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Differential Pattern of Arsenic Binding by the Cell Wall in Two Arsenite Tolerant *Bacillus* Strains Isolated from Arsenic Contaminated Soil

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Abstract

Arsenite binding was evaluated in two *Bacillus* strains i.e., *B. megaterium* and *B. pumilus*, isolated from arsenic contaminated soil of Unnao district of Uttar Pradesh (India). Initial results showed that more than 90% of arsenite was removed by surface binding by the cell wall component in both the tested species of bacteria. Results on the concentration dependent arsenic binding in bacterial strains exhibited higher effciency of arsenite binding in *B. megaterium* (q_{max} - 1000 mg g^{-1} protein) than *B. pumilus* (q_{max} - 666.7 mg g^{-1} protein). The pH optima for arsenic (As) binding in both *B. megaterium* (pH 6.0) and *B. pumilis* (pH 8.0) were found to be different. Results on temperature dependent arsenite binding by *B. megaterium* showed maximum binding at 30ºC, while arsenic binding maxima in *B. pumilus* showed a broad temperature range (25ºC to 35ºC). The kinetic parameters on arsenite binding revealed that both the bacterial strains followed pseudo-second order kinetics. The As adsorption behavior of the bacterial strains was better explained by Langmuir isotherm rather than Freundlich model. Results of FTIR spectra on surface binding of As revealed major spectral changes in the band region of 1600 cm^{-1} to 800 cm^{-1} in case of *B. megaterium*, indicating involvement of mainly amines, alkenes and C-N functional groups. Whereas FTIR spectrum of *B. pumilus* showed changes in the band region of 3433 cm^{-1} to 2924 cm^{-1} indicating the involvement of hydroxyl, alkanes, alkenes, amides and aromatic functional groups in the arsenic binding. A corollary of these results indicated differential binding of arsenite in both the *Bacillus* strains was on account of different arsenite binding ligands on cell surface as evident from the FTIR results as well as different pH and temperature optima.

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ReceivedE0.:i 1 E0.54 Tc (M4 Td(E-)Tj150.91 324ngh03 164.57 Uttar Pr)Tj1himra2 324ngl

(27 40' , 80 00') ... 15 ... 40 ... 40 ...

... 17 ... 16 ... *Bacillus megaterium* (A ... C633281) ... *Bacillus pumilus* (A ... C633283).

Arsenic biosorption experiment

... 100 ... 20 ... (3000 , 15 ...), ... (0-100 ...$^{-1}$) ... 30 C ... 120 ... 10 ... (1500 , 10 ...). ... 2 ... 10 ... 60 C ... 10:1 ... 18 ...

... 4, 5, 6, 7, 8 ... 9. ... 25 C, 30 C, 35 C, 40 C, 45 C. ... 50 ...$^{-1}$ ...

Determination of adsorption isotherms

...

Langmuir isotherm

... 19 ...

$$= \; _A.C + \; _A.C$$

...

$$\frac{C_e}{q_e} = \frac{1}{q_{max} b} + \frac{C_e}{q_{max}}$$

..., C ... (...$^{-1}$), ... (...$^{-1}$), ...

Freundlich isotherm

...

$$= C^{1/n}$$

... (...$^{-1}$), C ... (...$^{-1}$), ... (...$^{-1}$), ... 20 .

$$\text{Log } q_e = (1/n) \log C_e + \log K_f$$

... C ... 1/n ... C ...

Determination of adsorption kinetics

... 50 ...$^{-1}$ ... (5, 15, 30, 45, 60, 90, 180 ... 240 ...) ...

... 21 .

$$\log(q_e - q_t) = \log Q_e - \frac{k_1}{2.303} t$$

... (...$^{-1}$) ... $_1$ (...$^{-1}$) ... $_1$.

... 22 .

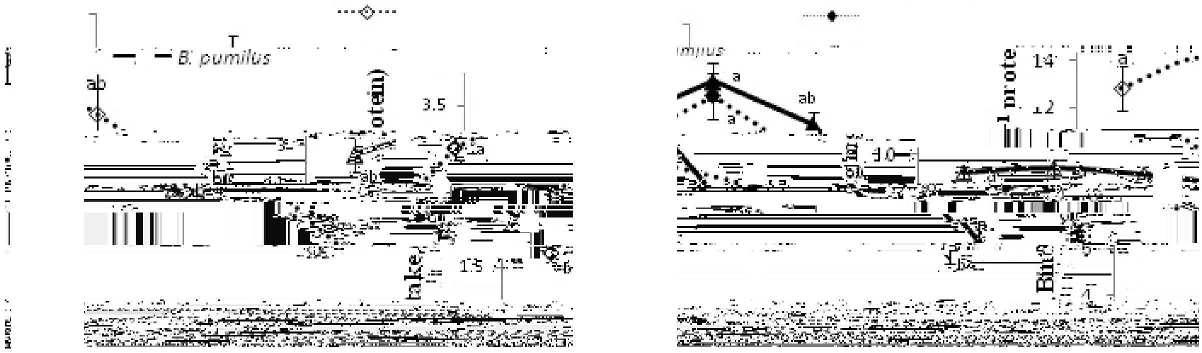
$$\frac{t}{q} = \frac{1}{k_2 q_e^{\,2}} + \frac{1}{q_e} t$$

Fourier transforms infrared (FTIR) spectroscopy

Statistical analysis

Results and Discussion

E ect of pH on surface binding and intracellular uptake



Bacillus ... A ... 30 C ... 35 C ... 31 ...

E ect of As concentration

... (10 ... -1 ... 100 ... -1) ... 3. C ... 50 ... -1. ... A ...

... 32,33 . A ... A ... A 34 . ... (76%) ... (12%) ... *B. megaterium*. ... 6 ... A ... 26,35-37 . A ... / ... 38,39 .

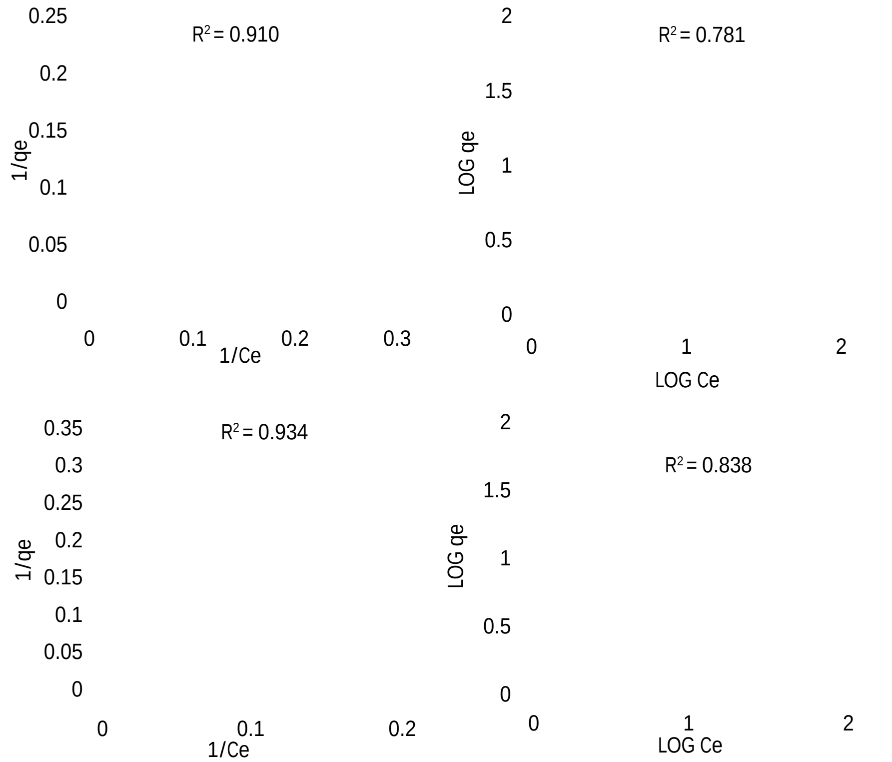


Figure 4: Langmuir and Freundlich isotherm for the adsorption of Arsenic by *Bacillus megaterium* (A) and *Bacillus pumilus* (B).

Bacterial Strains	Langmuir Constant			Freundlich Constant		
	Max. adsorption capacity 'q_{max}' (mg g^{-1}protein)	Adsorption affinity '*b*' (L mg^{-1})	Regression Coefficient 'R^2'	Adsorption coefficient 'K_f' (mg g^{-1} protein)	Adsorption intensity '*n*'	Regression Coefficient 'R^2'
B. megaterium	1000.0	0.0013	0.910	-0.499	1.17	0.781
B. pumilus	666.7	0.0008	0.934	-3.0	1.12	0.838

Table 1: Langmuir and Freundlich isotherm constants for the adsorption of Arsenic by *B. megaterium* and *B. pumilus.*

Bacterial Isolates	Q (Experimental)	Pseudo Second Order Kinetic Constants			Pseudo First Order Kinetic Constants		
		Q_e(Calculated) (µg/mg) mg)	K_2(gmg^{-1} min^{-1})	R^2	Q_e(Calculated) (µg/mg)	K (min^{-1})	R^2
B. megaterium	35.62	38.46154	0.006318	0.999	-0.08197	0.006909	0.591
B. pumilus	26.76	28.57143	0.007206	0.999	-0.0846	0.009212	0.702

Table 2:

Biosorption isotherms

[illegible]

Biosorption kinetics

[illegible]

[illegible] 50 [illegible] $^{-1}$ ([illegible] 5) [illegible] / *vs* [illegible] (-) *vs* [illegible] 2. [illegible] (^{2}) [illegible] $_{2}$ [illegible]

1604 [illegible] A [illegible] 1428 [illegible] 1429 [illegible] 888 [illegible] 885 [illegible] (3).

[illegible] 3433 [illegible] 3426 [illegible] *B. pumilus* [illegible] A [illegible] 3080 [illegible] 3066 [illegible] C [illegible] 2924 [illegible] C- C [illegible] 2926 [illegible] (2960 [illegible] 970 [illegible]) [illegible] 1460 [illegible] 1453 [illegible] (3).

[illegible] 45 [illegible] C [illegible] C- [illegible] 46-49 [illegible] 50,51 [illegible] C [illegible] C- [illegible] ( ) [illegible] *E. coli* 16 [illegible]

Conclusion

B [illegible] (90%) [illegible] *Bacillus* [illegible]

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