

Supplementary Material

A Protocol for Elucidating the Mechanisms of Biosorption: Uptake of Cu(II) and Pb(II) by Viable *Pseudomonas aeruginosa* Cells

Clint Sutherland^{1,*} 

¹ Department of Project Management and Civil Infrastructure Systems, The University of Trinidad and Tobago, San Fernando, 602905, Trinidad and Tobago

*Corresponding Author: Clint Sutherland, E-mail: clint.sutherland@utt.edu.tt

This file contains supplementary material associated with the main article. All declarations are provided in the main article.

Steps To Determining R_{DC} and Plotting the Characteristic Curve

Step 1: Determine diffusion-chemisorption model parameters

Using data from columns 1 and 2, solve for q_e and K_{DC} by applying non-linear regression (Curve Expert Professional Software Ver 2.7.3 ©) to solve Eq. (4).

Table S1. Experimental data for *Pseudomonas aeruginosa* biosorption of Cu(II) ions

Column 1	Column 2	Column 3	Column 4	Column 5
t (min)	q_t (mg/g)	$q_{t, cal}$ (mg/g)	$(t/t_{ref})^{0.5}$	$Q_t = (q_t/q_{tref})$
0	0	0.00	0.00	0.00
2	12.40	13.95	0.18	0.58
10	21.60	19.54	0.41	0.81
20	22.40	21.59	0.58	0.89
30	22.60	22.65	0.71	0.94
45	22.90	23.58	0.87	0.98
60	23.40	24.18	1.00	1.00

$t_{ref} = 60$ min and $q_{tref} = 24.183$

Table S2. Curve Expert output for diffusion-chemisorption model

Equation name	DC-Model
Equation:	$(q_e * K_{DC} * t^{0.5} / (q_e + K_{DC} * t^{0.5}))$
Parameters:	
$q_e =$	28.925
$K_{DC} =$	19.045
Coefficient of Determination(R^2):	0.9819

Step 2: Calculated values of $q_{t, cal}$ from diffusion-chemisorption model parameters and varying values of time using Eq. 4 to produce column 3

$$q_{t,cal} = \frac{q_e K_{DC} t^{0.5}}{q_e + K_{DC} t^{0.5}} = \frac{28.925 * 19.045 * t^{0.5}}{28.925 + 19.045 * t^{0.5}} \quad (S1)$$

Step 3: Calculate values of $(t/t_{ref})^{0.5}$ and $Q_t = (q_t/q_{tref})$ using $t_{ref} = 60$ min and $q_e = 24.183$ to produce columns 4 and 5

Step 4: Determine R_{DC} graphically - Using Eq. 15-17 and applying non-linear regression, determine R_{DC} using data from columns 4 and 5.

Table S3: Curve Expert output for R_{DC} equation

Equation name	R_{DC} Equation
Equation:	$T^*(1+R_{DC})/(T+R_{DC})$
Parameters:	
$R_{DC} =$	0.196
Coefficient of Determination (R^2):	1.0000

Step 5: Alternatively, R_{DC} can be calculated from the diffusion-chemisorption model parameters using Eq. 14 as follows:

$$R_{DC} = \frac{q_e}{K_{DC} t_{ref}^{0.5}} = \frac{28.925}{19.045 * 60^{0.5}} = 0.196 \quad (S2)$$

Table S4. Data for plotting the standard characteristic curve

	$R_{DC}=0.01$	$R_{DC}=0.1$	$R_{DC}=1$	$R_{DC}= >>> 1$
T	Qt			
0.0000	0.0000	0.0000	0.0000	0.0000
0.0010	0.0918	0.0109	0.0020	0.0010
0.0050	0.3367	0.0524	0.0100	0.0050
0.0100	0.5050	0.1000	0.0198	0.0100
0.0200	0.6733	0.1833	0.0392	0.0200
0.0400	0.8080	0.3143	0.0769	0.0400
0.0600	0.8657	0.4125	0.1132	0.0600
0.1000	0.9182	0.5500	0.1818	0.1000
0.2000	0.9619	0.7333	0.3333	0.2000
0.3000	0.9774	0.8250	0.4615	0.3000
0.4000	0.9854	0.8800	0.5714	0.4000
0.5000	0.9902	0.9167	0.6667	0.5000
0.6000	0.9934	0.9429	0.7500	0.6000
0.7000	0.9958	0.9625	0.8235	0.7000
0.8000	0.9975	0.9778	0.8889	0.8000
0.9000	0.9989	0.9900	0.9474	0.9000
1.0000	1.0000	1.0000	1.0000	1.0000

Step 6: Graphical representation of characteristic curve for experimental data

The characteristic curve was generated using Eq. 15 for any value of T and R_{DC} values of 0.01, 0.1, 1.0 and $\gg 1.0$. The data for the characteristic curves are presented in Table S4.

Step 7: Plot the characteristic curve for *Pseudomonas aeruginosa* biosorption of Cu(II) ions using data from Table S1 (Columns 4 and 5) and superimposed onto the standard characteristic plot

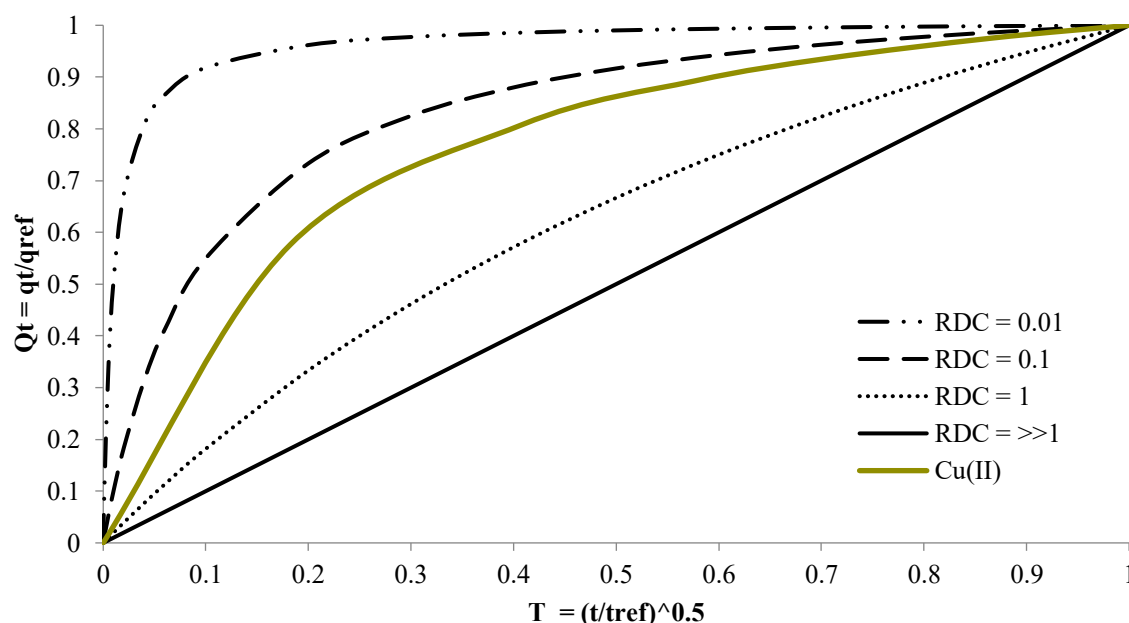


Figure S1. Plot of characteristic curve for *Pseudomonas aeruginosa* biosorption of Cu(II) ions superimposed onto the standard characteristic plot

Table S5. Background of kinetic models used in this study

Model Name	Background
Lagergren pseudo-first-order model	The first-order kinetic model, introduced by Sten Lagergren in 1898 to describe adsorption on charcoal [S1], assumes that each adsorption site binds a single molecule. As an empirical model, it has proven effective in simulating physisorption processes [S2].
Pseudo-second-order	Originally developed for modelling the sorption of divalent metal ions onto peat moss [S3], the pseudo-second-order model assumes that each adsorbate molecule interacts with two binding sites, following a chemisorption mechanism [S4].
Diffusion-chemisorption	The diffusion-chemisorption model, developed by Sutherland [S5], describes heavy metal sorption onto heterogeneous media as a process governed by both diffusion and chemisorption. It is based on equating the rate of change in solid-phase concentration to mass transfer, equilibrium capacity and a time exponent $n=0.5$, indicating parabolic time dependence typical of intraparticle diffusion [S6].
Weber and Morris Intraparticle diffusion	Weber and Morris [S7] proposed that intraparticle diffusion rates are proportional to the square root of time. As noted by Ofomaja [S8], a linear plot passing through the origin suggests intraparticle diffusion control, with the intercept, c reflecting boundary layer effects.

Table S6. Background of equilibrium models used in this study

Model Name	Background
Langmuir	The Langmuir isotherm [S9], introduced in 1916, assumes a chemisorption driven process with uniform adsorption sites, each binding a single molecule to form a monolayer.
Freundlich	Firth, as cited in Swan and Urquhart [S10], noted that De Saussure first used the expression $x=kc^{1/n}$ in 1814 for gas adsorption, with Boedecker later applying it to solutions in 1859. Freundlich [S11] formalized the equation to describe reversible, non-ideal adsorption on heterogeneous surfaces with varying energy sites.
Sips	Sips [S12] proposed an empirical isotherm assuming that each adsorbate molecule can bind to two distinct sites, making it suitable for characterizing heterogeneous surfaces with varying adsorption affinities [S13].

Table S7. Comparative sorption capacities of various microbial biosorbents for Cu(II) and Pb(II) removal

Biosorbent	Contaminant	pH	Sorption capacity, q_m (mg/g)	Reference
Geobacillus toebii	Cu(II)	4.0	48.5	[S14]
Bacillus coagulans	Cu(II)	6.0	75.3	[S15]
Trichoderma spp.	Cu(II)	6.5	19.6	[S16]
Pseudomonas sp.	Cu(II)	6.0	12.6	[S17]
Ochrobactrum intermedium	Cu(II)	6.0	49.9	[S18]
Pseudomonas aeruginosa	Cu(II)	5.5	82.5	This study
Curtobacterium sp.	Pb(II)	6.0	186.6	[S19]
Stenotrophomonas maltophilia	Pb(II)	5.0	133.3	[S20]
Bacillus subtilis	Pb(II)	5.0	17.3	[S21]
Bacillus coagulans	Pb(II)	6.0	118.3	[S15]
Lactobacillus brevis	Pb(II)	6.0	53.6	[S22]
Pseudomonas aeruginosa	Pb(II)	5.5	32.5	This study

This supplementary material provides supporting tables and figures referenced in the main text. References cited only within the supplementary material are listed separately under Supplementary References.

Supplementary References

- [S1] W. J. Weber, Jr. and C. T. Miller, "Modeling the sorption of hydrophobic contaminants by aquifer materials—I. Rates and equilibria," *Water Research*, vol. 22, no. 4, pp. 457-464, 1988, doi: [https://doi.org/10.1016/0043-1354\(88\)90040-1](https://doi.org/10.1016/0043-1354(88)90040-1).
- [S2] H. K. Boparai, M. Joseph, and D. M. O'Carroll, "Kinetics and thermodynamics of cadmium ion removal by adsorption onto nano zerovalent iron particles," *Journal of Hazardous Materials*, vol. 186, no. 1, pp. 458-465, 2011, doi: <https://doi.org/10.1016/j.jhazmat.2010.11.029>.
- [S3] Y. Ho and G. McKay, "Sorption of dye from aqueous solution by peat," *Chemical Engineering Journal*, vol. 70, no. 2, pp. 115-124, 1998, doi: [https://doi.org/10.1016/s1385-8947\(98\)00076-x](https://doi.org/10.1016/s1385-8947(98)00076-x).
- [S4] A.-B. Al-Odayni, F. S. Alsubaie, N. A. Y. Abdu, H. M. Al-Kahtani, and W. S. Saeed, "Adsorption kinetics of methyl orange

- from model polluted water onto N-doped activated carbons prepared from N-containing polymers," *Polymers (Basel)*, vol. 15, no. 9, 2023, doi: <https://doi.org/10.3390/polym15091983>.
- [S5] C. Sutherland, *Removal of Heavy Metals from Waters Using Low-Cost Adsorbents: Process Development [Doctoral Dissertation]*, The University of the West Indies. Trinidad, 2004.
- [S6] C. Sutherland and C. Venkobachar, "A diffusion-chemisorption kinetic model for simulating biosorption using forest macro-fungus, *Fomes fasciatus*," *International Research Journal of Plant Science*, vol. 1, no. 4, pp. 107-117, 2010.
- [S7] W. J. Weber, Jr. and J. C. Morris, "Kinetics of adsorption on carbon from solution," *Journal of the Sanitary Engineering Division*, vol. 89, no. 2, pp. 31-59, 1963, doi: <https://doi.org/10.1061/jsedai.0000430>.
- [S8] A. E. Ofomaja, "Intraparticle diffusion process for lead(II) biosorption onto mansonia wood sawdust," *Bioresource Technology*, vol. 101, no. 15, pp. 5868-5876, 2010, doi: <https://doi.org/10.1016/j.biortech.2010.03.033>.
- [S9] I. Langmuir, "The adsorption of gases on plane surfaces of glass, mica and platinum," *Journal of the American Chemical Society*, vol. 40, no. 9, pp. 1361-1403, 1918, doi: <https://doi.org/10.1021/ja02242a004>.
- [S10] E. Swan and A. R. Urquhart, "Adsorption equations," *The Journal of Physical Chemistry*, vol. 31, no. 2, pp. 251-276, 1927, doi: <https://doi.org/10.1021/j150272a008>.
- [S11] H. Freundlich, "Über die adsorption in lösungen," *Zeitschrift für Physikalische Chemie*, vol. 57U, no. 1, pp. 385-470, 1907, doi: <https://doi.org/10.1515/zpch-1907-5723>.
- [S12] R. Sips, "On the structure of a catalyst surface," *The Journal of Chemical Physics*, vol. 16, no. 5, pp. 490-495, 1948, doi: <https://doi.org/10.1063/1.1746922>.
- [S13] N. Tzabar and H. J. M. ter Brake, "Adsorption isotherms and Sips models of nitrogen, methane, ethane, and propane on commercial activated carbons and polyvinylidene chloride," *Adsorption*, vol. 22, no. 7, pp. 901-914, 2016, doi: <https://doi.org/10.1007/s10450-016-9794-9>.
- [S14] S. Özdemir, E. Kilinc, A. Poli, B. Nicolaus, and K. Güven, "Biosorption of Cd, Cu, Ni, Mn and Zn from aqueous solutions by thermophilic bacteria, *Geobacillus toebii* sub.sp. decanicus and *Geobacillus thermoleovorans* sub.sp. stromboliensis: Equilibrium, kinetic and thermodynamic studies," *Chemical Engineering Journal*, vol. 152, no. 1, pp. 195-206, 2009, doi: <https://doi.org/10.1016/j.cej.2009.04.041>.
- [S15] D.-Y. Lei, Z. Liu, Y.-H. Peng, S.-B. Liao, and H. Xu, "Biosorption of copper, lead and nickel on immobilized *Bacillus* coagulans using experimental design methodologies," *Annals of Microbiology*, vol. 64, no. 3, pp. 1371-1384, 2014, doi: <https://doi.org/10.1007/s13213-013-0782-y>.
- [S16] A. S. Y. Ting and C. C. Choong, "Bioaccumulation and biosorption efficacy of *Trichoderma* isolate SP2F1 in removing copper (Cu(II)) from aqueous solutions," *World Journal of Microbiology and Biotechnology*, vol. 25, no. 8, pp. 1431-1437, 2009, doi: <https://doi.org/10.1007/s11274-009-0030-6>.
- [S17] Y. Liang *et al.*, "Characterization of Cu and Cd biosorption by *Pseudomonas* sp. strain DC-B3 isolated from metal mine soil," *International Journal of Environmental Science and Technology*, vol. 16, no. 8, pp. 4035-4046, 2019, doi: <https://doi.org/10.1007/s13762-018-2011-5>.
- [S18] J. Fan, T. Onal Okay, and D. Frigi Rodrigues, "The synergism of temperature, pH and growth phases on heavy metal biosorption by two environmental isolates," *Journal of Hazardous Materials*, vol. 279, pp. 236-243, 2014, doi: <https://doi.org/10.1016/j.jhazmat.2014.07.016>.
- [S19] F. Masoumi *et al.*, "Nickel and lead biosorption by *Curtobacterium* sp. FM01, an indigenous bacterium isolated from farmland soils of northeast Iran," *Journal of Environmental Chemical Engineering*, vol. 4, no. 1, pp. 950-957, 2016, doi: <https://doi.org/10.1016/j.jece.2015.12.025>.
- [S20] S. Wierzba, "Biosorption of lead(II), zinc(II) and nickel(II) from industrial wastewater by *Stenotrophomonas maltophilia* and *Bacillus subtilis*," *Polish Journal of Chemical Technology*, vol. 17, no. 1, pp. 79-87, 2015, doi: <https://doi.org/10.1515/pjct-2015-0012>.

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- [S21] Y. Cai *et al.*, "A novel Pb-resistant *Bacillus subtilis* bacterium isolate for co-biosorption of hazardous Sb(III) and Pb(II): Thermodynamics and application strategy," *International Journal of Environmental Research and Public Health*, vol. 15, no. 4, 2018, doi: <https://doi.org/10.3390/ijerph15040702>.
- [S22] Q. H. Dai *et al.*, "Biosorption of lead(II) from aqueous solution by lactic acid bacteria," *Water Science and Technology*, vol. 79, no. 4, pp. 627-634, 2019, doi: <https://doi.org/10.2166/wst.2019.082>.