أيونات الرصاص بواسطة الخلايا البكتيرية المجففة بالتجميد للعزلة المنتخبة على أن الحد الأقصى من الأمتصاص تم تحقيقه خلال 30 دقيقة عند الرقم الهيدروجيني 6. وأن قدرة الأمتصاص القصوى لإزالة الأيونات بواسطة الكتلة الحيوية المجففة بالتجميد من لهذة البكتيريا هو 114.94 جرام/مجم. وكانت معاملات الإنحدار هي 0.8847 و 0.9751 من نماذج الأيسوسيرم لكلا من لانجيومير وفرينديش، على التوالي، لذلك تبين أن الأمتصاص الحيوي لأيونات الرصاص على الكتلة الحيوية لبكتيريا *بيسيدوموناس اريوجونوزا* رقم 2103 أكثر تناسبا مع نموذج الأيسوسيرم لفرينديش من نموذج الأيسوسيرم لانجيومير.