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Nonlinear Isotherm Reassessment of Malathion Biosorption by Dried *Bacillus* sp. S14 Biomass

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Abstract

Malathion residues presence in water remains of concern as the parent insecticide and its transformation products can be toxic to non-target organisms. The present study reanalyzed published equilibrium data of sorption of malathion by the dried bacterium *Bacillus* sp. S14 biomass using nonlinear regression instead of linearized regression to avoid distortions in errors in data structure. Eight models (Henry, Langmuir, Freundlich, Brunauer–Emmett–Teller (BET), Toth, Sips, Fritz–Schlunder IV and Fritz–Schlunder V) were evaluated. Statistical selection of the best model was carried out using root mean square error (RMSE), adjusted R^2 , corrected Akaike information criterion (AICc), Bayesian information criterion (BIC), Hannan–Quinn criterion (HQC), bias factor and accuracy factor. The four-parameter Fritz–Schlunder model had the lowest RMSE (0.0363) and the highest adjusted R^2 (0.9984) and bias and accuracy factors close to unity (1.007 and 1.016). However, the three-parameter Sips model had the lowest AICc (–24.46) and gave an interpretable saturation capacity of 2.640 mg g^{–1} (95% CI 2.503–2.777) with $K_s=0.114$ L mg^{–1} (95% CI 0.071–0.158) and $n_s=0.351$ (95% CI 0.286–0.417). Thus, no single model was the best for all criteria, with the Fritz–Schlunder IV model minimizing residual error and the AICc favoring the more parsimonious Sips equation. The wide confidence intervals and unstable capacity estimates for the higher-parameter models suggest overparameterization in this small, digitized dataset. The reanalysis is consistent with heterogeneous, saturable binding, but does not itself establish a molecular mechanism, and more independent experiments with replicate measurements are required for mechanistic evaluation.

INTRODUCTION

Malathion [diethyl (dimethoxyphosphorothioylthio)succinate] is a broad spectrum organophosphate insecticide used in crop protection, stored product management, veterinary practice and vector control. Its insecticidal activity is due to metabolic activation to malaaxon, a potent inhibitor of acetylcholinesterase, whereas hydrolytic and carboxylesterase-mediated pathways produce less active products [1–4]. Malathion is less persistent than many organochlorine pesticides but repeated application, runoff, spray drift and improper disposal can introduce it into surface waters, ground waters, sediments and waste waters. Environmental risk is not only determined by the parent compound but rather by dose, formulation, transformation products, duration of exposure and the vulnerability of the receiving ecosystem [1–6]. The water treatment methods for streams contaminated with pesticides include activated-carbon

adsorption, advanced oxidation, membrane separation, constructed wetlands and biological treatment [7–12]. Adsorption is attractive for dilute effluents because it can be operated with comparatively simple equipment and does not necessarily produce reactive transformation products. The major drawbacks are the cost of the adsorbent, the competition of dissolved organic matter, regeneration requirements and the transfer of the pollutant to a spent solid phase [7,9,13].

Biosorption is the replacement of conventional sorbents (living or inactive cells, cell-wall fractions, algae, fungi, or agricultural residues) with biological materials and may reduce costs associated with the material, when biomass is available locally [14–19]. Dried bacterial cells are of particular importance for biosorption because the surface groups such as carboxyl, phosphoryl, hydroxyl, amino, etc. can interact with solutes by electrostatic attraction, hydrophobic association, hydrogen

bonding, ion exchange or surface complexation [14–18]. Inactive biomass does not require nutrients or controlled growth conditions during treatment and is less vulnerable than living cells to toxicant inhibition. Members of *Bacillus* are also easy to culture, form robust cell structures and have a long history of environmental and industrial use [20–23]. However, a good batch test does not necessarily show selectivity, regeneration, safety or effectiveness in real water matrices.

Equilibrium isotherms are a summary of the relationship between the concentration dissolved (C_e) and the amount sorbed (q_e) at a given temperature. The Langmuir equation assumes equal energetics of the sites and a finite monolayer capacity, while the Freundlich equation represents an empirical distribution of affinities without an explicit saturation limit [24–26]. The Sips and Toth equations introduce heterogeneity, but still assume a finite capacity; Fritz-Schlunder forms provide additional flexibility [27–31]. These are descriptive equations. A close fit cannot prove a particular adsorption mechanism without spectroscopic, thermodynamic, or kinetic evidence [28,32–35].

Linear transformation of nonlinear formulas modifies the error structure and weights observations unequally. When the experimental errors are associated with the response variable, direct nonlinear regression is preferred [32–38]. Model selection should not be based only on R^2 , but also on parameter uncertainty and complexity. Information criteria, residual-error measures and multiplicative bias indices answer different questions and can rank the same models differently [36–42]. This distinction is particularly important when only a few equilibrium observations are available, because equations with four or five adjustable parameters can fit noise and give poorly identified constants.

Adhikari et al. reported malathion biosorption by dried cells of *Bacillus* sp. S14 and interpreted linearized Langmuir and Freundlich fits [43]. The present study revisits the published equilibrium curve using nonlinear regression and a wider set of isotherms. The objectives were to (i) compare one- to five-parameter models using multiple fit criteria, (ii) quantify parameter uncertainty for the leading models, and (iii) distinguish statistical fit from mechanistic interpretation. This work is a secondary analysis of published graphical data; it does not replace validation with the original numerical dataset or new experiments.

MATERIALS AND METHODS

Data source and digitization

Equilibrium data were extracted from figure 5 of Adhikari et al. [43] using WebPlotDigitizer. The axes were calibrated using the published scale, and the points plotted were digitized one by one. Source values were extracted from a figure rather than original authors, so the resulting data set has rounding, graphical resolution and calibration error. Therefore, the analysis should be considered a reproducibility-directed re-evaluation rather than an independent adsorption experiment.

Isotherm equations

Eight equilibrium models were fitted in their nonlinear forms (Table 1), wherein C_e is the equilibrium concentration (mg L^{-1}), and q_e is equilibrium uptake (mg g^{-1}), q_m is a fitted saturation capacity.

The remaining constants are model-specific affinity or heterogeneity parameters, and parameter units depend on the exact equation and exponent; they should not be compared across models without dimensional normalization [24–31].

Table 1. Nonlinear adsorption-isotherm models evaluated in the reassessment [24–31].

Isotherms	p	Formula	Primary interpretation
Henry's law	1	$q_e = HC_e$	Linear partitioning
Langmuir	2	$q_e = \frac{q_m b_l C_e}{1 + b_l C_e}$	Finite homogeneous sites
Freundlich	2	$q_e = K_F C_e^{\frac{1}{n_F}}$	Empirical heterogeneity
Bet	3	$q_e = \frac{q_{mBET} \alpha_{BET} C_e}{(1 - \beta_{BET} C_e)(1 - \beta_{BET} C_e + \alpha_{BET} C_e)}$	Multilayer-type empirical fit
Toth	3	$q_e = \frac{q_{mT} C_e}{(K_T + C_e^{n_T})^{1/n_T}}$	Heterogeneous finite capacity
Sips	3	$q_e = \frac{K_s q_{mS} C_e^{\frac{1}{n_S}}}{1 + K_s C_e^{\frac{1}{n_S}}}$	Heterogeneous finite capacity
F4	4	$q_e = \frac{A_{FS} C_e^{\alpha_{FS}}}{1 + B_{FS} C_e^{\beta_{FS}}}$	Flexible empirical equation
F5	5	$q_e = \frac{q_{mFS5} K_1 C_e^{\alpha_{FS}}}{1 + K_2 C_e^{\beta_{FS}}}$	Flexible capacity equation

Nonlinear regression and model evaluation

Models were fitted by least squares in CurveExpert Professional 2.6.5. Goodness of fit was evaluated using RMSE, adjusted R^2 , AICc, BIC, HQC, bias factor (Bf) and accuracy factor (Af). Lower values of RMSE, AICc, BIC and HQC represent better performance for the same dataset. Values of adjusted R^2 closer to 1 represent higher explained variation after adjusting for the number of parameters. Preferably, the multiplicative indices Bf and Af should be close to 1 [36–42,44–46].

AICc was emphasized for small-sample comparison, because it makes a stronger correction when n is small relative to the number of fitted parameters. The results of the nonlinear fitting gave approximate 95% confidence intervals. Information criteria were calculated consistently from the residual sum of squares and the number of parameters. Models were not ranked by any single statistic, because residual indices and information criteria penalize complexity differently [36–42].

RESULTS AND DISCUSSION

Comparative model performance

The one- and two-parameter equations did not reproduce the sharp rise followed by the apparent plateau as closely as the flexible heterogeneous models (Figs. 1–8). Henry was the weakest conventional model (RMSE 0.3846; adjusted R^2 0.8556), while Freundlich, Langmuir, BET, and Toth gave intermediate fits. Sips, Fritz–Schlunder IV (F–S IV), and Fritz–Schlunder V (F–S V) followed the data closely across the observed range.

Table 2. Error function analysis for the fitting of the isotherm of Malathion adsorption onto *Bacillus sp.* S14 biomass.

Model	<i>p</i>	RMSE	adR ²	AICc	BIC	HQC	BF	AF
Henry	1	0.38	0.86	-7.96	-14.28	-14.89	1.34	1.71
Langmuir	2	0.25	0.92	-8.41	-20.25	-21.48	1.14	1.21
Freundlich	2	0.31	0.88	-5.25	-17.09	-18.32	1.14	1.25
BET	3	0.28	0.90	2.92	-18.17	-20.02	1.14	1.21
Toth	3	0.21	0.94	-1.53	-22.63	-24.48	1.14	1.17
Sips	3	0.05	1.00	-24.46	-45.56	-47.40	0.77	1.40
F4	4	0.04	1.00	-10.62	-50.30	-52.76	1.01	1.02
F5	5	0.04	1.00	47.38	-48.22	-51.29	1.01	1.02

Note: RMSE Root mean Square Error
adR² Adjusted Coefficient of determination.
p no of parameters
AF Accuracy factor
BF Bias factor
BIC Bayesian Information Criterion
AICc Adjusted Akaike Information Criterion
HQC Hannan–Quinn information criterion

There was no clear winner on the criteria. F–S IV had the lowest RMSE and the highest adjusted R² and most favorable BIC, HQC and Bf/Af values close to 1. In this eight-point dataset, the model with the lowest AICc that strongly penalized the fourth parameter of F–S IV was Sips. F–S V added a fifth parameter, but did not improve the fit of the residual and was penalized with an extreme AICc. It should therefore not be favored. These results amend the internally inconsistent claim in the previous manuscript of Sips minimizing all criteria. F–S IV can be considered as the best fit to the sampled points, while Sips provides a better compromise between parsimony, saturation behavior and interpretable parameters under AICc [36–42].

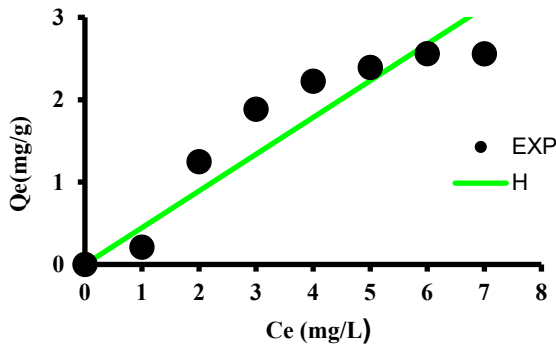


Fig. 1. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the Henry model.

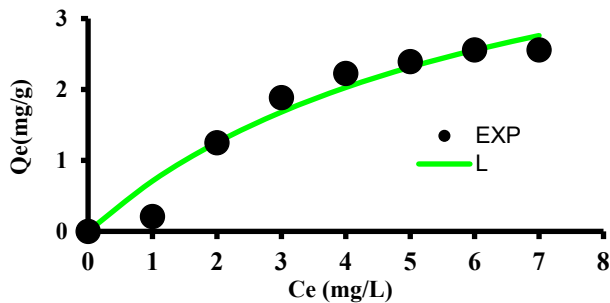


Fig. 2. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the Langmuir model.

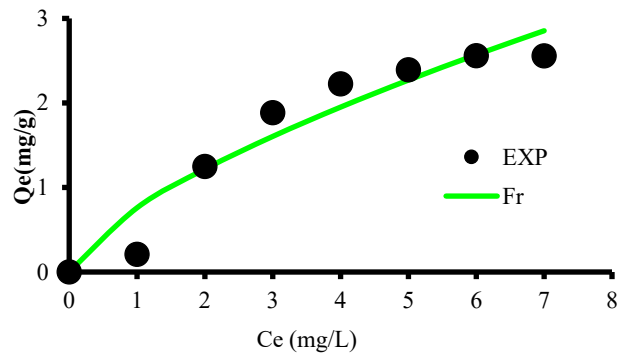


Fig. 3. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the Freundlich model.

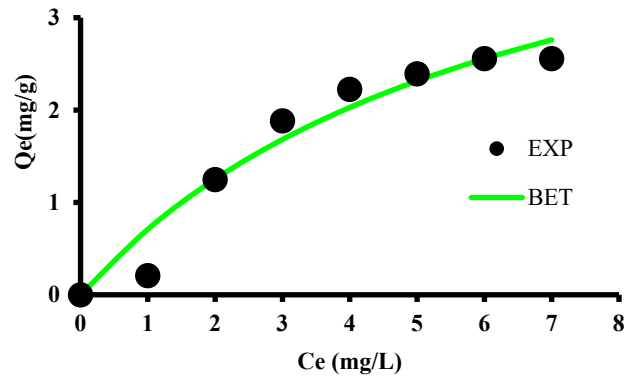


Fig. 4. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the BET model.

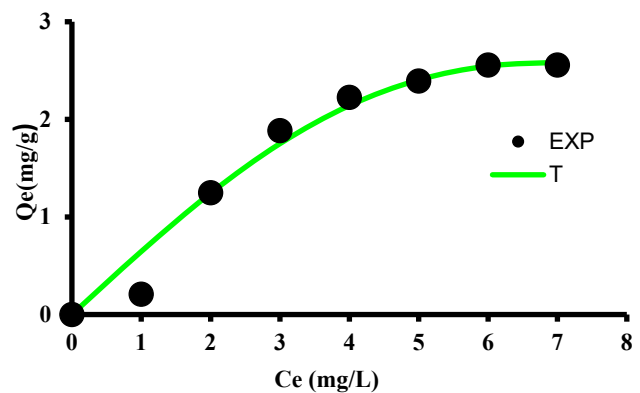


Fig. 5. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the Toth model.

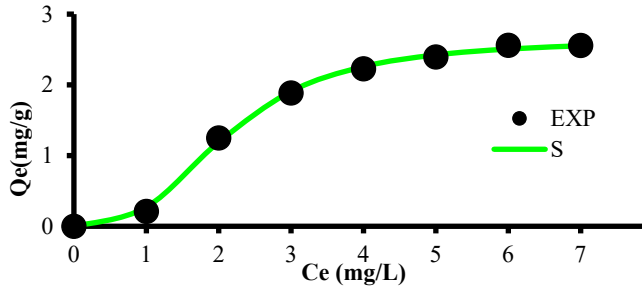


Fig. 6. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the SIPS model.

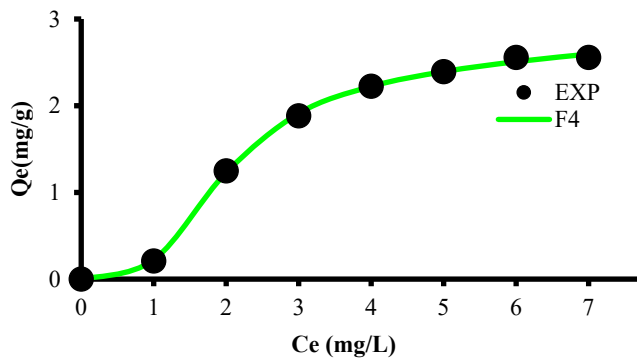


Fig. 7. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the Fritz Schlunder IV model.

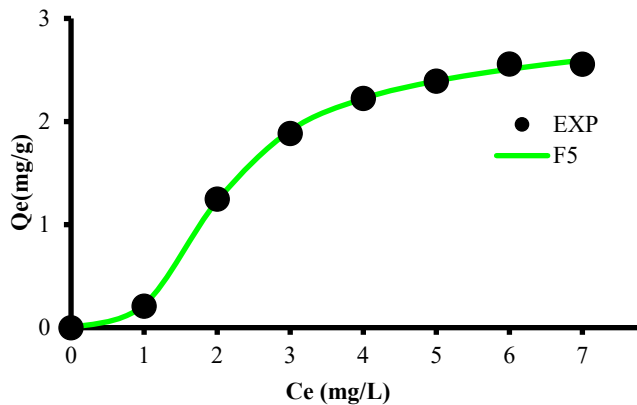


Fig. 8. Adsorption isotherm of Malathion onto *Bacillus sp.* S14 biomass as modelled using the Fritz Schlunder V model.

Parameter estimates and identifiability

The Sips fit gave $q_m=2.640 \text{ mg g}^{-1}$ (95% CI 2.503–2.777), $K_s=0.114 \text{ L mg}^{-1}$ (95% CI 0.071–0.158), and $n_s=0.351$ (95% CI 0.286–0.417) where exponent below 1 is consistent with an empirical distribution of site affinities. Within the observed range, the finite q_m agrees with the visual plateau near 2.6 mg g^{-1} but this parameter should not be treated as an independently measured maximum, however, because its value depends on the selected equation and the concentration range [27–35].

Table 3. Parameter estimates for the four leading nonlinear models.

Model		Unit	Value	95% Confidence interval
Sips	qmS	mg g^{-1}	2.640	2.503 to 2.777
	KS	L mg^{-1}	0.114	0.071 to 0.158
	nS		0.351	0.286 to 0.417
Fritz-Schlunder IV	AFS	mg g^{-1}	0.253	0.133 to 0.373
	VaFS		3.553	2.554 to 4.553
	BFS		0.136	0.077 to 0.196
	bFS		3.376	2.501 to 4.251
Fritz-Schlunder V	$qmFS$	mg g^{-1}	0.651	-1785513.747 to 1785515.0487
	K1	L mg^{-1}	0.389	-1067104.210 to 1067104.987
	aFS	L/g min	3.553	2.232 to 4.875
	k2		0.136	0.057 to 0.216
	bFS		3.376	2.219 to 4.533
Toth	qmT		22465.553	-772511.826 to 817442.933
	kT		165.461	-1349.875 to 1680.798
	nT		2.044	-1.312800 to 5.401633

Note

Isotherms that have no direct way in estimating the maximum adsorption capacity (mg g^{-1}).

Direct evidence of poor parameter identifiability is represented by the very large confidence intervals on the Toth capacity and two F–S V constants (Table 3). The individual constants are unstable, while the curve can be close to every observation. The small number of reconstructed observations and their limited range of concentrations cannot support reliable estimation of five free parameters, and such uncertainty is important for engineering extrapolation: a capacity predicted well beyond the measured range may be numerically convenient but physically unlikely [32–38,47–50].

Interpretation, Limitations and implications

The success of Sips and F–S IV in fitting these data suggests that a heterogeneous saturating curve is a better description than either linear partitioning or the two-parameter forms tested. It does not allow for multilayer adsorption, a specific cell-wall binding group or a single rate-limiting mechanism. *Bacillus* surfaces are chemically diverse polymers, and malathion is relatively hydrophobic, so there may be several interactions occurring simultaneously [14–18,20–23]. At a minimum, confirmation would require surface spectroscopy before and after uptake, zeta-potential measurements across pH, desorption tests, and competition experiments with environmentally relevant solutes.

A caveat is this study has four important limitations. The first being the observations were first digitized from a published figure, and so graphical error is added to experimental error. Secondly, the dataset only consists of eight concentration points and the replicate-level variances are not available. This makes it impossible to perform weighted regression and lack-of-fit test. Thirdly, the confidence intervals from local nonlinear optimization can be unreliable with strongly correlated parameters. Fourth, that the equilibrium is fit does not prove detoxification. Biosorption involves the transfer of malathion to the biomass, whereas biodegradation requires chemical evidence of transformation and reduced toxicity [14–19,43]. Future experiments should be based on the original numerical data or on new replicate measurements, extend the concentration range on both sides of the plateau, report analytical recovery and uncertainty, and compare models by residual diagnostics, AICc and cross-validation.

Tests in natural or wastewater matrices should include measurements of dissolved organic matter, ionic strength, pH, competing pesticides, and biomass regeneration. Breakthrough behavior, mass balance, pressure drop, leaching and safe management of malathion-loaded biomass should be studied in continuous-column [7–19,43,47–50].

CONCLUSION

The use of nonlinear reassessment in this study substantially alters the interpretation of the previously published malathion–*Bacillus* sp. S14 equilibrium curve. Despite the F–S IV having the lowest residual error and the best BIC, HQC, bias factor, and accuracy factor, and the three-parameter Sips model was preferred by AICc and provided a plausible saturation capacity of 2.640 mg g⁻¹. The choice for the best model must not only rely upon statistical results alone, but other factors such as parameters 95% confidence interval range, and number of data points. F–S V and Toth showed parameter instability, demonstrating the danger of fitting highly flexible equations to a small digitized data set. The data are consistent with heterogeneous, saturable biosorption as a descriptive pattern, but do not establish a molecular mechanism or process-scale performance. This biomass requires further replication of experiments and validation in complex water matrices before it can be recommended for treatment applications.

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interests or personal relationships.

ETHICS APPROVAL

Not applicable

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AUTHORS' CONTRIBUTION

The conceptualization of the study was led by I.A.S. Data analysis was conducted by I.A.S. with the remaining authors contributing to the review and editing.

DATA AVAILABILITY STATEMENT

The data analyzed in this study were reconstructed from the published figure cited as reference [43].

AI USAGE DECLARATION

During the preparation of this work, the authors used generative AI technologies solely for language editing and refinement. All of the authors reviewed and edited the content. The authors take full responsibility for the final content of the publication.

CONFLICTS OF INTEREST

The authors declare no competing interests exist.

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