Biosorption of Heavy Metal Ions on *Rhodobacter Sphaeroides* and *Alcaligenes eutrophus* H16

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(Abbreviated title: BIOSORPTION OF HEAVY METALS ON BACTERIA)

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ABSTRACT

A fundamental study of the application of bacteria to the recovery of toxic heavy metals from aqueous environments was carried out. The biosorption characteristics of cadmium and lead ions were determined with purple non-sulfur bacteria, *Rhodobacter sphaeroides* and hydrogen bacteria, *Alcaligenes eutrophus* H16 that were inactivated by steam sterilization. A simplified version of the metal binding model proposed by Plette *et al*. was used for the description of metal binding data. The results showed that the biosorption of bivalent metal ions to whole cell bodies of the bacteria was due to monodentate binding to two different types of acidic sites: carboxilic and phosphatic-type sites. The number of metal binding sites of *A. eutrophus* was 2.4-fold lager than that of *R. sphaeroides*.

Key Words: biosorption; heavy metals; *Rhodobacter sphaeroides*; *Alcaligenes eutrophus*

INTRODUCTION

The existence of toxic heavy metals and/or radionuclides in the environment, whether derived from natural or industrial processes, has attracted much attention in recent years. The metal recovery from dilute aqueous solutions by biosorption is an emerging field of interest, from both a resource conservation standpoint and an environmental remediation standpoint. Unlike conventional sorptive resins or organic solvents, biosorption employs inexhaustible, inexpensive, nonhazardous materials that exhibit significant specificity for the targeted contaminants, thus generating low volumes of nonhazardous waste. Secondary waste from the biosorption-based process is readily treated and can be easily disposed by incineration. Bacteria, algae, and other types of biomass have been investigated for use in this application (1-9).

In the earlier studies (2-5), the determination of adsorption parameters using the Langmuir adsorption model was attempted to assist in the comparison of biosorption performance. However, the adsorption models used in these studies could not predict the influence of pH, which may be one of the most important influencing factor, on biosorption. Only a few studies dealt with the effect of pH on metal binding to bacteria. Plette *et al*. (10) investigated the binding of calcium, cadmium, and zinc to the isolated cell

walls of the Gram-positive soil bacterium *Rhodococcus erythropolis* A177 at different concentrations of protons and competing metal ions. It is important to elucidate the metal binding mechanism to the bacterial cell walls since it is the first step in the biosorption of metal ions to bacteria. They proposed a multicomponent competitive binding model based on the NICA (non-ideal competitive adsorption) model (11,12). The model gave good results for the description of metal ion binding data.

In the present study, the biosorption characteristics of toxic heavy metals, cadmium and lead, were investigated with purple non-sulfur bacteria, *Rhodobacter sphaeroides* and hydrogen bacteria, *Alcaligenes eutrophus* H16 that were inactivated by steam sterilization. Panti *et al*. (13) have shown that the uptake of heavy metal cations by microorganisms is a rapid and reversible reaction that is not necessarily mediated by metabolic processes. In fact, it has been demonstrated that nonliving cells accumulate heavy metals to the same or greater extent than living cells (14-16). The use of nonliving bacteria for biosorption process has the obvious advantages of being inexpensive and easy to handle (17). In addition, purple bacteria have recently become very promising tools for purifying waste waters and gases (18-21), and hydrogen bacteria have been used for the production of biodegradable polyester (22-25). The residual waste biomass resulting from water treatment and polyester

production systems is economically attractive materials for biosorbents. In this paper, potentiometric and conductometric titrations were performed to determine the acid-dissociation characteristics of the acidic sites on/in *R. sphaeroides* and *A. eutrophus*. Then we tried to apply the metal binding model proposed by Plette *et al*. (10) to the biosorption of lead and cadmium ions to the whole cell bodies of *A. eutrophus* and *R. sphaeroides*. Based on a simplified metal binding model, the biosorption mechanism of these bacteria will be discussed.

MATERIALS

Cadmium nitrate, lead nitrate and sodium nitrate were obtained from Wako Pure Chemical Industries (Japan). All chemicals were of reagent-grade quality.

Rhodobacter sphaeroides and *Alcaligenes eutrophus* H16 were obtaind from the Institute for Fermentation, Osaka (Osaka Japan) and the Institute of Applied Microbiology, Tokyo University (Tokyo Japan), respectively. They were grown in a batch to the late-exponential growth stage in nutrient rich medium (23). The cells were harvested, inactivated in an autoclave at 120℃ for 15 min, and washed three times with distilled water.

EXPERIMENTAL METHODS

Conductometric Titration of Cells

The total number of acidic sites of bacteria was determined by conductometric titrations (26). For this purpose a certain amount of bacteria was suspended in 0.3 dm^3 of distilled water and mechanically stirred at 30℃. To eliminate CO_2, N_2 gas was continuously bubbled through the system. After reaching thermal equilibrium, the bacteria suspension was titrated with a volumetric standard solution of HNO_3 or NaOH (0.1 mol dm^{-3}). The conductivity of the suspension was measured with a conductometer (TOA Digital Conductometer CM-15A).

Potentiometric Titration of Cells

A suspension (0.3 dm^3) containing a certain amount of bacteria and $NaNO_3$ (0.1 mol dm^{-3}) was titrated with a standard solution of HNO_3 or NaOH (0.1 mol dm^{-3}) at 30℃. The pH of the suspension was measured by a pH meter (Oiron Research 520-A). The number of protonated acidic sites of bacteria was determined from the difference between the bulk proton concentrations in the presence and in the absence of bacteria. The other experimental procedures and

conditions were almost the same as those in the conductometric titration.

Adsorption of Metal Ions onto Cells

A suspension (90 cm^3) of $NaNO_3$ (0.1 mol dm^{-3}) containing a certain amount of bacteria was prepared. The pH of the solution was adjusted to the desired value with HNO_3. After the thermal equilibrium was reached at 30℃, 10 cm^3 of a solution (10 g dm^{-3}) containing a certain amount of $Cd(NO_3)_2$ or $Pb(NO_3)_2$ was added to the suspension. The suspension was stirred for the time necessary to attain the adsorption equilibrium, and then bacteria were separated from the liquid phase in a centrifuge (Kokusan H-1500F) at 10,000 rpm for 20 min. The pH and metal ion concentration of the supernatant were measured. The metal ion concentration was determined with an atomic absorption spectrophotometer (Hitachi A-1800). The amount of metal ion complexed with bacteria was determined from the difference between the metal ion concentrations in the initial and the equilibrium states.

RESULTS AND DISCUSSION

Acid-Base Properties of Cells

Figures 1 and **2** show the protonation characteristics of the acidic sites of *R. sphaeroides* and *A. eutrophus* obtained from potentiometric titration, respectively. The ordinate of the figures, X_H, represents the equilibrium number of protons bound to acidic sites. The titration curves of bacteria are broad and ill-defined, thus reflecting the diversity in acidic sites. Plette *et al*. (27) investigated the acid-base properties of isolated cell walls of soil bacterium, *Rhodococcus erythropolis*, and determined three acid-dissociation constants. The three acidic sites are amino, carboxylic and phosphatic groups. Assuming that the similar relation holds in the present bacteria, the acid-dissociation reactions of *R. sphaeroides* and *A. eutrophus* can be written as

$$-S_1H \leftrightarrow -S_1^- + H^+ \qquad ;K_1 \qquad [1]$$

$$-S_2H \leftrightarrow -S_2^- + H^+ \qquad ;K_2. \qquad [2]$$

$$-S_3H^+ \leftrightarrow -S_3 + H^+ \qquad ;K_3. \qquad [3]$$

-S_1, -S_2, and -S_3 represent the carboxylic, phosphatic, and amino-type sites of bacteria, respectively. The acid-dissociation constants K_i are defined as

$$K_i = \alpha_i[\mathrm{H}^+]/(1-\alpha_i) \qquad (i = 1, 2 \text{ or } 3) \qquad [4]$$

where α_i is the degree of dissociation of type i acidic sites. The number of protons bound to acidic sites on 1 dry-g of bacteria, X_H, can be expressed as

$$X_\mathrm{H} = N_1 \frac{[\mathrm{H}^+]}{K_1 + [\mathrm{H}^+]} + N_2 \frac{[\mathrm{H}^+]}{K_2 + [\mathrm{H}^+]} + N_3 \frac{[\mathrm{H}^+]}{K_3 + [\mathrm{H}^+]}, \qquad [5]$$

where N_1, N_2, and N_3 are the number of carboxylic, phosphatic, and amino-type sites, respectively. A nonlinear least-squares method was applied to find six constants, K_1, K_2, K_3, N_1, N_2, and N_3, in Eq. [5]. These constants for *R. sphaeroides* and *A. eutrophus*, which gave the best fit with the experimental data (in Figs.1 and 2), were listed in **Tables 1** and **2**. The solid lines in Figs. 1 and 2 represent the theoretical curves calculated from Eq. [5] using the constants listed in Tables 1 and 2. The experimental data agreed well with the theoretical curves. From the results listed in Tables 1 and 2, the acid-base

properties of three acidic sites of the bacteria can be clarified: the sum of the number of carboxylic and phosphatic sites, which are electrostatically capable of binding heavy metal ions (10), of *A. eutrophus* is 2.4-fold larger than that of *R. sphaeroides*. The acid-dissociation constants K_1 and K_2 of *R. sphaeroides* are larger than those of *A. eutrophus*.

The total numbers of acidic sites of *R. sphaeroides* and *A. eutrophus* were confirmed by the conductometric titration. **Figures 3a** and **3b** show the results of conductometric titration of *R. sphaeroides* with 0.1M HNO_3 (○) and 0.1M NaOH (□), respectively. The results of *A. eutrophus* are presented in **Figs. 4a** and **4b**. In the titration curves the end point is the intersection of two straight lines (26). The total numbers of acidic sites on *R. sphaeroides* and *A. eutrophus* were determined from these titration curves to be 1.39×10^{-3} and 2.73×10^{-3} mol g^{-1}, respectively. The total number of acidic sites obtained from the conductometric titration were in fair agreement with the number determined from the model calculation, 1.37×10^{-3} mol g^{-1} (*R.*. *sphaeroides*) and 2.70×10^{-3} mol g^{-1} (*A. eutrophus*).

Biosorption of Bivalent Metal Ions to Bacteria

Plette *et al*. (10) investigated the binding of calcium, cadmium and zinc ions to the cell wall materials of Gram-positive soil bacterium *R. erythropolis*.

They proposed a multicomponent metal binding model based on the following assumptions:

(a) Two site types are involved in metal ion binding to the cell wall material: the carboxylic sites (site-type 1) and the phosphatic sites (site-type 2).

(b) Biosorption of bivalent metal ions is due to monodentate binding to all sites. Thus, the adsorption maxima both for protons and for bivalent cations are equal to the number of available binding sites.

Furthermore, they selected the NICA (non-ideal competitive adsorption) model (11,12), that can account for both ion-specific non-ideality and heterogeneity, for the quantitative description of the metal binding data over a wide range of coverage with bivalent ions. The model gave good results for the description of metal ion binding data. Assuming that the similar relation holds in the present bacteria, the biosorption reaction of bivalent metal ions to *R. sphaeroides* and *A. eutrophus* can be written as

$$-S_i^- + M^{2+} \leftrightarrow -S_iM^+ \qquad ; K_{Mi} \qquad (i = 1 \text{ or } 2) \qquad [6]$$

$-S_i^-$ and M^{2+} in Eq. [6] represent the type *i* acidic site and the bivalent metal ions, respectively. In the present study, metal adsorption experiments were conducted at rather low coverage region. Therefore we assumed that the

electrostatic effects and other non-ideal behavior of adsorbate metal ions are negligible, and simplified their model by setting the heterogeneity and nonideality parameters equal to one. Thus the metal binding constants K_{Mi} (dm^3 mol^{-1}) in Eq. [6] is defined as

$$K_{Mi} = \theta_i / \{(1-\theta_i)\alpha_i[M^{2+}]\}$$

$$= \frac{\theta_i}{\{(1-\theta_i)K_i / (K_i + [H^+])\}[M^{2+}]} \qquad [7]$$

where θ_i represents the fraction of type i sites occupied by metal ions. The number of metal ions bound to 1 dry-g of bacteria, X_M, can be expressed as

$$X_M = N_1 \frac{K_1 K_{M1}[M^{2+}]}{K_1 K_{M1}[M^{2+}] + K_1 + [H^+]} + N_2 \frac{K_2 K_{M2}[M^{2+}]}{K_2 K_{M2}[M^{2+}] + K_2 + [H^+]} \qquad [8]$$

Figures 5 and **6** show the pH dependence of the number of lead bound to *R. sphaeroides* and *A. eutrophus*, respectively. The ordinate, X_M, represents the equilibrium number of lead ions bound to the bacteria. These figures show that a pH increase in the adsorption system resulted in more metal-bacteria

complexes. A nonlinear least-squares method was applied to find two constants, K_{M1} and K_{M2}, using Eq. [8]. The obtained constants are listed in **Tables 3**. The solid lines in Figs. 5 and 6 represent the theoretical curves calculated from Eq. [8] using the constants listed in Tables 1-3. Figures 5 and 6 demonstrate a good agreement of the experimental data with the theoretical curves. **Figures 7** and **8** show the pH dependence of the number of cadmium bound to *R. sphaeroides* and *A. eutrophus*, respectively. The two constants, K_{M1} and K_{M2}, were determined in the same manner as those for lead adsorption, and they are listed in **Table 4**. The theoretical curves in Figs. 7 and 8 calculated from Eq. [8] with the values in Tables 1, 2 and 4 demonstrate a good agreement of the experimental data with the model.

CONCLUSION

Purple non-sulfur bacteria, *Rhodobacter sphaeroides* and hydrogen bacteria, *Alcaligenes eutrophus*, which have been used for water treatment and biodegradable polyester synthesis processes, were applied to the recovery of cadmium and lead ions. The bacteria were inactivated by steam sterilization at 120℃ for 20 min and were used as an adsorbent material. Potentiometric and conductometric titrations were performed on *R. sphaeroides* and *A. eutrophus* to determine the number and acid-dissociation constants of carboxylic, phosphatic, and amino-type sites on/in the bacteria. The number of metal binding sites (carboxylic and phosphatic-type sites) of *A. eutrophus* is 2.4-fold larger than that of *R. sphaeroides*. A simplified version of the metal binding model proposed by Plette *et al*. was used to determine the biosortion characteristics of cadmium and lead ions to whole cell bodies of *R. sphaeroides* and *A. eutrophus*. The metal binding model proposed by Plette et al. was applicable to the biosorption of bivalent metal ions to whole cell bodies of the bacteria, and the simplified model gave good results for the description of metal binding data at low coverage with bivalent metal ions.

APPENDIX A: NOMENCLATURE

K_i = acid dissociation constant of type *i* acidic sites (mol dm^{-3})

K_{Mi} = metal binding constant of type *i* acidic sites (dm^3 mol^{-1})

N_i = number of type *i* acidic sites (mol g^{-1})

X_H = number of proton bound to bacteria (mol g^{-1})

X_M = number of metal ions bound to bacteria (mol g^{-1})

α_i = degree of dissociation of type *i* acidic sites (-)

θ_i = fraction of type *i* acidic sites occupied by metal ions (-)

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Figure Captions

FIG. 1. Number of protonated acidic sites of *R. sphaeroides* as a function of pH. The number of protonated acidic sites was determined by potentiometric titrations for 300 cm^3 of a solution containing 0.38 g (acidic region) or 0.52 g (alkaline region) of *R. sphaeroides* and $NaNO_3$ (0.1 mol dm^{-3}) with 0.1 mol dm^{-3} HNO_3 at 30℃. The solid line represents the theoretical curve calculated from Eq. [5].

FIG. 2. Number of protonated acidic sites of *A. eutrophus* as a function of pH. The number of protonated acidic sites was determined by potentiometric titrations for 300 cm^3 of a solution containing 0.20 g (acidic region) or 0.14 g (alkaline region) of *A. eutrophus* and $NaNO_3$ (0.1 mol dm^{-3}) with 0.1 mol dm^{-3} HNO_3 at 30℃. The solid line represents the theoretical curve calculated from Eq. [5].

FIG. 3. Conductometric titration curves for 300 cm^3 of a solutions containing 0.27 (a) or 0.54 (b) g of *R. sphaeroides* at 30℃.

FIG. 4. Conductometric titration curves for 300 cm^3 of a solutions containing 0.43 (a) and 2.16 (b) g of *A. eutrophus* at 30℃.

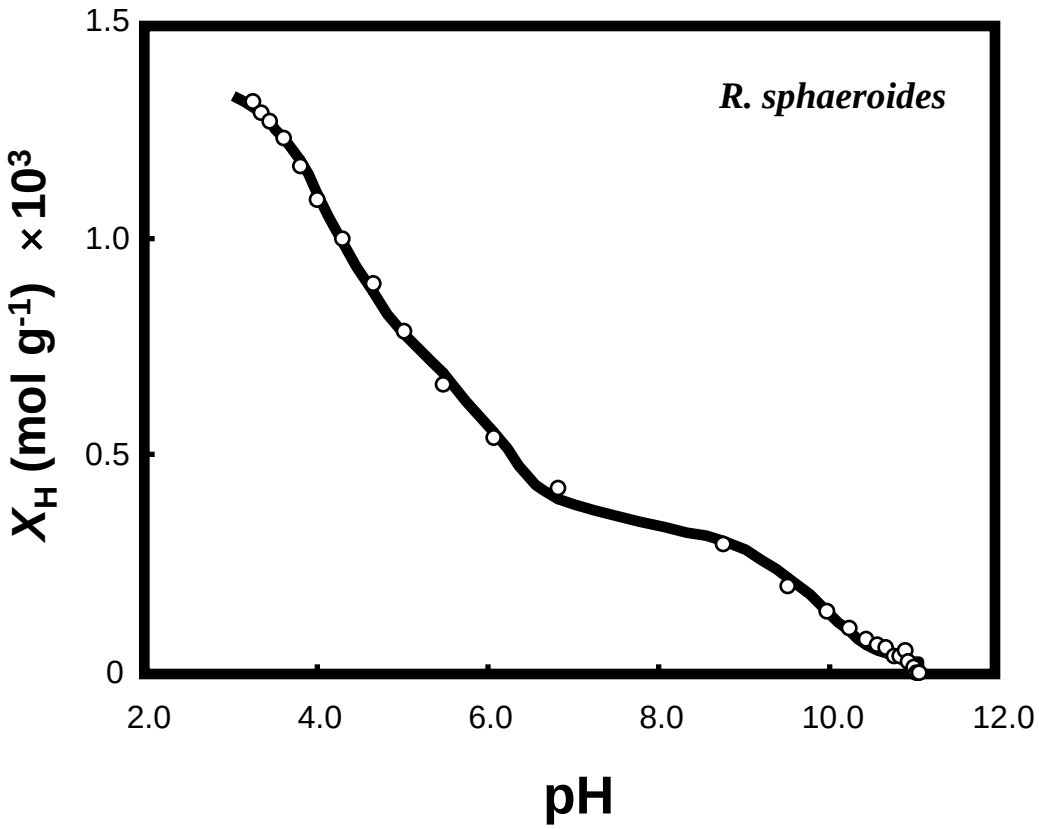
FIG. 5. pH dependence of lead adsorption to *R.sphaeroides* at 30 ℃. Concentrations of *R. sphaeroides* and $NaNO_3$ were 0.48 g dm^{-3} and 0.1 mol dm^{-3}, respectively. The solid lines represent the theoretical curve calculated from Eq. [8].

FIG. 6. pH dependence of lead adsorption to *A.eutrophus* at 30 ℃. Concentrations of *A. eutrophus* and $NaNO_3$ were 0.48 g dm^{-3} and 0.1 mol dm^{-3}, respectively. The solid lines represent the theoretical curve calculated from Eq. [8].

FIG. 7. pH dependence of cadmium adsorption to *R.sphaeroides* at 30℃. Concentrations of *R. sphaeroides* and $NaNO_3$ were 0.40 g dm^{-3} and 0.1 mol dm^{-3}, respectively. The solid lines represent the theoretical curve calculated from Eq. [8].

FIG. 8. pH dependence of cadmium adsorption to *A.eutrophus* at 30℃. Concentrations of *A. eutrophus* and $NaNO_3$ were 0.45 g dm^{-3} and 0.1 mol dm^{-3}, respectively. The solid lines represent the theoretical curve calculated from Eq. [8].

Original Figures
&
Tables



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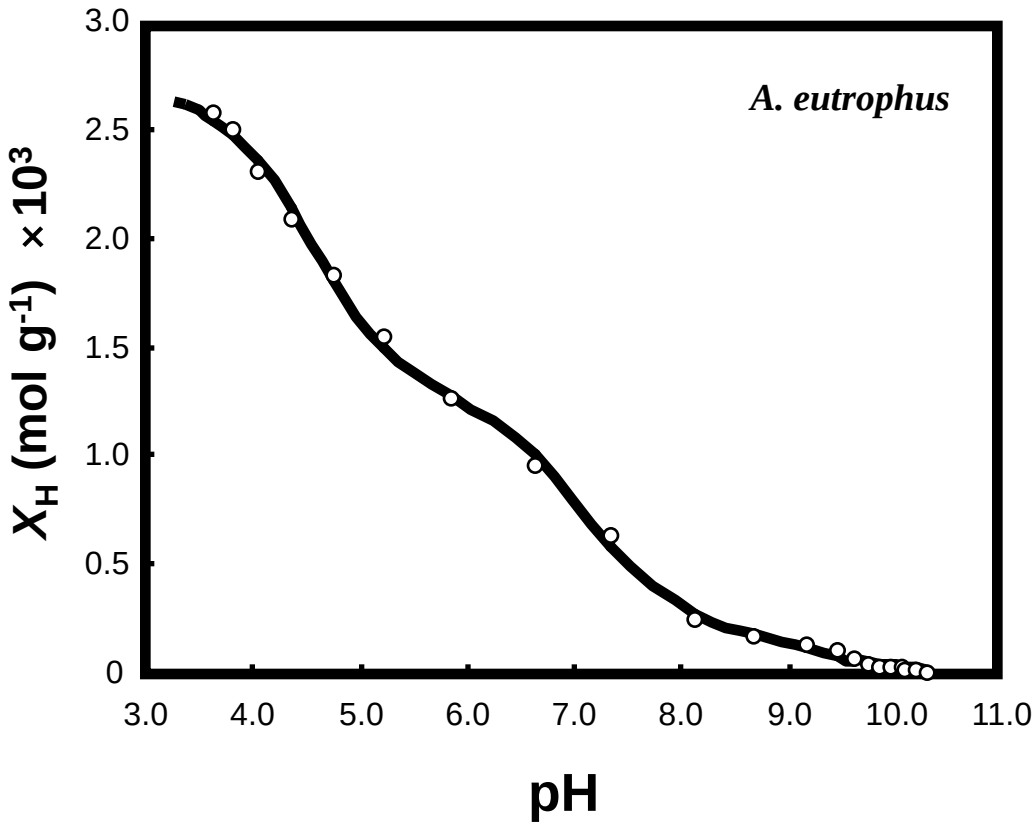

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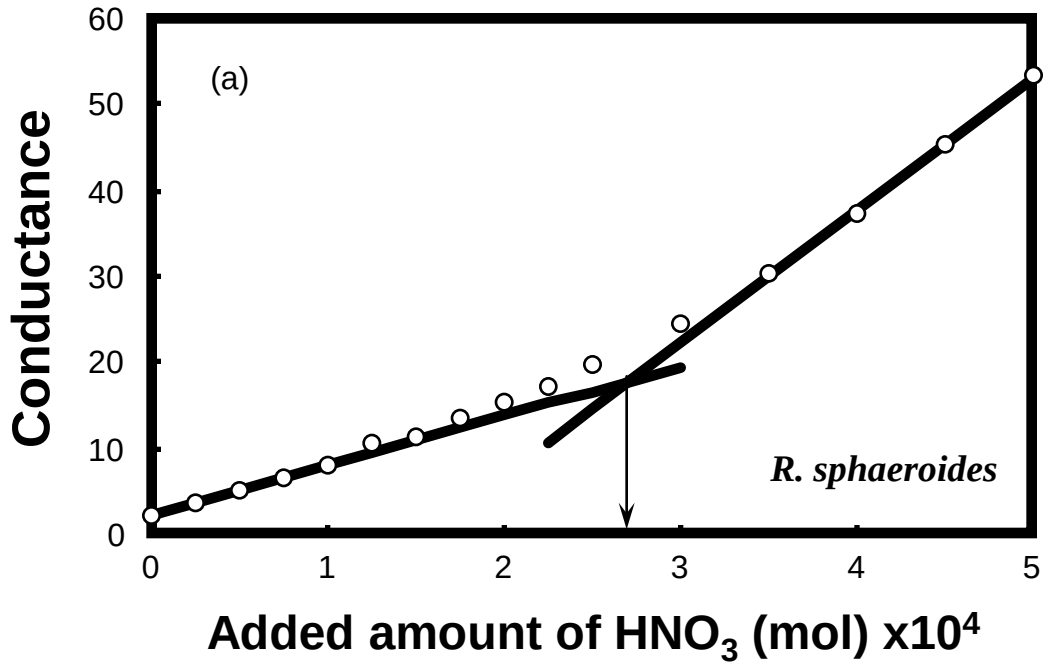

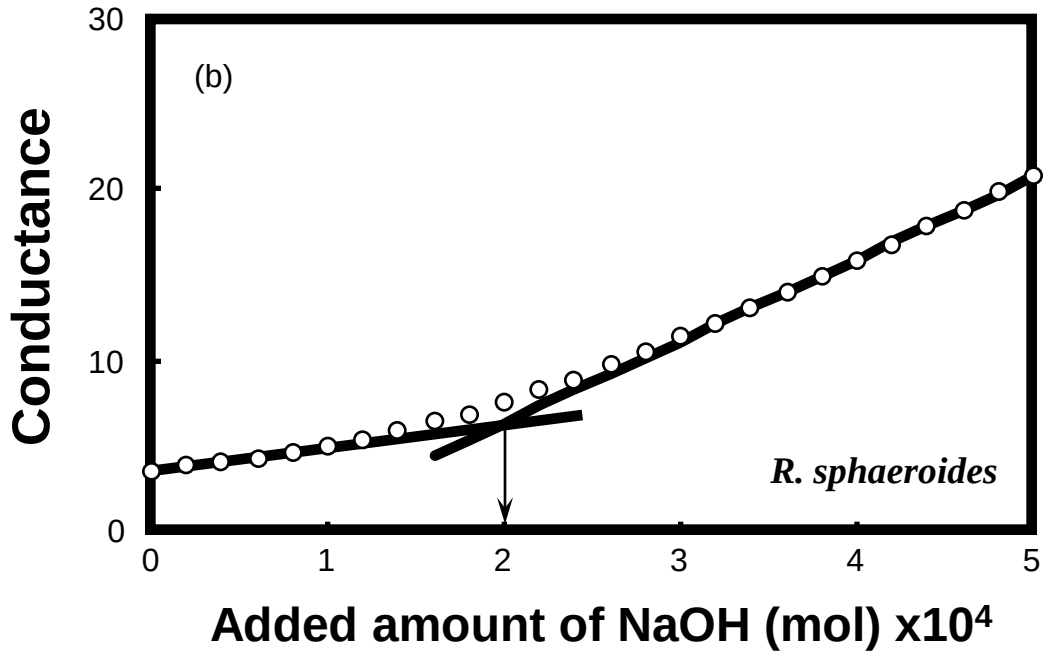

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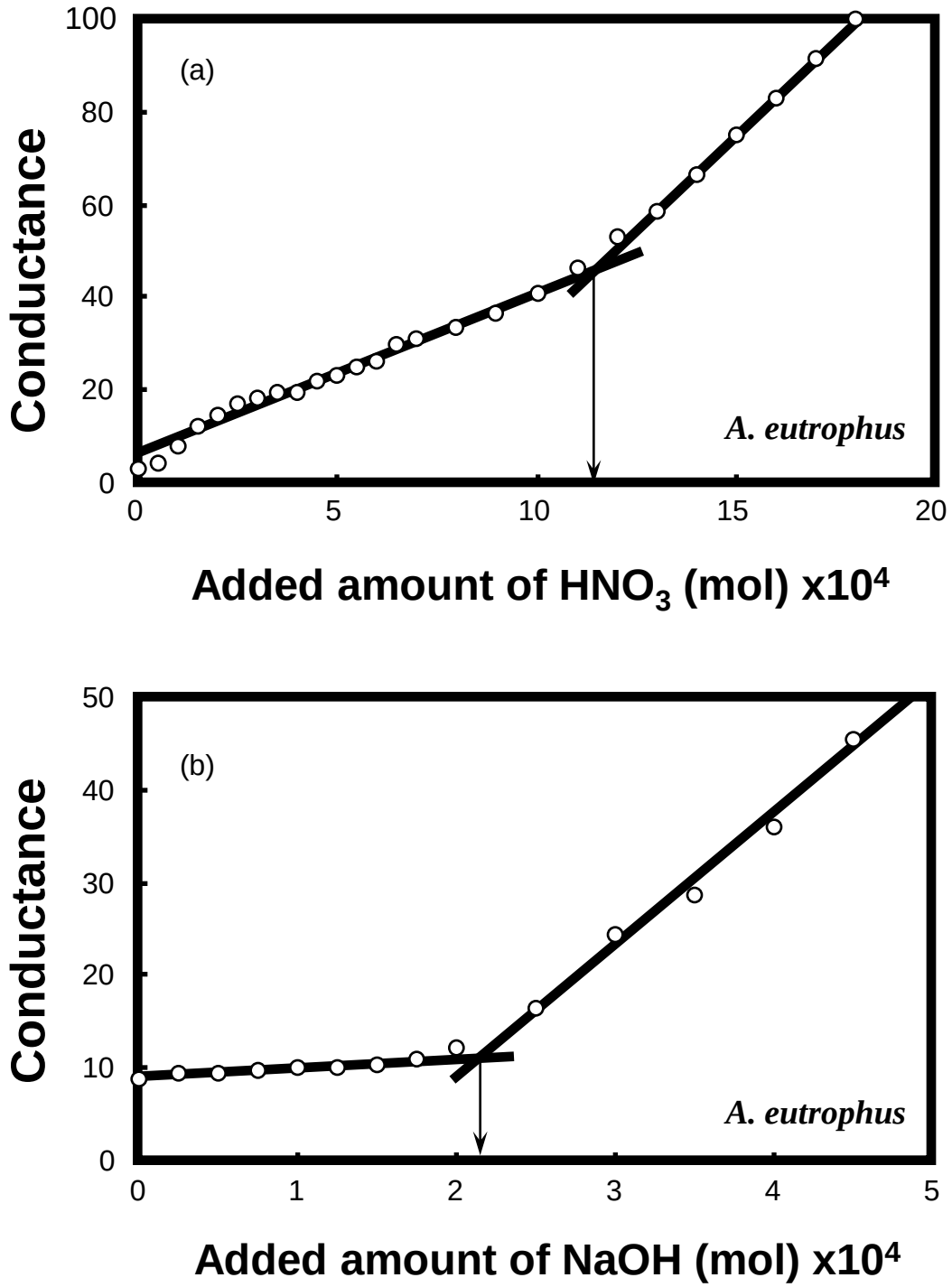

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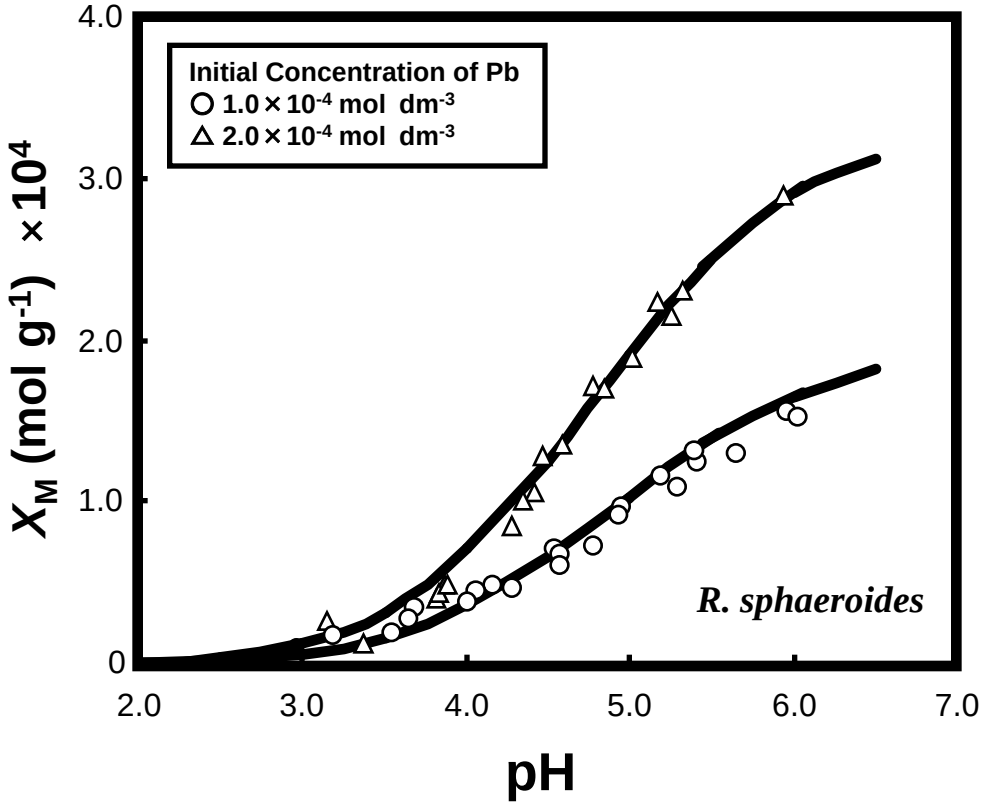

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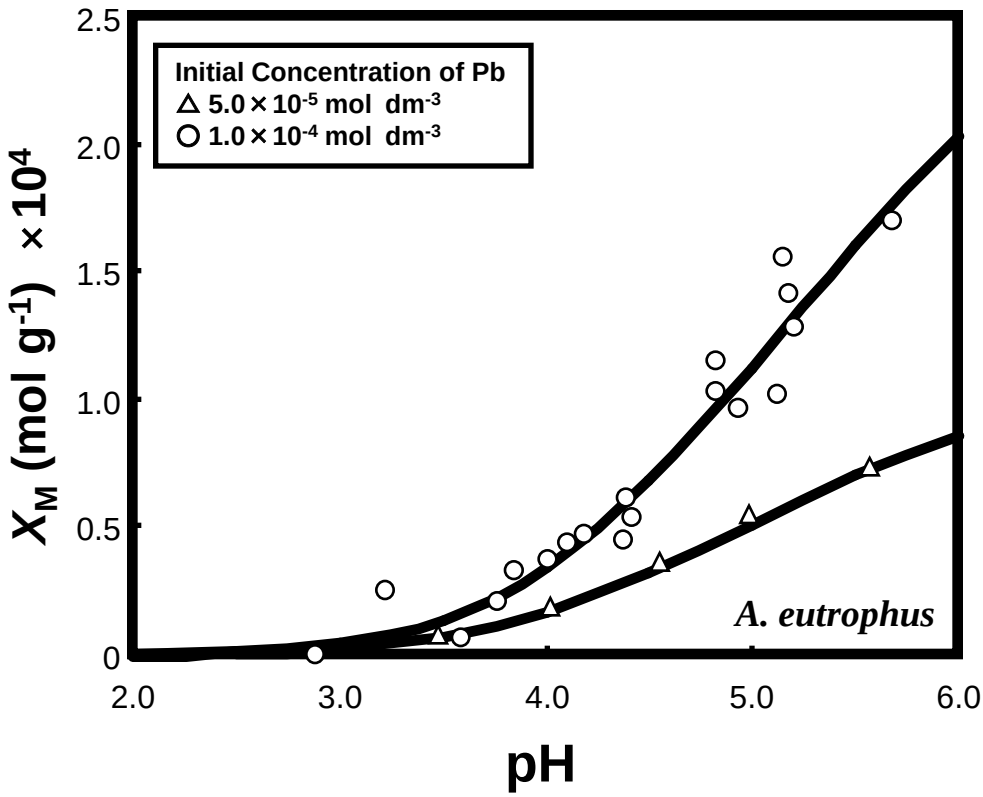

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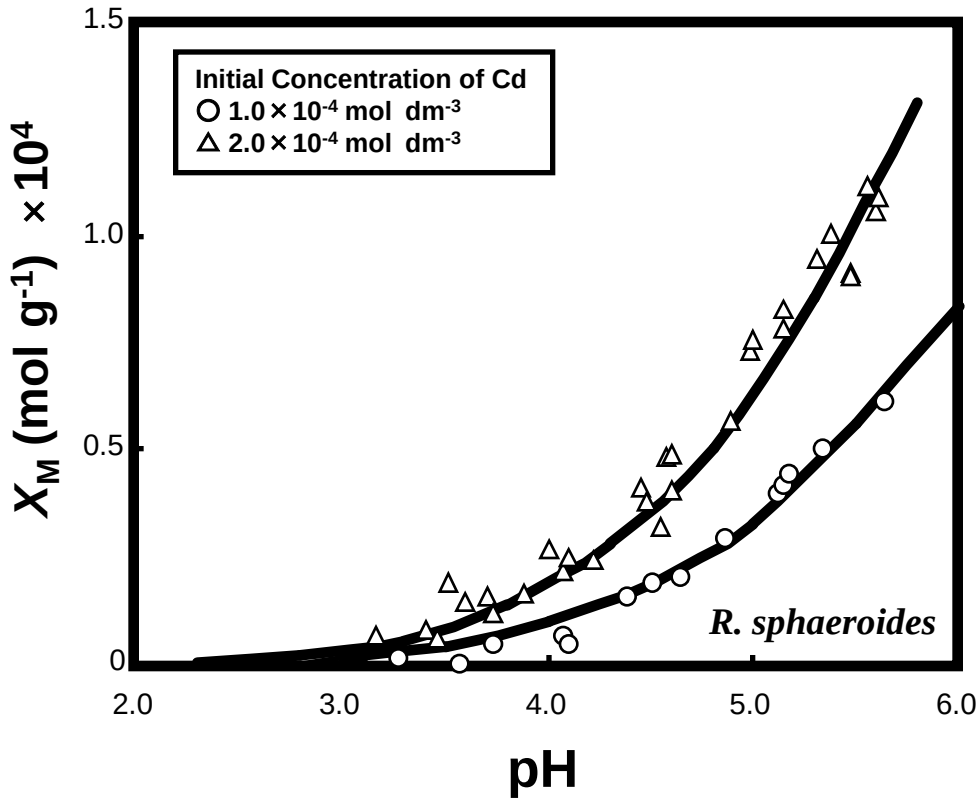

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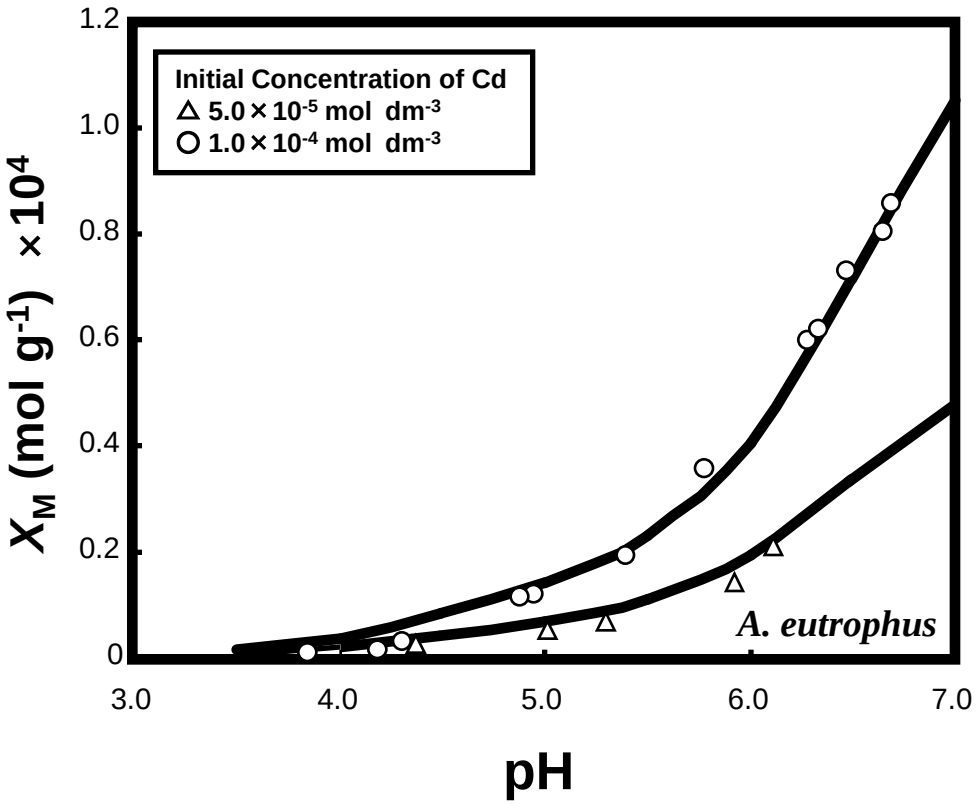

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Table 1. Equilibrium Parameters for the Acid Dissociation of *R. sphaeroides.*

	carboxylic	phosphatic	amino
pK	4.17	6.14	9.85
N	6.43×10^{-4}	4.01×10^{-4}	3.27×10^{-4}

Table 2. Equilibrium Parameters for the Acid Dissociation of *A. eutrophus.*

	carboxylic	phosphatic	amino
pK	4.53	7.08	9.26
N	1.43×10^{-3}	1.08×10^{-3}	1.92×10^{-4}

Table 3. Metal Binding Constants for Pb Biosorption.

	pK_{M1}	pK_{M2}
R. sphaeroides	-3.00	-4.82
A. eutrophus	-2.92	-5.13

Table 4. Metal Binding Constants for Cd Biosorption.

	pK_{M1}	pK_{M2}
R. sphaeroides	-2.58	-3.85
A. eutrophus	-2.03	-3.65