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Thermochimica Acta 269/270 (1995) 393–404

thermochimica acta

Calorimetric studies on interactions of divalent cations and microorganisms or microbial envelopes ☆

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Received 16 September 1994; accepted 2 May 1995

Abstract

The biosorption of heavy metal ions has attracted serious attention as a biotechnological approach to the removal of these elements from effluents or to the recovery of precious metals and radionuclides. We compared the metal sequestering capability of *Bacillus subtilis*, *Saccharomyces cerevisiae* and other strains, and determined sorption equilibria of the reaction $M + L \rightleftharpoons ML$ and the corresponding heats of reaction. The strains showed typical capacities and affinities to a series of metal ions but no clear selectivities. Adsorption was slightly endothermic and very fast and was driven by large $T\Delta S$ contributions. The data provide a critical view of the technical applicability of biosorption.

Keywords: Biosorption; Heavy metal ion; Microbial envelope; Microorganism

1. Introduction

The accumulation of heavy metals by a microbial biomass is a phenomenon which has increasingly attracted research activities. The scope of research covers aspects of new materials for environmental applications, e.g. wastewater purification, recovery of rare elements or precious metals, and detoxification of contaminated residues from the nuclear industry and metallurgy. Bioaccumulation is ascribed to several different

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☆ Presented at the 6th European Symposium on Thermal Analysis and Calorimetry, Grado, Italy, 11–16 September 1994.

SSDI 0040-6031(95)02515-4

mechanisms which involve living or dead biomass and active, i.e. metabolically driven, or passive mechanisms. The latter are promising with regard to industrial application as dead biomass products can be stored and handled like shelf chemicals [1]. Unfortunately the mechanisms of passive biosorption are little understood and sometimes affinity, selectivity and performance criteria are overestimated.

It has been shown that the equilibria constants of metal ion exchange in the cell walls of *Nitella flexilis* are almost independent of temperature [2]. The authors calculated ΔH values from very small temperature coefficients of K_{ex} values by means of the Van't Hoff equation and estimated an enthalpy change of reaction close to zero.

When technical applications of biosorption are considered, the strains have to be already available at low costs, and have to show extraordinary properties, i.e. affinity, capacity or selectivity towards the metals concerned [3].

In order to validate older estimates or to review earlier findings, we determined the enthalpy change of biosorption ΔH_{ads} experimentally. We used the LKB 2277 microcalorimeter equipped with a flow-mixing measuring cylinder. The data of chemical equilibria ($M_{dissolved}$, M_{bound}) were also assayed. We selected several strains according to these guidelines and produced crude cell-wall preparations and deactivated cells because biosorption very often is discussed as a surface-related phenomena. Large metabolic enthalpy changes caused by living cells [4] would disrupt the calorimetric determination of the heat of adsorption (ΔH_{ads}). Biomass or cell walls of *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Halococcus morrhuae* were converted to the so-called homoionic ion exchange materials and were countercharged with K^+ ions prior to calorimetric investigations. We hope our results will assist the discussion of the mechanisms concerning the interaction of metal ions (M) and binding sites of the biomass (L). The heterogeneity of ligands present in different microorganisms may lead to typical shapes of adsorption isotherms and ΔH_{ads} values. New criteria of technical applicability may arise from thermodynamic evaluations of biosorption.

2. Materials and methods

2.1. Selection of microorganisms

We selected *Saccharomyces cerevisiae* DSM 70451 (German type culture collection, Braunschweig), bakers yeast, which is already available at low prices in bulk and which is very often investigated with regard to metal biosorption [5]; *Bacillus subtilis* DSM 2109, a gram positive strain normally used to produce technical enzymes, also well studied [6], *Halococcus morrhuae* DSM 1307, a halophilic archaebacteria with uncommon cell wall properties [7], and several filamentous fungi.

2.2. Preparation of cell walls and whole cells, and chemical characterization

Strains of microorganisms were cultivated on a bench scale (5 L) utilizing appropriate media (DSM recommendations) and were harvested by spinning for 30 min at 3000 g and 277 K (Heraeus Labofuge RF). Raw biomass was resuspended, deactivated

at 353 K, washed three times in 5 mM PIPES-KOH-buffer pH 7.0 (piperazin-1,4-bis(2-ethane-sulphonic acid, Merck), and finally resuspended to 15–20% dry weight/vol.

To get crude cell walls the cells were mechanically disrupted in a swinging bucket mill (Retsch). A disruption effectiveness of $\geq$ 95% was achieved when 18 g glass beads, 0.25–0.5 mm diameter, and 10 ml of the supension were placed in an agate milling bucket of 25 ml working volume and ground for 60 min at a frequency of 1800 min^{-1} and 277 K. The suspension was diluted five times and the glass beads were allowed to settle. The supernatant was spun for 20 min at 3000 g and 277 K to harvest the cell walls. Cell walls were washed three times in reagent grade water (Millipore, Milli-Q-Water 18.2 MΩ), modified by acidic washing (acidified to pH 2–3 with HNO_3, Merck suprapur) and finally readjusted to pH 7 with KOH. The treatment replaced the majority of the common cations that were initially associated with the biomass. Removal of cations was monitored by AAS analysis. Raw cells of *Bacillus subtilis* DSM 2109 were treated as described except grinding, and were washed in different solutes as indicated when neccessary. Biomass was stored at 273–277 K until determination of adsorption equilibria or calorimetric data, respectively. The dry weight content of biomass or cell wall suspensions was assayed gravimetrically after membrane filtration (Sartorius, cellulose acetate 0.22 μm) of an aliquot that contained about 25–50 mg of solids. No corrections were made for ash content.

Isotherms were recorded at 298 K using the same ratios of biomass and metal solution as in the calorimetric analysis. Metals were analysed by AAS techniques appropriate to the element and its concentration. The spectrometer was calibrated with commercial standard solutions (Merck) using appropriate matrix modifiers. Solids were wet-digested prior to analysis. Small corrections were made for overestimates resulting from free solution which adhered to the pellets of biomass after spinning. The total amounts of metals were balanced with regard to the recovery of dissolved and bound metal ions. All metals were applied as analytical grade Me^{2+} nitrates.

2.3. Calorimetric investigations

The investigations were done at 298 K on an LKB 2277 BioActivity Monitor equipped with a flow-mixing cylinder 2277-204. Peristaltic pumps (LKB) under gravimetric flow control supplied reagents or cell wall suspension, respectively, to the flow-mixing cell. Each measurement lasted 15 min (Fig. 1). The sensitivity of the flow cell decreased with increasing flow rate of reactants (Fig. 2). When water (c_{p298K} = 4.1768 $J g^{-1} K^{-1}$) or ethanol (c_{p298K} = 1.4258 $J g^{-1} K^{-1}$) were used as fluids, the sensitivity was affected much less by the latter, indicating the reasonable effect of transportion of heat capacity through an isoperiobolic measuring cell that contributes to heat conductance and hence decreases sensitivity. A more comprehensive account of this is given by Poore and Beezer [8].

The accuracy of electric calibration was checked by the heat of protonation of TRIS–HCl buffer (pH 8) with HCl (Tris(hydroxymethyl)-aminomethane, Merck). This buffer shows a heat of protonation (ΔH_{prot}) of −46.442 kJ mol^{-1} [9]. Results remained unaffected by the flow rate within the working range of the instrument (Fig. 3). The

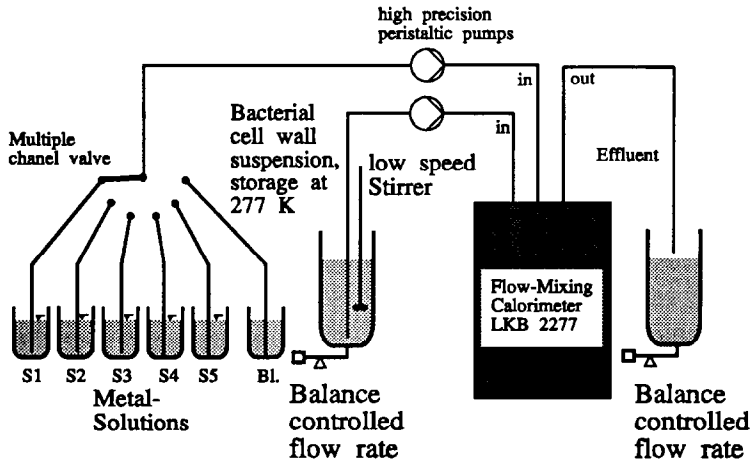

Fig. 1. Schematic drawing of the experimental setup. Calorimetry was performed by flow-mixing technique which allowed calculations of turnover and ΔH_{ads} as well as kinetic studies.

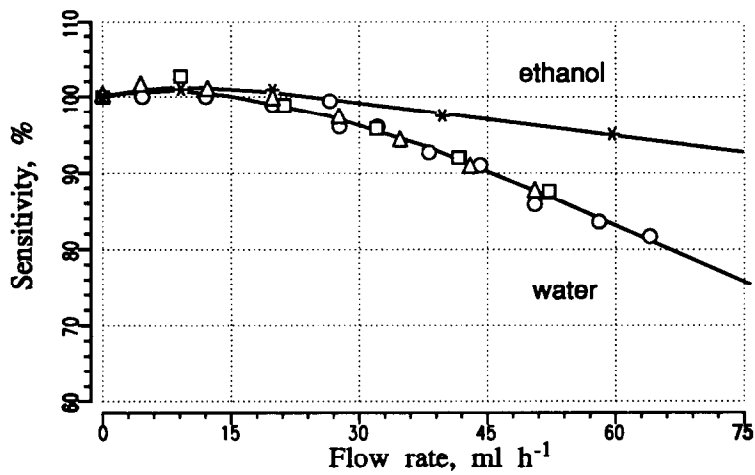

Fig. 2. Sensitivity of the calorimeter depending on liquid flow-rate and heat capacity of the liquid (electric calibration 30 uW, 100 uW, 300 uW).

accuracy of ΔH_{prot} determinations which served as a prototype of a fast chemical reaction guaranteed the applicability of this type of reaction calorimetry for kinetic investigations. The ΔH_{prot} of PIPES–KOH buffer was ascertained as -10.41 kJ mol^{-1} accordingly.

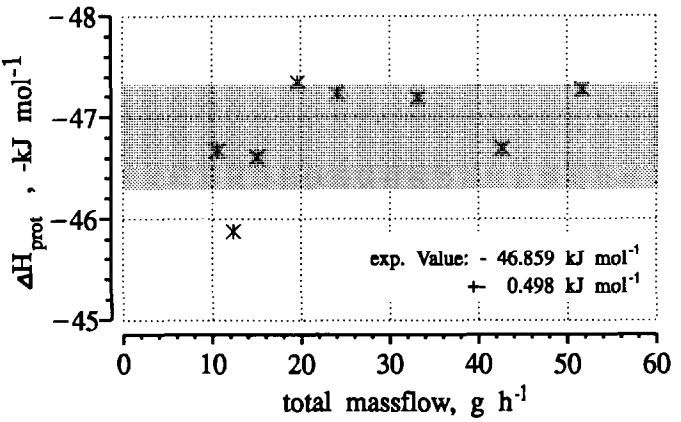

Fig. 3. Determination of ΔH_{prot} of Tris–HCl buffer at various flow rates of reactants. Values were calculated after electric calibration at the same flow rate.

3. Results

3.1. Biosorption characteristics and description of chemical equilibria

Sorbent materials were characterized: (i) by potentiometric titrations, and (ii) by experimental isotherms, in order to estimate appropriate description of the binding behaviour. The most commonly used descriptions of biosorption isotherms are the Langmuir and Freundlich formalism (Eqs. (1) and (2))

$$Q_{ads} = \frac{Q^*_{ads} K_L c_{free}}{(1 + K_L c_{free})} \Rightarrow c_M = \frac{Q^*_{ads} K_L c_{free}}{(1 + K_L c_{free})} c_{biosorb} + c_{free} \quad (1)$$

$$Q_{ads} = K_F c_{free}^{1/nF} \quad (2)$$

The Langmuir parameters Q^*_{ads} and K_L were calculated by non-linear regression analysis according to a Marquard algorithm and simple weighting of each data point (software package Enzfit, R.J. Leatherbarrow, Elsevier Biosoft 1987). When the degree of saturation of ligands is discussed, the values of Q^*_{ads} were used to calculate the partial saturation of ligands $\theta = ML/(L + ML) = Q_{ads}/Q^*_{ads}$. The optimized Langmuir constant K_L served to calculate $\Delta G^\circ_{ads} = -RT\,2.302 \log K_L$.

Calculations of ΔH_{ads} did not need any knowledge of a particular reaction mechanism or optimized parameters since all data were available experimentally. This guaranteed that assumptions made during calculations which have to be discussed later with the aid of resulting data, are not necessary. The flow-mixing calorimetry gave steady power signals when reactants were supplied at a constant flow-rate and concentration for more than 5τ of the instrument ($\tau_{inst} \approx 105$ s). Values of ΔH_{ads} were calculated by Eq. (3) from steady-state readings of the power dissipation P and chemical equilibria data. The fractional extent α of M, which underwent a reaction

$M + L \rightleftarrows ML$, was accessible from the experiments as $\alpha = (c_M - c_{free})/c_M$. The corresponding concentrations of the mixture in flow calorimetry were calculated using Eq. (4)

$$\Delta H_{ads} = \frac{P}{\dot{v}_{reac1} c_{reac1} \alpha} \tag{3}$$

$$c_M = c_{reac1} \frac{\dot{v}_{reac1}}{(\dot{v}_{reac1} + \dot{v}_{reac2})}$$

$$c_{biosorb} = c_{reac2} \frac{\dot{v}_{reac2}}{(\dot{v}_{reac1} + \dot{v}_{reac2})} \tag{4}$$

Acid-washed preparations were characterized by potentiometric titrations, when they were readjusted to pH 7 after acid treatment and water rinses. Titration curves were flat without sharp inflections (pK_a values), which indicated the multitude of different ligands (various types of carboxylic acid residues, etc.). Ligand concentrations of *B. subtilis* and *H. morrhuae* were sufficient to work at 1.25 g solids l^{-1}. The low concentration of ligands in yeast and filamentous fungi required a concentration of 5 g solids l^{-1} (see Table 1). Chemical equilibria data of the three biomaterials are shown in Fig. 4. *B. subtilis* removed Cd^{2+} most effectively from the solution, and the yeast removed low fractions of metal ions although a four-fold amount of the biosorbent was added.

Calorimetric data on formation of ML complexes of a variety of Me^{2+} and acid-treated cell walls of *B. subtilis* are shown in Fig. 5. The abscissa represents the feeding rate of M, $Mdt^{-1} = \dot{v}_{reac1} c_{reac1}$. Because of nearly constant flow rates through all the experiments, these data correspond to a concentration c_M between 0 and 1000 μ mol l^{-1}.

Inflections of the power vs. Mdt^{-1} curves resulted either from decreasing fractional extent of reaction (α) or alterations of ΔH_{ads} with increasing saturation of ligands (θ).

Table 1
KOH-titratible ligands of various acid-washed biosorbent materials

Species	DSM number	Pretreatment	Protonated Ligands mmol kg^{-1} [a]	Working concentration/ g l^{-1} [b]
Bacillus subtilis	2109	Acidic washes	900	1.25
Bacillus subtilis	2109	Wall prep., acid washes	1735	1.25
Halococcus morrhuae	1308	Wall prep., acid washes	770	1.25
Saccharomyces cerevisiae	70451	Wall prep., acid washes	156	5.00
Gliocladium flavo-fuscum	3500	Wall prep., acid washes	131	n.t.
Sclerotium glucamicum	2159	Wall prep., acid washes	110	n.t.

n.t. Not tested by flow-mixing calorimetry due to flocculation.
[a] Ligands HL were determined by titration of acid-washed cells or cell walls from pH 2 to pH 7.
[b] Effective concentration of cells or cell walls after mixing with metal solution.

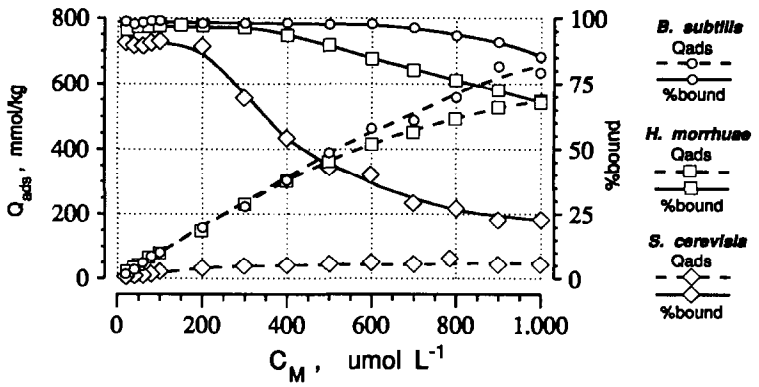

Fig. 4. Equilibria data of three biosorbents and Cd^{2+} ions. Q_{ads} of the biosorbent (left) and degree of elimination from the solution, α (right).

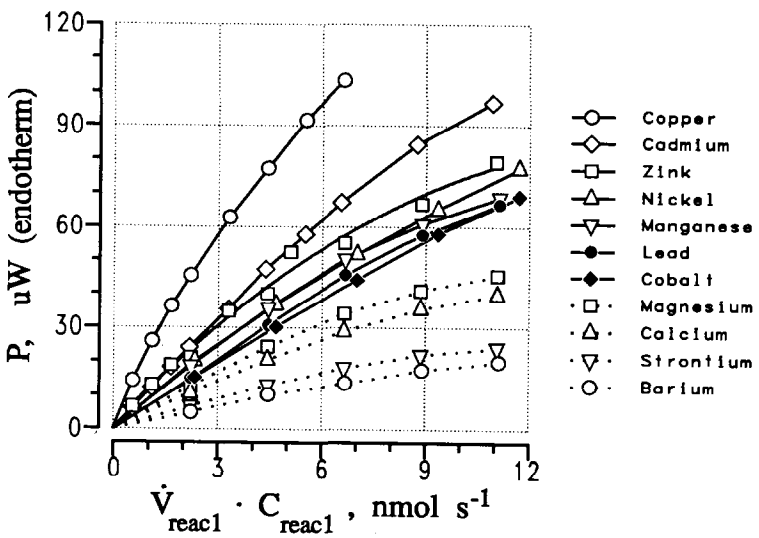

Fig. 5. Heat output P of biosorption of *Bacillus subtilis* cell walls exposed to $Me(NO_3)_2$ salt solutions in aqueous media at pH_{ini} 7.0.

Fig. 6 shows both phenomena, constant $\Delta H_{ads} = f(\theta)$ in most cases, and decreasing values when Cu^{2+} or Zn^{2+} are considered. The curvature of $\Delta H_{ads} = f(\theta)$ may originate from overlapping reactions of different ligands or the formation of different ML complexes but this remains speculative without further evidence. A survey of data is listed in Table 2.

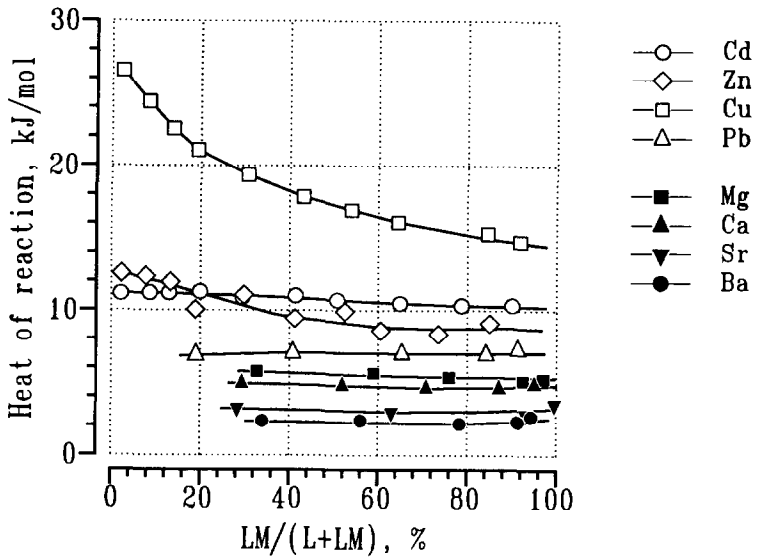

Fig. 6. ΔH_{ads} of biosorption on cell walls of *Bacillus subtilis*. For calorimetric data in Fig. 5.

Table 2
Characteristic data of biosorbents investigated in aqueous solution of Me^{2+}. ΔH_{ads} values correspond to ML/(L + ML) = 0.5 where the sorption capacity is half exhausted

Source of acid-washed cell walls	Me ion	Q^*_{ads}/mol kg^{-1}	CV	log K_L	CV	ΔG^0/kJ mol^{-1}	ΔH_{ads}/kJ mol^{-1}	$T\Delta S$/kJ mol^{-1}
Bacillus subtilis	Cd	702	25	4.00	0.047	−22.814	10.41	33.22
	Cu	775	38	3.83	0.063	−21.844	17.32	39.16
	Zn	742	53	3.59	0.084	−20.478	9.35	29.82
	Pb	721	22	3.99	0.041	−22.757	7.01	29.76
	Mg	708	9.4	4.07	0.021	−23.214	5.45	28.66
	Ca	459	15	3.99	0.046	−22.757	4.78	27.53
	Sr	353	43	4.22	0.16	−24.069	3.05	27.12
	Ba	512	25	4.03	0.078	−22.986	2.33	25.32
Saccharomyces cerevisiae	Cd	46.2	1.3	3.84	0.061	−21.901	7.65	29.55
Halococcus morrhuae	Cd	530	15	3.78	0.048	−21.560	7.70	29.26

3.2. *Kinetic investigations on native* Bacillus subtilis

Flow-mixing calorimetry offered the opportunity to monitor heats of reaction at various residence times, since the calorimetric readings were precise and reliable within narrow standard deviations (Fig. 3). Plug flow characteristics of the tubular path of the

mixing cell were assumed so the residence time Φ corresponded to $\Phi = v_{cell}/(\dot{v}_{reac1} + \dot{v}_{reac2})$. The working volume of the coiled tubular measuring cell was $v_{cell} = 0.67$ ml. The flow rates $\dot{v}_{reac}$ of both reactants were adjusted from 5 ml h^{-1} to 40 ml h^{-1} each. Fig. 7 shows the experimental results which are shown as ΔH_{exp} data and were calculated according to Eq. (3). They were not recalculated with regard to α, the fractional extent of metal ions which formed complexes ML from the supplied amounts of M. So the data can be interpreted as $\Delta H_{exp} = \eta\alpha\Delta H_{ads}$. The parameter η represents the progress of reaction kinetics and should increase as $f(\Phi)$ from 0 (initial state) to 1 (equilibria). The graphical presentation indicates progress of the reaction close to equilibria within the shortest period of time ($\approx$ 20s) accessible by this technique.

3.3. *Heat of biosorption in various solute systems*

Chemical investigations of biosorbents in aqueous media yielded data on cation and proton release (not shown here). Proton release and protonation of consitituents of experimental media or the biosorbent itself may also contribute to the experimental ΔH_{ads}. So we determined ΔH_{ads} of Me^{2+} on *B. subtilis* cells using three experimental media: (i) TRIS–HCl buffer which exerted high ΔH_{prot}; (ii) PIPES–KOH buffer which exerted low ΔH_{prot}; and (iii) water where the biosorbent itself may exert unknown contibutions due to protonation or deprotonation The value of ΔH_{prot} of acid-treated and neutralized *B. subtilis* cells was estimated as -12.3 kJ mol^{-1}.

Fig. 8 clearly shows the effect of buffer solution. The more exothermic reaction of TRIS compared to PIPES buffer gave clear evidence for a predominating H^+/Me^{2+} exchange especially when θ was small. From the difference $\Delta H_{prot-TRIS} - \Delta H_{prot-PIPES}$

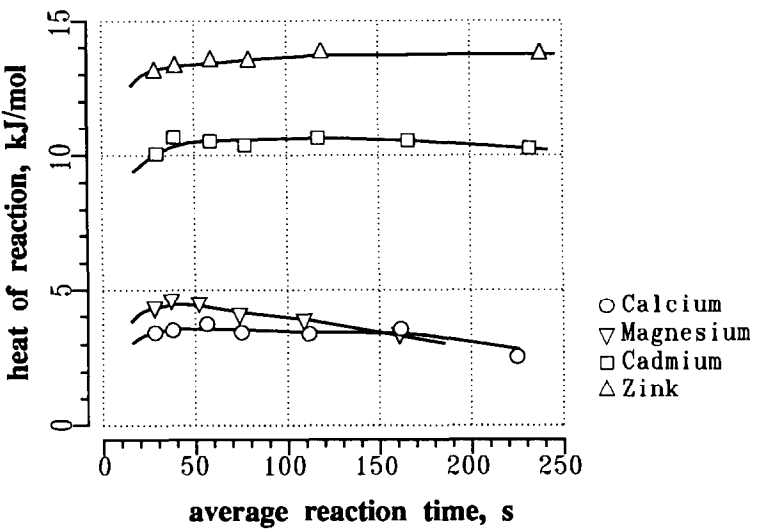

Fig. 7. Kinetic investigations of biosorption on *Bacillus subtilis* acid-treated cells. The shape of $\Delta H_{exp.} = f(\Phi)$ indicates complete equilibration within less than 20 s.

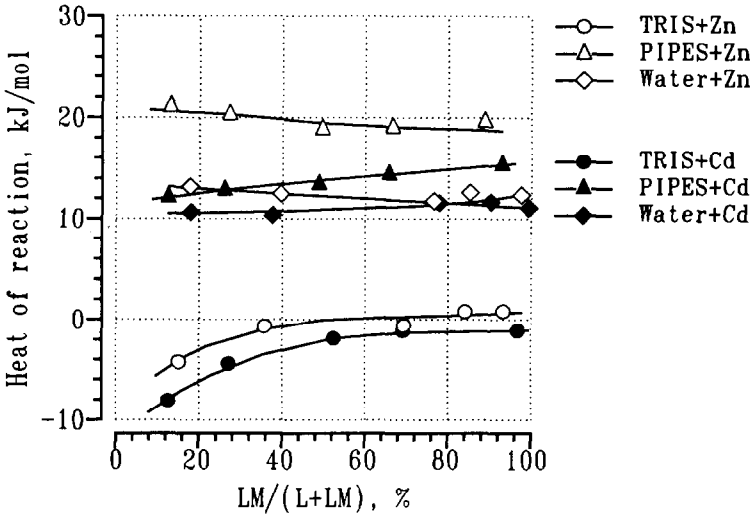

Fig. 8. ΔH_{ads} of *Bacillus subtilis* cells (K^+ homoionic) exposed to Me^{2+} ions in media of different ΔH_{prot} indicates a strong contribution from H^+/Me^{2+} exchange.

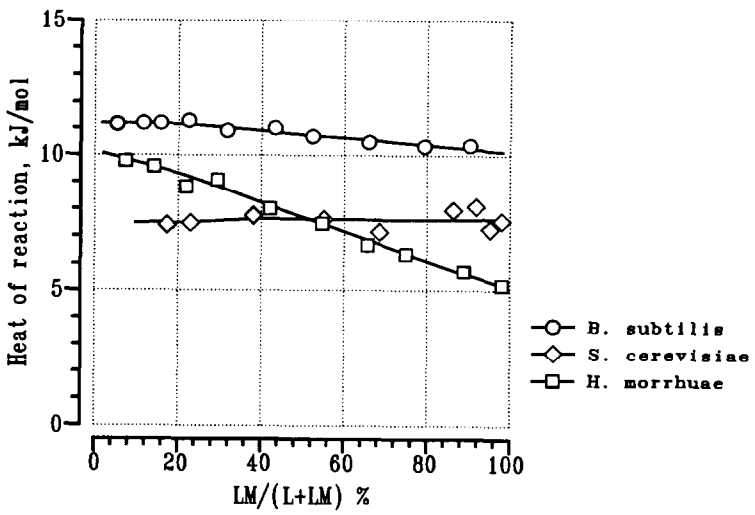

Fig. 9. Biosorption of Cd^{2+} by three microorganisms. Comparison of ΔH_{ads} indicates differences. *H. morrhuae* may carry two distinct ligands of high and low affinity.

(-38.17 kJ mol^{-1}), the H^+ exchange was calculated to contribute 0.65 to 0.33 H^+/Me^{2+} decreasing with increasing saturation of the biosorbent. This was supported by pH measurements of the biosorbent/water system, potentiometric titrations during the addition of metal ions and determination of ion releases (not shown here). The

charge balance was further maintained mainly by K^+ and to a minor degree by Mg^{2+} and Ca^{2+} release. The shape of ΔH_{ads} in the pure water solvent system is unexplained without further knowledge of the interactions.

3.4. Comparison of different types of cell walls

Not only did different biosorbents carry different numbers of ligands, but the formation of ML complexes was also accompained by different ΔH_{ads} values and $\Delta H_{ads} = f(\theta)$ curves. *B. subtilis* and *S. cerevisiae* walls showed constant endothermic values but ΔH_{ads} of *H. morrhuae* strongly depended on the degree of saturation. Its isotherm was also best fitted to experimental data when two types of ligands, and hence two types of reaction, were allowed to react simultaneously according to Langmiur formalisms, but the optimized parameters of the second, weakly bonding ligand were estimated within confidence limits that were too broad to calculate thermodynamic data.

4. Conclusions

Conventional analytical techniques has served to investigate the kinetics of biosorption in terms of minutes to hours [10]. The calorimetric technique facilitated the determination of biosorption kinetics within a time domain of several seconds to minutes and hence supported the prediction of requirements with regard to process engineering. Treated biomass reacted very rapidly, as expected, so water treatment with biosorbents may be done in very small apparatus with high throughput. But speed is not the only urgent demand in water purification techniques.

The mechanism of biosorption is ascribed to a multitude of ligands and depends on the chemical behaviour of the particular metal ion. Gadd distinguishes hard sphere (Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}) and soft sphere ions (Zn^{2+}, Cd^{2+}, Pb^{2+}, Ag^+) and transition metal cations (Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}) respectively [11]. Our study did not reveal strong indications of large differences in binding mechanisms when metal ions were added to acid-washed and neutralized biosorbents which functioned as an ion exchange resin. The reaction $M + L \rightleftarrows ML$ on a natural biosorbent may not be well characterized by capacity and Langmuir constants, but until a better description is available, these data help to evaluate the potential of biosorbents. We guess that H^+, and alkaline and alkaline earth ions seriously compete with divalent transition metal cations so biosorbents may not work well in media of high ionic strength. This conclusion is indicated by the very similiar K_L values of all the ions investigated here and also by the data which show a predominating and uniform contribution of $T\Delta S$ as the driving force (Table 2). Calorimetry may help with routine screening and development of novel biosorbents, either by flow-mixing calorimetry or by other techniques, without initial complex chemical analysis.

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