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COPPER REMOVAL FROM AQUEOUS SYSTEMS: BIOSORPTION BY *PSEUDOMONAS SYRINGAE*

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ABSTRACT

The potential of two strains of *Pseudomonas syringae* (Blue and Brown) to remove copper from aqueous solutions has been investigated and assessed against the synthetic Linde LZ-52Y aluminosilicate zeolite. The two bacterial strains were tolerant to copper and were able to grow in media doped with concentrations of up to 1000 ppm. The biosorptive capacity and the mechanism of copper uptake were investigated using "active" and "inactive" species grown in nutrient-rich and complex media. The degree of copper removal by ion exchange with the Y zeolite is reported and compared with that achieved when using the biosorbents under the same treatment conditions. The bacteria were harvested, freeze-dried, and used to adsorb copper under starved and glucose activated conditions. The need to distinguish between "bio-uptake" and the action of complexing agents that may be present are highlighted.

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The experimental data are fitted to standard Freundlich, BET, and Langmuir adsorption models where the latter yielded both meaningful theoretical maximum adsorption capacities and adsorption affinity coefficients. These values are discussed in terms of the sorbate/sorbent interactions, which are shown to involve a passive mechanism where the majority of the copper attaches to the outer cell wall.

Key Words: Adsorption; Copper uptake; Langmuir model; *Pseudomonas syringae*; Zeolite Y; Biosorption mechanism

INTRODUCTION

In order to preserve the ecology of aqueous environments the introduction of pollutants must be carefully controlled. Toxic metal ions are one of the many pollutants that can damage the environment. The removal of these metals from an aqueous pollution source is not only of benefit to the environment but recovery makes economical sense to commercial concerns such as the metallurgical and electronics industries. There are many established technologies (1) for the recovery of metals from wastewater including chemical precipitation, electrolytic recovery, membrane separation, adsorption, and evaporation. Synthetic aluminosilicate zeolites are often used in ion exchange (2) as the "compensating" zeolitic cations (normally alkali metal ions) are not rigidly fixed within the hydrated aluminosilicate framework and readily exchange with external heavy metal ions in solution (3). An alternative method for the removal of toxic metals and radionuclides is biosorption or bioaccumulation by microorganisms. The term *biosorption* is taken by Gadd and White (4) to mean "uptake by whole biomass (living or dead) via physicochemical mechanisms such as adsorption or ion exchange." Eccles (5) gives the definition of bioaccumulation as the "processes responsible for the uptake of metal ions by living cells."

The selective removal of specific metals by several single microorganism species has been reported in the literature (6–8). Of direct relevance to this study, *P. syringae* has been investigated to ascertain its metal ion adsorption potential (9,10) and has been found to contain copper resistant plasmids and genes (11,12). Original methods for growing *P. syringae* in copper doped media and its subsequent addition to an existing culture led to anomalous results (13) argely due to the difficulty in distinguishing between bacterial copper uptake and bio-unavailable or precipitated copper complexes. Azenha (14) has noted that the presence of ligands, and moreover the type of ligand, can considerably alter the toxicity of copper to *P. syringae*. A method is clearly needed to isolate the bacteria from any

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complexing agents that can interfere with the analysis. The complexation of copper is also a factor when considering a method to inactivate the bacteria without altering the cell wall. In this paper, we present a mechanism for copper biosorption by *Pseudomonas syringae* and assess its potential (against a synthetic Y zeolite) to remove copper from aqueous pollutant sources, possibly on a commercial scale.

EXPERIMENTAL

Materials

Zeolite

The zeolite used was a Linde LZ-52Y (Union Carbide), which has the nominal unit cell composition $Na_{58}(SiO_2)_{134}(AlO_2)_{58}(H_2O)_{238}$. In order to obtain, as far as possible, the homoionic sodium form the zeolite, as received, was contacted five times with 1 mol dm^{-3} $NaNO_3$. The zeolite was then washed briefly with deionized water, oven-dried (363 K), and stored over saturated NH_4Cl solution at room temperature. The theoretical maximum exchange capacity for Cu(II) (g/g anhydrous zeolite) = 0.14.

Bacteria

Two strains of *Pseudomonas syringae* were employed in this study. They are characterized by their pigmentation (Blue and Brown) when grown on mannitol/glutamic acid agar plates. The bacteria were donated by the University of Portsmouth.

Methods

Culture Conditions

Two media were employed: 1) Mannitol-glutamate (MG), containing (g dm^{-3}) Mannitol (10.0), L-glutamic acid (Na Salt) (2.0), KH_2PO_4 (0.5), NaCl (0.2) and $MgSO_4 \cdot 7H_2O$ (0.2); 2) Nutrient Broth (NB) (25.0 g dm^{-3}). The pH of the media were adjusted to 7 with 5M NaOH before autoclaving. If the solution was to be buffered, then 2-(*N*-morpholino)ethanesulfonic acid (12.5 g dm^{-3}) was added, and the pH was adjusted to 6.5 with 5M NaOH before autoclaving. The media were sterilized at 394 K for 15 min.

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Inactivation of Bacteria

A number of different methods proved unsuccessful for the inactivation of *P. yringae* and include: 1) the addition of NaN_3 (0.05% w/v), which was unsuitable as azide is a copper complexing agent; 2) Cl_2 gas, which oxidized the cell; 3) exposure to UV (at 254 nm) over a 24 h period, which made no significant impact on cell activity; 4) the addition of NaOH to raise the pH to 14 inhibited growth but, upon returning to neutral conditions, bacterial growth continued; and 5) the addition of chloroform (0.25% v/v) made no impact on cell activity until it was driven off at 329 K at which point the bacteria were inactivated. The method that was ultimately adopted for the inactivation of *P. syringae* involved a temperature treatment of the culture at 323 K for 5 h. This inactivation technique was the most suitable as it did not involve the use of extraneous material that could bind copper whereas protein denaturation at this temperature was kept to a minimum.

Copper Uptake by Zeolite

The hydrated zeolite ($0.1 \pm 5 \times 10^{-4}$ g) was treated with an aliquot (10.0 cm^3) of saline for 5 h. The resulting suspension was doped to the relevant copper concentration (in the range 100–800 ppm) with an aliquot (10 cm^3) of $CuSO_4$/saline solution and incubated on an orbital shaker (133 rpm, 301 K, 5 days). Seven replicate treatments were conducted at each copper concentration.

Copper Uptake by *Pseudomonas syringae*

Bacteria from 5-day cultures were harvested by centrifugation (3 g, 45 min, 277 K), washed with saline, and freeze-dried. The freeze-dried bacteria ($0.1 \pm 5 \times 10^{-4}$ g) were rehydrated with an aliquot (10.0 cm^3) of saline for 5 h. The resulting hydrated culture was doped to the relevant copper concentration with an aliquot (10 cm^3) of $CuSO_4$/saline solution and was then incubated on an orbital shaker (133 rpm, 301 K, 5 days). Ten replicate treatments were conducted at each concentration. In order to ensure that the active bacterial uptake was not adversely affected by starvation, a glucose rehydration experiment was conducted whereby the freeze-dried cultures were rehydrated with glucose (10 cm^3, 50 mmol) (15). In the case of the inactive uptake of copper, the 5-day cultures were thermally inactivated (323 K, 5 h) before repeating the procedure as above. At the end of the 5-day equilibration period, the samples were centrifuged (3 g, 45 min, 277 K). Aliquots (0.1 cm^3) of the supernatant were diluted with an aliquot of saline (9.9 cm^3) and analyzed for copper by Atomic Absorption Spectrophotometry (Pye Unicam SP9); the standards employed were prepared from stock solutions (Aldrich).

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pH Monitoring

In order to ascertain whether any changes in the pH of the copper/bacteria media affected copper uptake, the pH was monitored continuously using a Hanna Instruments (HI 931400 microprocessor) pH meter over a 5-day period. The 800 ppm copper solution concentration was chosen, as this was the most acidic test solution and acidic conditions are known to result in a removal of copper from the cell surface (8). In a series of bacterial screening tests we established the minimum inhibitory concentration (MIC) at 1000 ppm. At copper concentrations in excess of 1000 ppm, the medium was too acidic to support *P. syringae* growth.

Bacteria Treatment—Esterification

Methanol (200 cm^3) and H_2SO_4 (2.58 cm^3) were added to the freeze-dried bacteria (0.5 g). The mixture was heated under reflux for 4 h, the bacteria were collected by filtration (0.2 μm Millipore filter) and lyophilized. Copper uptake on these freeze-dried bacteria was determined as outlined previously.

Identification of Intercellular Copper Uptake

Samples of inactive and active freeze-dried bacteria (0.2 g) were rehydrated with aliquots of saline (10.0 cm^3) for 5 h. The resulting hydrated cultures were then doped to a copper concentration of 800 ppm with aliquots (10 cm^3) of $CuSO_4$/saline solution. The mixtures were incubated on an orbital shaker (133 rpm, 301 K, 5-days) and again centrifuged (3 g, 45 min, 277 K). The supernatants were discarded and the bacterial pellets washed with saline (2 $\times$ 10 cm^3) before being centrifuged (3 g, 45 min, 277 K). The saline supernatants were then removed, EDTA (0.5M, pH 7.0, 20 cm^3) was added, and the resulting mixture was left to incubate on an orbital shaker (133 rpm, 301 K, 5 days). The mixtures were centrifuged (3 g, 45 min, 301 K), the supernatant discarded, and the bacterial pellets washed with saline (2 $\times$ 10 cm^3) and centrifuged (3 g, 45 min, 301 K). The resulting bacterial pellets were treated with nitric acid (5 cm^3, 70% v/v) for 2 days and centrifuged (3 g, 45 min, 277 K). Saline (15 cm^3) was then added, and the samples were analyzed for copper; 10 replicate weights per concentration were examined.

Mathematical Modelling of Uptake Isotherms

The most commonly employed models are the standard Langmuir and Freundlich equations adapted for use in aqueous media. The Langmuir model

has been applied to the metal-induced sulphate adsorption by soils (16), the adsorption mechanism of hard and soft metal ions by *Saccharomyces cerevisiae* (17), the biosorption of Cu (II) on fungal mycelia (18), and the adsorption of a mixed metal solution by peat (19). Houng and Lee (20) and Mishra and Chaudhury (21) found that both the Langmuir and Freundlich models adequately fitted their uptake data, whereas Neufeld and Hermann (22) and Aksu et al. (23) found that only the Freundlich model was suitable. The Brunauer-Emmet-Teller (BET) model, more commonly used in determining the surface areas of solids, has been successfully adopted by Allison (24) in biosorption studies. The Langmuir adsorption model is based on the assumptions that every adsorption site is equivalent, and the ability to bind a molecule is independent of the condition of the neighboring sites. In this model, uptake is restricted to monolayer formation, and the equilibrium copper uptake (Q_{eq}) is related to the equilibrium concentration in solution (C_{eq}) according to

$$Q_{eq} = \frac{Q_0 b C_{eq}}{1 + bC_{eq}} \tag{1}$$

where Q_0 represents the maximum uptake of copper (mmol g^{-1}) and b is a constant related to the energy of adsorption (dm^3 $mmol^{-1}$). A commonly adopted linear form of the above is

$$\frac{1}{Q_{eq}} = \frac{1}{Q_0} + \left(\frac{1}{bQ_0}\right)\frac{1}{C_{eq}} \tag{2}$$

where a plot of $1/Q_{eq}$ vs. $1/C_{eq}$ results in a straight line (slope $= 1/bQ_0$, y-intercept $= 1/Q_0$) for systems conforming to the Langmuir model. The Freundlich model is based on the same assumptions, but acknowledges that different types of adsorption sites with different associated energies can exist. This model takes the general form

$$Q_{eq} = K_f C_{eq}^{1/n} \tag{3}$$

where K_f is a proportionality constant (having units of mmol g^{-1} $(\text{mmol dm}^{-3})^{1/n}$) and n is a dimensionless exponent that is related to the energy of adsorption. The linear form of the Freundlich equation is given by

$$\log Q_{eq} = \log K_f + 1/n \log C_{eq} \tag{4}$$

where adherence to the Freundlich model should yield a linear relationship between $\log C_{eq}$ and $\log Q_{eq}$; slope $= 1/n$, y-intercept $= \log K_f$. Although the Langmuir model precludes the possibility of multiplayer adsorption, the BET model allows for this and has the general form

$$\frac{C_{eq}}{(C_s - C_{eq})Q_{eq}} = \frac{1}{BQ_0} + \left(\frac{B-1}{BQ_0}\right)\left(\frac{C_{eq}}{C_s}\right) \tag{5}$$

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where B is a dimensionless constant related to the energy of adsorption and C_s represents the saturation concentration of the solute (mmol dm^{-3}). The linearized form is

$$\frac{C_{eq}}{(C_s - C_{eq})Q_{eq}} = \frac{1}{BQ_0} + \left(\frac{B-1}{BQ_0}\right)\left(\frac{C_{eq}}{C_s}\right) \quad (6)$$

According to the BET equation, a plot of $C_{eq}/(C_s - C_{eq})Q_{eq}$ vs. C_{eq}/C_s results in a straight line (slope $= B - 1/BQ_0$, y-intercept $= 1/BQ_0$) for systems conforming to this model. Although this model describes multilayer adsorption it reduces to the Langmuir equation when adsorption is limited to a single layer.

RESULTS AND DISCUSSION

Uptake Equilibria

The pretreatments that each sorbent was subjected to and the nature of copper uptake monitoring are listed in Table 1. The zeolite sample was more effective in copper removal than any of the bacterial samples at copper doping levels above 200 ppm, as is illustrated by the uptake isotherms in Figures 1 and 2. Periodic sampling of the solution phase revealed that contact times of up to 48 h were necessary in order to attain the equilibrium copper concentration. Bacterial samples grown in Nutrient Broth (NB) and *P. syringae* Blue grown in Mannitol Glutamate (MG) exhibited higher copper uptakes at the lowest doping concentration that was considered. Certain bacterial samples exhibited a downward trend in terms of uptake at higher initial concentrations, and this is shown in Figure 2. The bacteria treated with glucose removed the least copper from solution where the Brown variety was more efficient than the Blue variety. In the case of the samples grown in NB, the inactive bacteria removed less copper at lower concentrations (<400 ppm) and more at the higher concentrations when compared with the active species, albeit the differences are not appreciable. The inactive samples grown in MG removed equivalent or more copper than the active forms. Samples of *P. syringae* Brown grown in nutrient broth exhibited an uptake in excess of 20 mg Cu per gram of bacteria (2% of the cell dry weight). This level of uptake is higher than that found in previous work (25) where 198 strains of bacteria, molds, and yeasts were observed to remove less than 0.3% of the cell dry weight, but our value is similar to the removal efficiencies quoted by Cabral (26). It should be noted that these studies differed experimentally in terms of the media used, solution pH, and the copper doping concentrations that were considered.

Application of the Langmuir model to the experimental equilibrium isotherm data yields the linear plots presented in Figures 3 and 4. The correlation coefficients (R^2 values) given in Table 2 attest to the equivalent applicability of the

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Table 1. The Nature of the Adsorbent Pretreatments and Uptake Monitoring Employed in This Study

Sorbent	Standard Copper Adsorption	Glucose Pretreatment	pH Monitoring	Esterification	Identification of Intercellular Uptake
Y zeolite	YES	–[a]	YES	–[a]	–[a]
P. syringae Blue, active, MG	YES	NO	NO	NO	YES
P. syringae Blue, inactive, MG	YES	NO	YES	NO	YES
P. syringae Brown, active, MG	YES	NO	NO	NO	YES
P. syringae Brown, inactive, MG	YES	NO	YES	NO	YES
P. syringae Blue, active, NB	YES	YES	NO	YES	YES
P. syringae Blue, inactive, NB	YES	NO	YES	YES	YES
P. syringae Brown, active, NB	YES	YES	NO	YES	YES
P. syringae Brown, inactive, NB	YES	NO	YES	YES	YES

[a]Not applicable.

Langmuir and BET models to the majority of the systems that were examined. When the Langumuir and BET models are both satisfied, the adsorption can be described as being restricted to a monolayer of specific noninteracting sites having the same enthalpy of adsorption. The overall agreement is illustrated in Figure 1 where the Langmuir equation lines of best fit are plotted along with the experimental isotherm data. The correlation coefficients for the Freundlich fit are indicative of a markedly poorer fit to the experimental data when compared with the Langmuir/BET treatment. As the Freundlich expression has little predictive value and the fitting parameters do not correlate well with the experimental values, there is little of value to be extracted from this approach. The Langmuir treatment yields two significant parameters, that is, maximum uptake (Q_0) and b, a constant related

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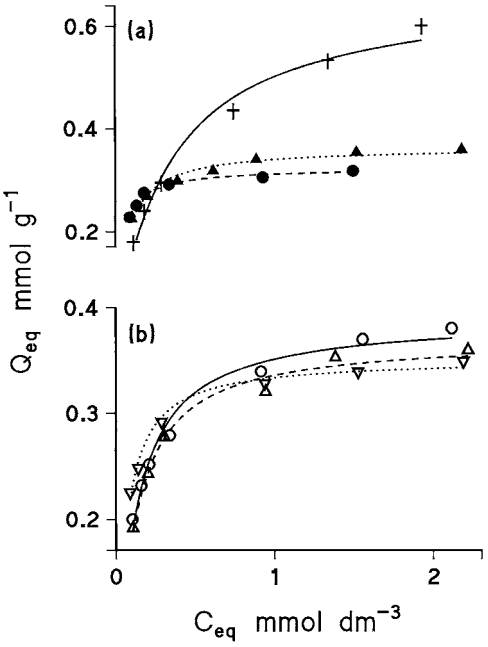

Figure 1. Equilibrium uptake isotherms for those sorbents that exhibited increasing copper uptake at increasing copper doping concentrations where the lines represent the best Langmuir fit: a) +, zeolite Y (solid line); ●, *P. syringae* Brown, active, NB (dashed line); ▲, *P. syringae* Blue, active, NB (dotted line); b) ○, *P. syringae* Brown, inactive, NB (solid line); △, *P. syringae* Blue, inactive, NB (dashed line); ▽, *P. syringae* Brown, inactive, MG (dotted line); $T = 301$ K; weight of sorbent = 0.1 g.

to the energy of adsorption. The Q_0 value is important in identifying which sorbent has the highest uptake capacity and, as such, is useful in scale-up considerations. Taking b as indicative of the affinity the adsorbent has for the adsorbate, lower values suggest weaker interactions where the copper ions can be easily displaced by a competing ion or affected by a temperature or concentration gradient. A high value of b is diagnostic of a strong affinity for copper and thus a more stable adsorption product. Application of the Langmuir model to the zeolite system generated the highest Q_0 value of all the adsorbents that were tested, but this level of uptake is still lower than the theoretical maximum exchange capacity. The exchange system in Y zeolite can be represented by the equilibrium (27)

$$Cu_s^{2+} + 2Na_z^{+} \rightleftharpoons Cu_z^{2+} + 2Na_s^{+} \tag{7}$$

where s and z represent the solution and zeolite phases, respectively. From a consideration of the charge concentration in the equilibrium solutions, exchange

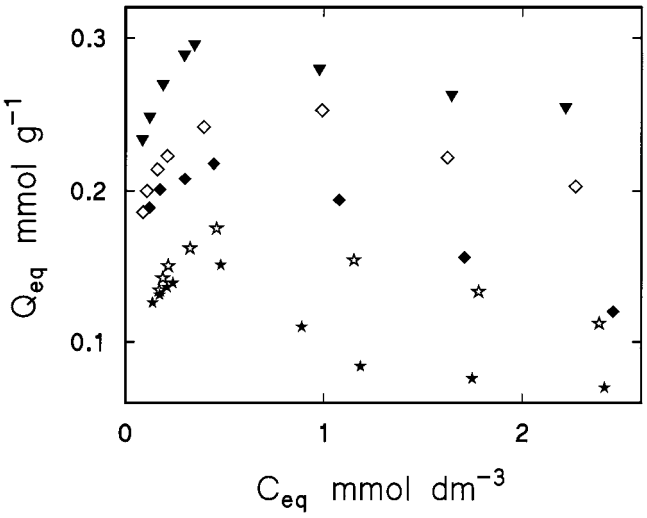

Figure 2. Equilibrium uptake isotherms for those sorbents that exhibited decreasing copper uptake at increasing copper doping concentrations: ★, *P. syringae* Blue, active, NB + glucose; ☆, *P. syringae* Brown, active, NB + glucose; ◇, *P. syringae* Brown, inactive, MG; ▼, *P. syringae* Blue, active, MG; ◆, *P. syringae* Brown, active, MG; $T = 301$ K; weight of sorbent = 0.1 g.

was essentially stoichiometric with two Na^+ ions replacing one Cu^{2+} cation. The forward exchange was indeed reversible and a back exchange of the copper loaded zeolite with external Na^+ was readily achieved (3). The exchangeable sodium ions are located at different sites within the zeolite framework with each site having different associated energetics and ease of access for the entering hydrated copper species. The applicability of both the Langmuir and the BET models suggests the involvement of the more accessible supercage sites that are of equivalent energy, an assertion that is supported by previous work (3,27).

Those bacterial samples (see Fig. 2) that exhibited a decrease in copper uptake at higher copper doping concentrations were also subjected to the standard Langmuir treatment (see Fig. 4). We limited the Langmuir regression to the solute concentration boundaries wherein uptake increased to yield the positive b values recorded in Table 2. The observed decline in uptake may be attributed to a pH shift caused by the copper binding in the double layer surrounding the bacteria. As more copper binds to the cell wall, the hydrogen ions associated with the cell wall components are exchanged into the diffuse region of the double layer. The resultant pH decrease (at the solid/liquid interface) may then interfere in two ways: a) the equilibrium of ion exchange is reversed and b) the aggressively low pH alters the structure of cell wall proteins and polysaccharides, transforming possible binding

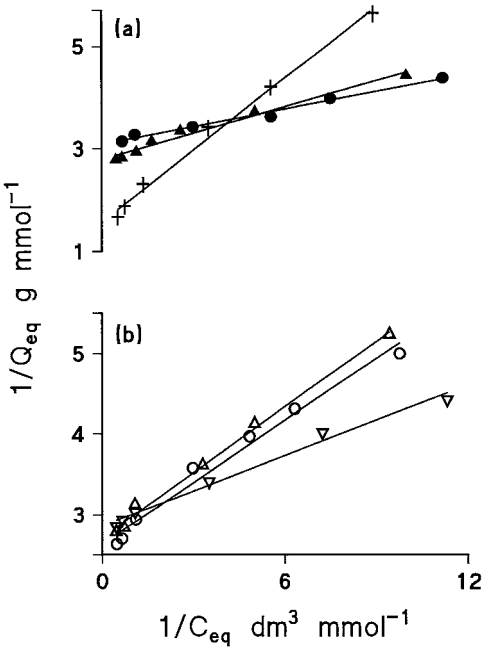

Figure 3. Linearized Langmuir plots for the uptake of copper on: a) zeolite Y (+); *P. syringae* Brown, active, NB (●); *P. syringae* Blue, active, NB (▲); b) *P. syringae* Brown, inactive, NB (○); *P. syringae* Blue, inactive, NB (△); *P. syringae* Brown, inactive, MG (▽).

sites. The results from the pH monitoring (of the bulk solution) experiments are presented in Figure 5. As the copper ions move towards the bacteria the associated hydration sheath is lost. This loss means that the hydrogen ions generated when the copper sulphate was dissolved are rebound with a resultant increase in pH. However, as the adsorption of copper moves towards equilibrium, the exchanged hydrogen ions diffuse into the bulk solution returning the pH to the initial level, as is shown by the profiles in Figure 5. The zeolite, however, exchanges copper in solution for sodium, and the pH does not return to its initial value.

A glucose treatment of both active strains served to lower both the maximum uptake capacity and the affinity for copper binding (as reflected in the magnitude of the *b* parameter). These results differ from those generated for glucose pretreated *Saccharomyces cerevisiae* cells (15), which showed a significant increase in heavy metal adsorption. The authors attributed the increased uptake as confirmation of the partial energy-dependent nature of metal accumulation. It should be pointed out that different experimental conditions (namely a different microorganism and a mixed metal effluent) were used in the latter study, and the results are

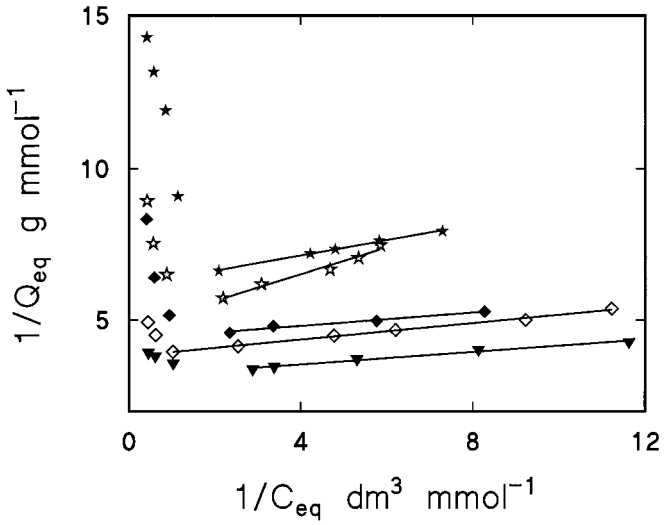

Figure 4. Linearized Langmuir plots for the uptake of copper on: *P. syringae* Blue, active, NB glucose (★); *P. syringae* Brown, active, NB + glucose (☆); *P. syringae* Brown, inactive, MG (◇) *P. syringae* Blue, active, MG (▼); *P. syringae* Brown, active, MG (◆).

not directly comparable. However, it can be postulated that the uptake of copper by *P. syringae* under our conditions proceeds via an inactive mechanism that is not energy dependent. The low uptake may be due to the release of extracellular polymeric substances by the metabolically active bacteria that bind the copper ions (28). Alternatively the incubation temperature of 301 K is sufficient to desorb the weakly bound copper. Indeed, the lower b values for the glucose-treated samples lends credence to a possible copper desorption. It is also possible that the bacterial copper resistant gene has been activated, which then protects the cell by releasing specific copper binding materials that inhibit copper adsorption. This is, however, contrary to previous work (9), which showed that "*P. syringae* PS61 containing cloned *cop* operon also accumulated more copper than PS61 lacking the operon."

In general, the active bacteria exhibited lower Q_0 and higher b values than the corresponding inactive species. The bacteria grown in NB are characterized by similar Q_0 values for both bacterial varieties, regardless of any pretreatment. The temperature and duration of the inactivation step could conceivably result in a denaturing of some proteins responsible for copper adsorption. These conformational changes may be considered to allow more binding groups to become exposed to the copper ions as, according to Voet and Voet (29), the low conformational stabilities of proteins make them susceptible to modifications when the proteins are heated in solution. The Q_0 values extracted for the bacteria grown in the MG media indicate that the uptake capacity of the Blue variety is appreciably

Table 2. Correlation Coefficients (R^2) for the Three Adsorption Models and the Q_0 and b Parameters Extracted from the Langmuir Treatment

Adsorbent	R^2			Q_0 (mmol g^{-1})	b ($dm^3 mmol^{-1}$)
	Freundlich	BET	Langmuir		
Y zeolite	0.99	0.99	0.99	0.63	4
P. syringae Brown, active, NB	0.87	0.99	0.99	0.33	27
P. syringae Blue, active, NB	0.95	0.98	0.98	0.35	18
P. syringae Brown, active, MG[a]	0.97	0.97	0.98	0.23	38
P. syringae Blue, active, MG[a]	0.98	0.99	0.98	0.31	30
P. syringae Brown, inactive, NB	0.97	0.99	0.98	0.39	11
P. syringae Blue, inactive, NB	0.94	0.98	0.99	0.38	10
P. syringae Brown, inactive, MG[a]	0.91	0.98	0.99	0.26	28
P. syringae Blue, inactive, MG	0.95	0.99	0.99	0.35	20
P. syringae Brown, active, NB + glucose[a]	0.94	0.97	0.98	0.17	16
P. syringae Blue, active, NB + glucose[a]	0.96	0.97	0.98	0.19	13

[a]Cu uptake decreased at higher initial doping concentrations.

higher than the Brown strain. This difference may be due to the pigmentation of the bacteria grown in these conditions as it has been seen elsewhere that melanin pigments are responsible for an increase in copper uptake by fungi (30,31). The synthetic Y zeolite exhibited a higher maximum copper uptake than any of the

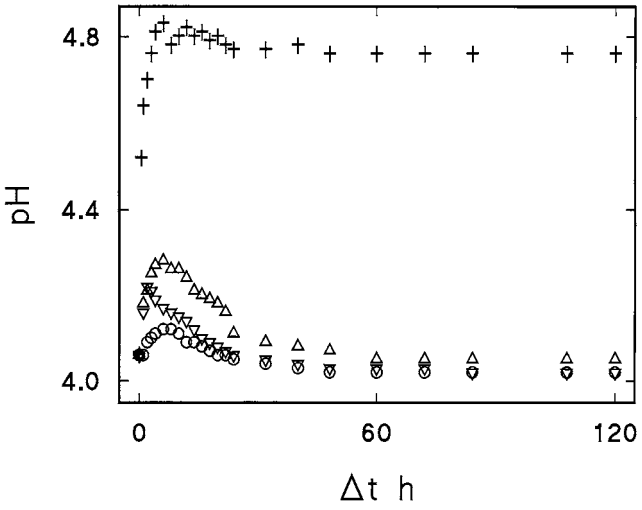

Figure 5. Variation of solution pH with contact time for the uptake of copper (800 ppm) on: zeolite Y (+); *P. syringae* Brown, inactive, NB (○); *P. syringae* Blue, inactive, NB (△); *P. syringae* Brown, inactive, MG (▽); $T = 301$ K; weight of sorbent = 0.1 g.

bacterial samples. However, on the basis of the extracted Langmuir *b* parameter, the affinity that the zeolite exhibits for copper is lower than all the bacterial samples. The commercial application of the glucose-treated strains and the bacteria grown in MG (with the exception of inactivated *P. syringae* Blue) is not really feasible considering the observed copper release at higher doping concentrations. The Blue and Brown strains grown in NB show definite potential and although the removal efficiencies are lower than those recorded for the zeolite (see Fig. 6) at higher initial copper concentrations, the costs involved in zeolite synthesis far exceed the expenses associated with growing bacteria.

Mechanism of Copper Biosorption

The mean intercellular uptake demonstrated by (the EDTA treated) active and inactive *P. syringae* Blue and Brown grown in both MG and NB media was 0.30 ± 0.01 mg Cu per gram of freeze-dried bacteria. When this value is compared with the uptake of copper by untreated freeze-dried bacteria (under the same conditions), an extracellular uptake to intracellular uptake ratio of 66:1 is observed; that is, in excess of 98% of the uptake occurs extracellularly. The level of intracellular uptake was the same for each bacterial activity suggesting the involvement of a passive mechanism. It should, however, be stressed that the EDTA (0.5 mol dm^{-3})

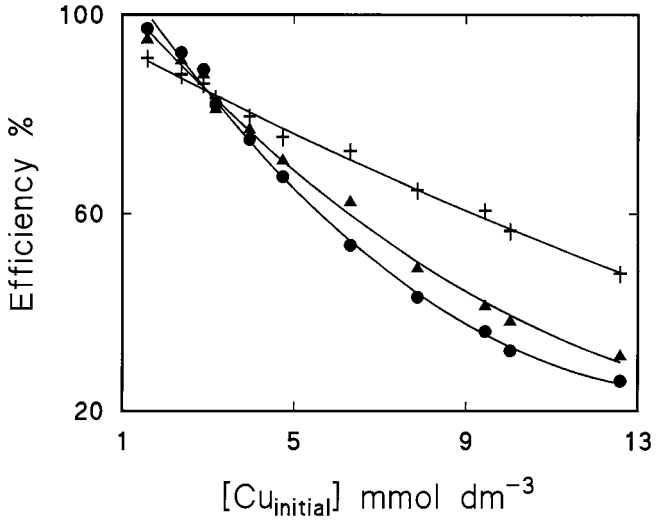

Figure 6. Copper removal efficiency as a function of initial copper concentration in solution ($[Cu]_{initial}$) for zeolite Y (+); *P syringae* Brown, active, NB (●); and *P. syringae* Blue, active, NB (▲): $T = 301$ K; weight of sorbent = 0.1 g.

treatment may have disrupted the cell wall in such a way that the intracellularly bound copper was released. However, in a study by Yoshinobu et al. (33) *Pseudomonas putida* cells suspended in tris hydrochloride (10 mmol dm^{-3}) and EDTA (2 mmol dm^{-3}) needed a further pressure treatment (20,000 lb/in^2) in order to disrupt the cell, and even under these conditions some cells remained intact. Although it is also possible that weakly (and reversibly) held copper on the intracellular sites may have been removed by the copper sparse wash solution, this did not occur for copper bound to outer cell wall sites and can be discounted as a significant effect. The bacteria were esterified in order to probe the possible involvement of carboxylic groups, present on the outer cell surface, in copper uptake. Repeated FTIR analyses (33) of the alcohol treated and untreated bacterial samples revealed a marked increase (in the former case) in the relative intensity of the characteristic bands for the carbonyl function in esters. The "esterification" treatment led to a decrease in copper adsorption when compared with the results generated for the untreated bacteria as shown in Table 3. The untreated active bacteria show as much as 11 time (and the inactive bacteria as much as 8 times) the copper uptake of that demonstrated by the esterified bacteria samples (at 100 ppm initial doping concentration). The treated samples also exhibited an increase in copper uptake as the initial doping concentration was increased. We accept that the alcohol treatment (in an acidic medium) can also lead to peptide cleavage, transesterification, and ether formation as well as the intended esterification, and it is unfeasible to explicitly

Table 3. Effect of Esterification on Copper Uptake by Active and Inactive Bacteria at Different Initial Copper Concentrations in Solution ($[Cu]_{initial}$)

$[Cu]_{initial}$ ppm	Biosorbent	$(Q_e)_{unreacted}/Q_e)_{esterified}$
100	Blue, active	7.5
	Brown, active	11.0
	Blue, inactive	7.8
	Brown, inactive	7.4
200	Blue, active	8.6
	Brown, active	3.4
	Blue, inactive	4.4
	Brown, inactive	4.0
400	Blue, active	2.0
	Brown, active	1.6
	Blue, inactive	1.9
	Brown, inactive	2.4
600	Blue, active	2.5
	Brown, active	2.1
	Blue, inactive	1.8
	Brown, inactive	2.3
800	Blue, active	2.1
	Brown, active	1.3
	Blue, inactive	3.3
	Brown, inactive	3.3

assign a particular functionality as the principal binding site. Nonetheless, as the esterification step did not completely suppress all metal adsorption, the uptake of copper by the bacteria does not merely involve an interaction with carboxyl groups and other groups that were unaffected by the alcohol treatment (such as SH, CN, and NH_2) may also be involved.

CONCLUSIONS

The results presented in this paper indicate that a nutrient-rich environment produces bacterial cells with a high copper biosorptive capacity that is nonetheless lower than the uptake recorded for a synthetic Y zeolite. Treatment of the biosorbent has a marked effect on the ability of the cells to adsorb copper where an increase in efficiency was observed with thermal inactivation and a decrease with glucose treatment. Cell pigmentation also appears to have a bearing on the biosorption characteristics under certain growth conditions. Both Langmuir and BET models

adequately represent the experimental uptake isotherms, whereas the Freundlich treatment generated poorer fits. The mechanism of bio-uptake can be described as passive with the majority (≥98%) of the copper being adsorbed on the external surface of the cell. Samples of *P. syringae* grown in Mannitol-glutamate (with the exception of the inactive Blue strain) exhibit a decrease in uptake at higher initial copper concentrations in solution. The inactive *P. syringae*, grown in a nutrient rich environment, has a significant biosorptive capacity and uptake characteristics that warrant further study in a commercial application for the removal and recovery of copper from high and low concentrated aqueous streams.

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