

Selective biosorption of heavy metals by algae*

A. Wilke¹, R. Buchholz², G. Bunke³

¹Hochschule Offenburg, University of Applied Sciences, Badstraße 24, D-77652 Offenburg, Germany

²Friedrich-Alexander-University of Erlangen-Nuremberg, Institute of Bioprocess Engineering, Paul-Gordan-Str. 3, D-91052 Erlangen, Germany

³Technical University Berlin, Institute of Biotechnology, Department of Bioprocess Engineering, Ackerstraße 71-76, D-13355 Berlin, Germany
Corresponding author e-mail: andreas.wilke@fh-offenburg.de; phone: +49 781 205 118; fax: +49 781 205 111

ABSTRACT

Thirty strains of algae were examined for their biosorption abilities for the uptake of cadmium, lead, nickel and zinc from aqueous solutions. A wide range of adsorption capacities was observed between the different strains of algae and between the four metals. The cyanophyceae *Lyngbya taylorii* exhibited high uptake capacities

for the four metals. It was confirmed by using instrumental analysis including EDX that the resulting capsules of the immobilization process are pervious to the lead. In simultaneous sorption processes of the four metals lead, cadmium, zinc and nickel, the following order of selective sorption was observed: Pb >> Ni > Cd > Zn. In contrast to a commercial ion-exchange material, the alkaline metal ions Na, Ca, K, and Mg showed only a small competitive effect on the adsorption abilities of the immobilized *L. taylorii*.

INTRODUCTION

The removal of heavy metals from aqueous solutions is an important issue faced by industries discharging wastewater containing heavy metals. Because of rapid industrialization, an alarming amount of toxic heavy metals has been released into the environment, endangering natural ecosystems and public health. Thousands of tons of lead are discharged from electric battery manufacturing, lead smelting, internal combustion engines fuelled with leaded petroleum, and mining activities (Cho and Kim 2003). Lead affects the human central nervous system, the blood pressure and the reproduction (Gower 1993).

Standard methods, such as chemical precipitation and reverse osmosis, are associated with high reagent consumption, high energy requirement and the production of sludge containing metals, from which it is difficult to remove the water and which also requires careful disposal (Aderhold et al. 1996; Wilde and Benemann 1993; Winter et al. 1994). The search for an effective treatment technology for removing heavy metals from industrial effluents includes the use of microorganisms. Algae biomass and especially microalgae show good adsorption properties for heavy metal uptake (Bedell and Darnall 1990; Veglio and Beolchini 1997, Volesky and Holan 1995).

The use of the dead cells offers several advantages: (a) metal removal system is not subjected to metal toxicity

limitations, (b) there is no requirement for growth media and nutrients, and (c) adsorbed heavy metals can easily be desorbed and the biomass can be reused. Because the fragile biomass of the microalgae is not suitable for the use in fixed bed columns (Chu et al. 1997), many immobilization methods have been developed to incorporate the cells into porous beads, which are more appropriate for operation in adsorption columns (Tsezos et al. 1989; Vannela and Verma 2006).

Natural and synthetic polymers such as Ca-alginate (Sag et al. 1995) or polyacrylamide have been widely used as a matrix system in some immobilization techniques. These techniques are mainly characterized by low algae content in the beads, low mechanical strength or slow adsorption kinetics. Considering these disadvantages it is important to improve existing matrix systems for immobilization of the biomass to extend their use in technical applications.

Wastewater effluents from some industries (e.g. galvanic industry) often contain a mixture of heavy metals and additionally alkali and alkaline-earth metals. To predict the efficiency of heavy metal uptake it is necessary to examine the selectivity of the uptake process. Moreover, the concentration and nature of light metals may influence the biosorptive capacity of heavy metals. It is important to elucidate the role of alkali and alkaline-earth metals on heavy metal uptake since they are always present in industrial effluents.

* Presented at The First International Environmental Best Practices Conference, 07-10 August 2006, Olsztyn, Poland

MATERIAL AND METHODS

Biomass

30 strains of microalgae were examined towards their adsorption abilities for heavy metal capacity and selectivity for Pb, Cd, Ni, and Zn (Klimmek et al. 2001). In this screening the cyanophyceae *Lyngbya taylorii* was characterized by its high adsorption capacity, simple cultivation, easy separation and by high biomass yield. This strain was originally isolated from the Seas of Korea.

Cultivation and preparation of biomass

Seawater Medium 5 (Schlösser 1994) with 5 fold KNO_3 concentration was used for cell cultivation. A sufficient quantity of algae material for experimental purposes was produced in three loop reactors (10 L, 20 L, 100 L fermentation volume) developed at the Institute of Biotechnology, Department of Bioprocess Engineering, TU Berlin (Walther 1999). In these reactors a mono-septic cultivation with high biomass yield is possible. The loops are made of glass and are illuminated by external light sources. An outline of the 100 L loop reactor is presented on Figure 1.

The biomass production in the respective cultivation systems was carried out under constant light conditions (the same light spectrum and intensity), constant temperature, and the same aeration rate. Before reaching the stationary growth phase, the biomass was separated from the cultivation medium by centrifugation. In order to achieve the same conditions for the following sorption experiments, the biomass was washed several times with deionized water until the conductivity became constant. This procedure was important because the ionic compounds of the cultivation media could have a significant influence on the biosorption process. Then the biomass was freeze-dried, ground and sieved ($250 \mu\text{m}$) in order to obtain a uniform particle size. The powdered *L. taylorii* was stored in air tight containers prior to the sorption experiments. No significant changes in the biosorption properties were observed between the different batches of cultivation.

Biomass entrapment was carried out according to Wilke et al. (1999) using the anionic cellulose polymer sodium cellulose sulphate (SCS) and the cationic agent polyethyleneimine (PEI) as the matrix system for encapsulation of the biomass. For the immobilization procedure, a cellulose polymer-solution (3.0-3.5%) was mixed with freeze-dried microalgae. The resulting suspension was pumped through the inner capillary of the immobilization tool, as shown on Figure 2. At the end of the inner capillary, the liquid forms a drop of variable diameter, depending on the flow rate of compressed air pumped through the outer capillary. Higher flow rates of compressed air result in smaller diameter of capsules. The diameter of the resulting particles ranged from 1.0 to 3.2 mm. After falling into a precipitation bath containing PEI (2.5%, pH 7.0), it precipitated immediately since the ionic character of SCS is negatively charged, while PEI is positively charged. A thin membrane thus surrounded the resulting hollow sphere. After stirring for one hour the immobilization process was completed. The biosorbents were finally washed three times with deionised water before further use.

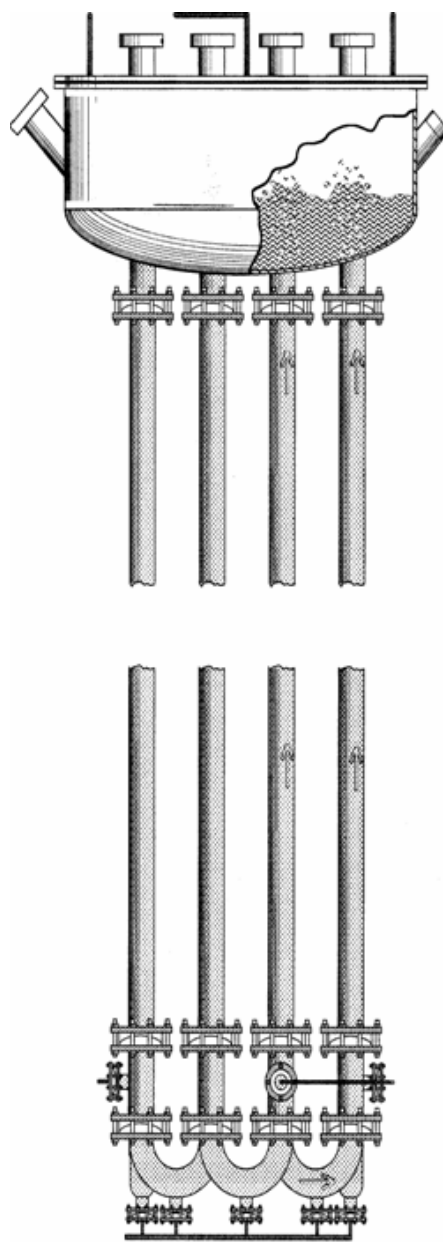


Figure 1. 100 L-loop-reactor for mono-septic cultivation of microalgae. The principle is based upon an airlift loop reactor with U-shaped, transparent loops. The loops are combined at the head of the reactor. A detailed description of the system is given by Walther 1999.

For raising the biomass content in the biosorbent material, the SCS/biomass suspension and the immobilization tool were heated up to 70-80°C before encapsulation started. For reducing the bulk density of an adsorption column, the biosorbent material was dried at 90-100°C for 24 h after the immobilization procedure has been completed (Wilke et al. 2000).

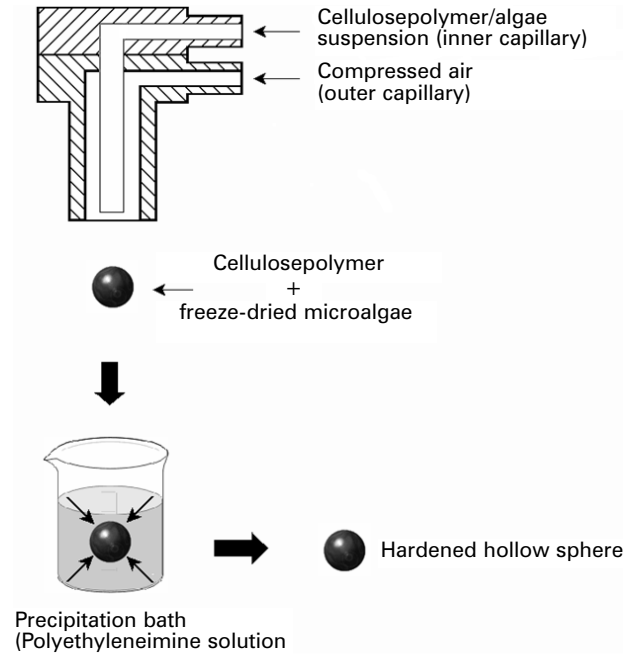


Figure 2. Immobilization process of *Lyngbya taylorii*.

Sorption experiments

Metal solutions were prepared by dissolving $Pb(NO_3)_2$ stock solution in doubly deionised water. The initial pH of the lead solution was adjusted to pH 5 by using either 0.1 M HCl or 0.1 M NaOH in order to prevent precipitation. Sorption equilibrium experiments were carried out using 500 mL shake flasks containing 100 mL lead solution of desired concentration. After the addition of the immobilized *L. taylorii*, the flasks were shaken in an overhead shaker at room temperature (20-22°C). In case of the equilibrium experiments, the flasks were shaken until adsorption equilibrium has been achieved (minimum 24h). The metal concentration in the bulk solution was determined by atomic absorption spectroscopy (Zeiss AAS 4, Analytik Jena AG) (Klimmek et al. 2001). With the help of the mass balance the adsorption capacity was calculated by the equilibrium concentration in the liquid phase.

Data analysis

Regarding the adsorption isotherms, the experimental data were fitted to the Langmuir adsorption model using a nonlinear regression software (SigmaPlot). Regression procedures use the values of one or more independent variables (concentration c) to predict the value of a dependent variable (uptake q). The SigmaPlot curve fitter uses the Marquardt-Levenberg algorithm

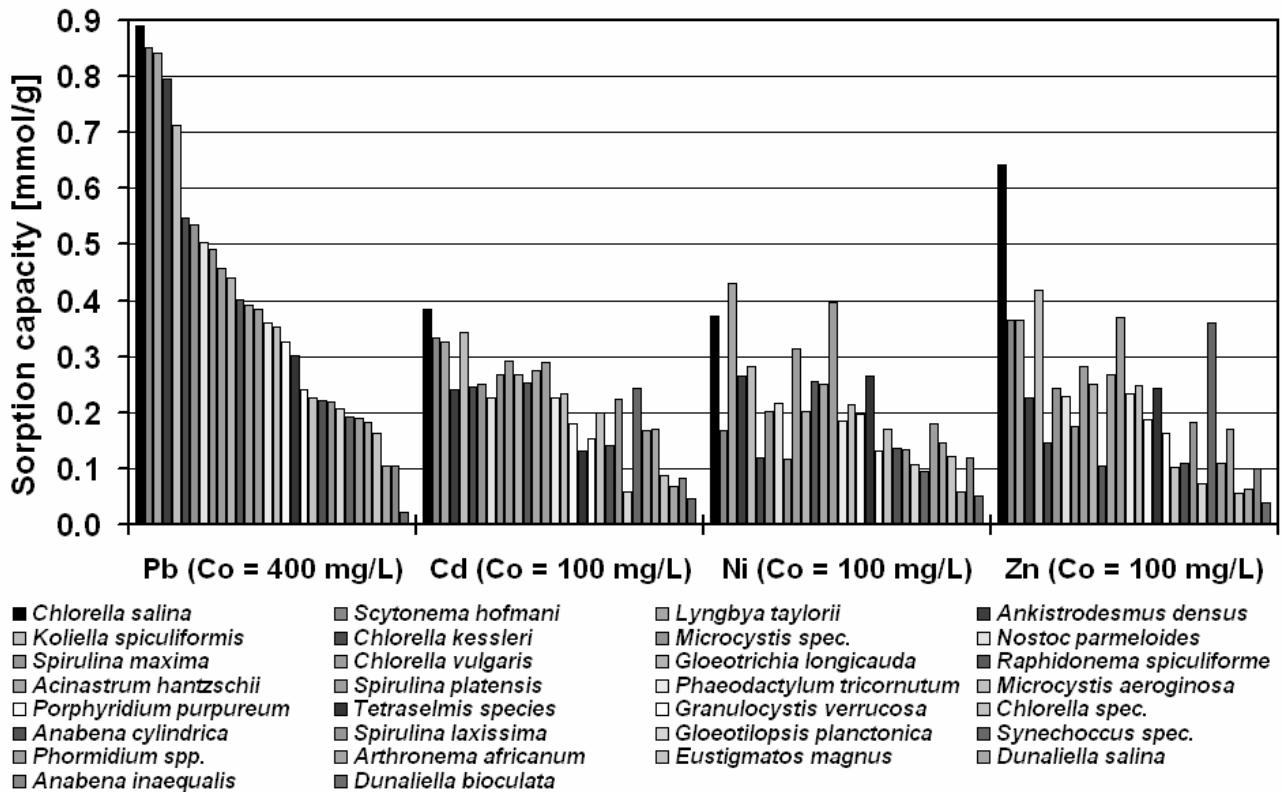


Figure 3. Results of the heavy metal screening of the lead, cadmium, zinc and nickel (c_0 = initial concentration). The algae species read in the legend from left to right correspond with the bars in the figure (also from left to right) for each heavy metal.

to find the coefficients (parameters: q_{\max} , b) of the independent variables resulting in the best fit between the equation and the data. This algorithm seeks the values of the parameters that minimize the sum of the squared differences between the observed and predicted values of the dependent variable.

Scanning electron microscopy (SEM) and energy dispersive X-ray microanalysis

Scanning electron microscopy in combination with energy dispersive X-ray microanalysis (EDX) was performed with a Hitachi S-2700 in conjunction with a Kevex Energy dispersive System (EDS) detector. Before the investigations the samples were coated with a thin layer of carbon.

RESULTS

Heavy metal screening

In the heavy metal screening 30 different algae strains were examined for their heavy metal capacities. The results are summarized on Figure 3. The examined algae strains showed a very heterogeneous capability of the uptake of lead, cadmium, zinc and nickel.

Most of the examined algae strains exhibited remarkably preferential adsorption of the metal lead. The chlorophyceae *Chlorella salina* and the cyanophyceae *Scytonema hofmanii* as well as *L. taylorii* showed highest metal uptake.

Equilibrium studies of the matrix material (SCS) and the non-optimized biosorbent

The matrix material sodium cellulose sulphate (SCS) had an advantage of its high adsorption capacity of heavy metals (Figure 4). The reason for the metal uptake could be explained by free sulphate groups of the SCS-molecule, which do not have a linkage to the positively charged polyethyleneimine and, on the other hand, by a series of hydroxyl groups acting as potential binding sites of the monomer glucose units.

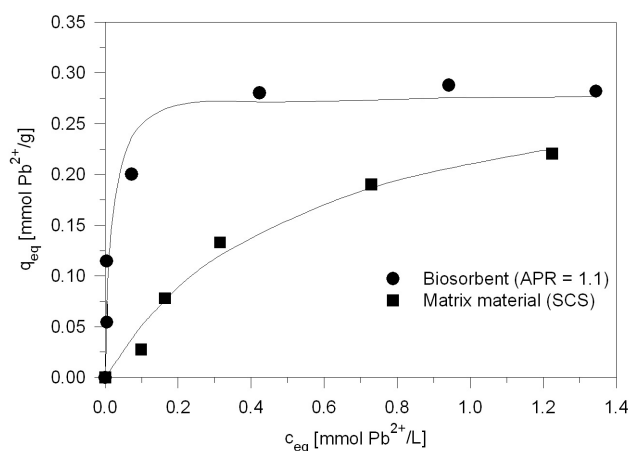


Figure 4. Lead-isotherms of the pure matrix material (SCS) and non-optimized biosorbent with low algae content (APR=1.1); q_{eq} represents the equilibrium uptake and c_{eq} the equilibrium concentration.

The immobilization process at room temperature lead to a maximum algae content of 48%. Biosorbents based on this algae to SCS ratio (APR=1.1) represented a Langmuir parameter b higher (76.20 L/mmol) than that of the matrix material (1.88 L/mmol) (Table 1). This reflected the higher binding strength of the heavy metal towards the biosorbent. High affinity of the metal lead to a higher sorbent equilibrium capacity q_{eq} at low equilibrium concentration c_{eq} in the liquid phase.

Optimization of the immobilization procedure

Encapsulation of *L. taylorii* by means of the described procedure produced biosorbents showing low algae content and low mechanical strength. Because the quantity of heavy metal uptake depended on the algae content of the capsules, different modifications of the immobilization method were tried in order to increase the density of biomass in an adsorption column.

The method of immobilization described above resulted in a maximum algae content of 48% inside the capsules (APR=1.1). Further increase of biomass content also raised the viscosity of the algae/cellulose polymer-suspension (Figure 2). At room temperature, it was consequently impossible to form capsules with this suspension due to the high viscosity. By heating the suspension with the consequent reduction of the viscosity it was possible to increase the APR up to 3.2, which corresponded to a content of 76% algae dry mass in the biosorbent (Wilke 2000).

By raising the algae content up to 76% (APR=3.2) the lead uptake of the biosorbent was raised up to 0.91 mmol lead per gram of biosorbent as a maximum uptake q_{max} (Table 1 and Figure 4). Comparing these results to unimmobilized cells of *L. taylorii*, q_{max} was reduced from 1.47 mmol lead per gram biomass (Klimmek et al. 2001) in case of biosorbent (APR=3.2) by 38%.

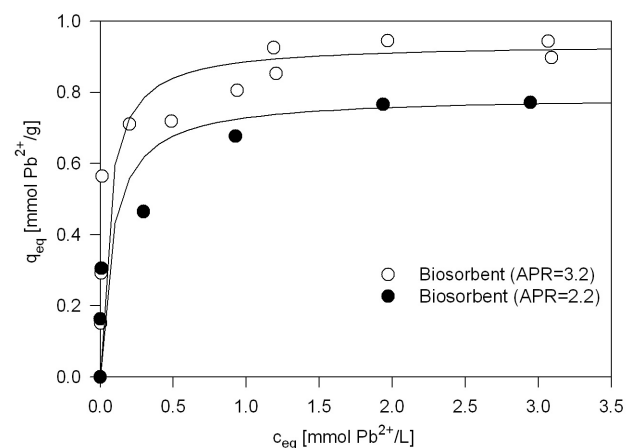


Figure 5. Lead-isotherms of the optimized biosorbent with high algae content (APR=2.2 or 68.8% algae dry weight and APR=3.2 or 76.2% algae dry weight); q_{eq} represents the equilibrium uptake and c_{eq} the equilibrium concentration.

Table 1. Langmuir parameters q_{\max} and b Langmuir isotherm constant of the pure matrix material SCS and *Lyngbya taylorii* immobilized particles on the basis of SCS.

Sorption material	APR	Langmuir constants			
		q_{\max}		b	
		[mmol/g]	CV [%]	[L/mmol]	CV [%]
Matrix material SCS	0	0.32	5.89	1.88	37.86
Non-optimized biosorbent	1.1	0.28	12.15	76.20	27.27
Optimized biosorbent *	3.2	0.91	5.07	26.00	-

* Curve fit based on fixed Langmuir constant b. Only the parameter q_{\max} was fitted.

Optimization of the bulk density

Besides the algae content, other parameters are important for scaling up the experiments to an industrial application, e.g. the bulk density of a fixed bed unit. Low densities of the biosorbent material increase the operational and investment cost due to larger hardware that is needed (columns, pumps etc.). To reduce the bulk density of the particles in an adsorption column, the wet capsules were dried at 90-100°C for 24 hours after the immobilization process. The capsules shrunk due to the humidity loss. When such treated capsules were placed back into an aqueous environment, their swelling capability was considerably reduced. As a result, the density of adsorption material in fixed bed reactors and its effectiveness increased (Figure 6).

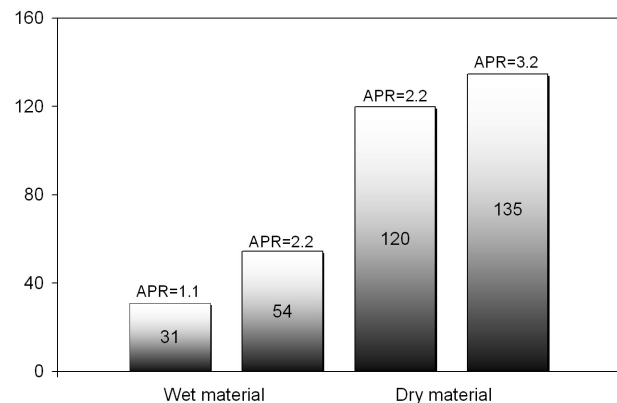


Figure 6. Influence of the algae to polymer (SCS) ratio (= APR) and the drying step after the immobilization procedure on the bulk density in an adsorption column.

By raising the APR from 1.1 to 2.2, the bulk density in an adsorption column increased by 74% up to $\rho_{\text{column}}=54$ g/L. Due to the drying step during the immobilization procedure, the bulk density raised up to $\rho_{\text{column}}=120$ g adsorbent material per L of reactor volume. The maximum density, now attainable for this system, was $\rho_{\text{column}}=135$ g biosorbent per L.

Characterization of the biosorbents

Thermal treatment after the immobilization process entailed an irreversible change of the membrane structure and caused a considerable increase of the biosorbents stability (Wilke et al. 2000). The SEM pictures demonstrated the results of the drying process. In order to check whether the membrane was still permeable for the metal ions, backscattered scanning electron microscopy and image analysis were made by the dried particles. With the help of this technology, elements with high atomic number can be visualised. On account of the strong electron dispersion they appeared as white spots on such pictures (Figure 7).

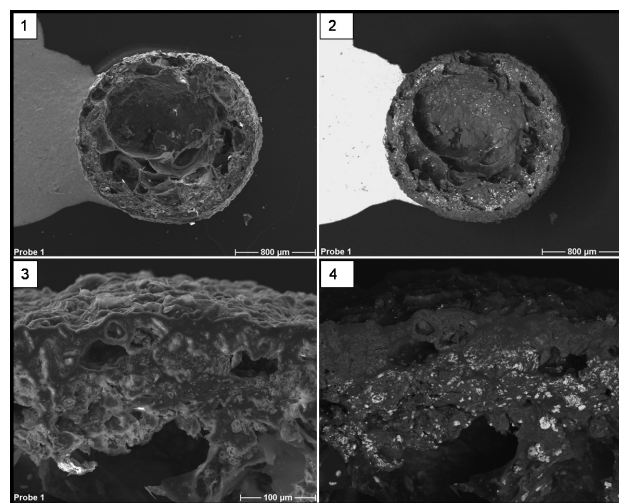


Figure 7. Scanning electron microscopy (1, 3) and backscattered radiation photos (2, 4) of immobilized *Lyngbya taylorii*. Sectional view (1, 2) and membrane area of the biosorbent (3, 4).

Regular distribution of the white spots over the whole cross section of the biosorbent could be recognised well. Another fact was the low uptake of lead in the membrane region. It can be explained with the blocked functional groups of the cellulose by polyethylenimine as essential membrane building component.

The proof that the white spots on the Figure 7 were caused by the lead could be verified by the help of the energy dispersive X-ray technique (EDX). The results of the spectrum (Figure 8) were based on the integral section inside of a biosorbent particle.

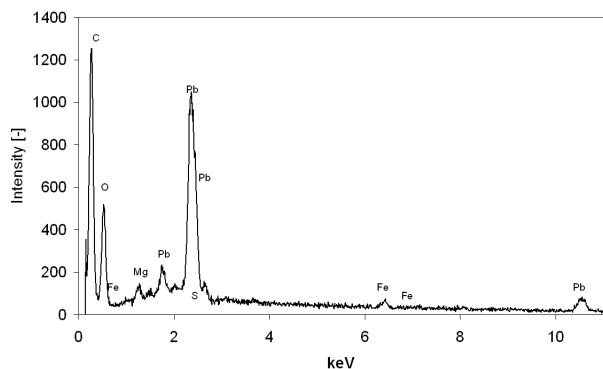


Figure 8. EDX spectrum of the sectional area of an biosorbent particle loaded with lead.

One can assign the element lead with the help of the typical band in the spectrum at 2.2 and 10.55 keV and therefore confirm that ions of lead can pass the membrane of the dried biosorbents. Other occurring elements in the spectrum were either components of the matrix system (C, O, S), the biomass (C, O, S, Fe, Mg), or impurities of the SCS production process (Fe, Mg).

Selective uptake of lead, cadmium, nickel and zinc

To estimate the selectivity of the heavy metal uptake in the presence of lead, cadmium, nickel and zinc, multimetal isotherms were determined. The practical procedure to measure multimetal isotherms is comparable to that for single isotherms. The basic difference was, that in the beginning of the experiment, equal molar metal concentration in the liquid phase were used. Figure 9 shows multimetal isotherms of *L. taylorii* biosorbents.

Multimetal isotherms (Figure 4) demonstrated preferential uptake of the metal lead. While the maximum uptake decreased only for approximately 25% comparing to the single isotherm, other metals showed a markedly reduced binding behaviour.

Studying selective uptake of nickel, cadmium and zinc, three- and two metal isotherms were additionally examined (Figure 9). In result the selectivity has been determined as:

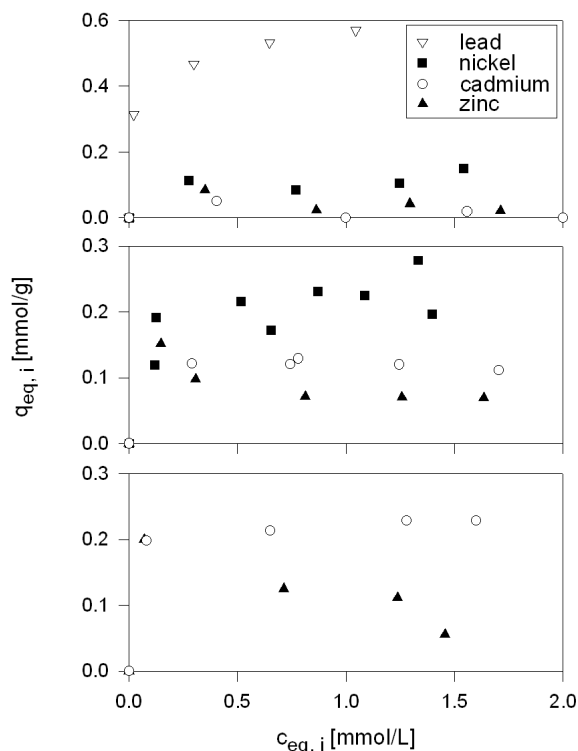
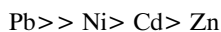


Figure 9. Multimetal isotherms of the metals lead, cadmium, nickel and zinc using immobilized *Lyngbya taylorii* as biosorbent (APR=3.2); $q_{eq,i}$ represents the equilibrium uptake and $c_{eq,i}$ the equilibrium concentration.

Influence of competing ions on the sorption capacity of lead

In the metal-processing industry the prevailing proportion of the liquid sewage has an acidic pH and is neutralized by the use of sodium hydroxide, calcium oxide hydrate, lime-milk, sodium carbonate or magnesite (Hartinger 1991). Nevertheless, after precipitation of the metal hydroxides, the metal concentration in the liquid phase often exceeds the legal threshold. This often results in a very high concentration of competing ions.

To examine the influence of the co-ions sodium, potassium, magnesium and calcium on the uptake of lead, several experiments were carried out using the following method. The co-ions were added to each batch at different concentrations. The ratio of biosorbent to the liquid volume was calculated with the help of the single isotherm of lead (Figure 10) to get an equilibrium concentration c_{eq} close to the maximum uptake q_{max} .

During the experiments, the starting concentration of the co-ions c_0 were increased from 0 up to 12 g/L. After reaching equilibrium the lead concentration was analysed and the resulting metal uptake was calculated using the mass balance.

The bivalent co-ions magnesium and calcium show no significant influence on the lead uptake of the biosorbents. In the presence of sodium (>4 g/L) and potassium (>8 g/L) the monovalent ions caused slight reduction of the lead uptake.

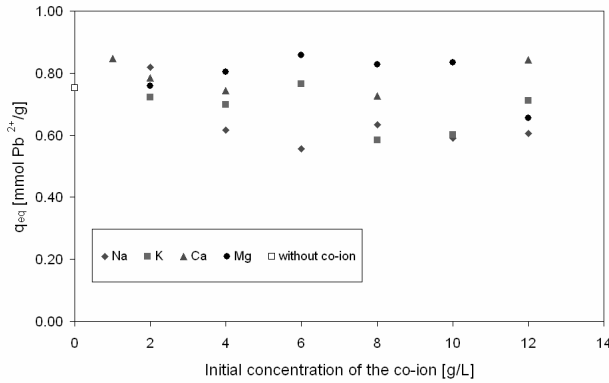


Figure 10. Influence of the co-ions sodium, potassium, magnesium and calcium on the lead uptake of immobilized *Lyngbya taylorii* as biosorbent (APR=3.2); q_{eq} represents the equilibrium uptake.

Influence of co-ions on the Langmuir parameters b and q_{max}

In order to check the influence of the co-ions on the shape of the Langmuir adsorption isotherm, two sets of experiments have been carried out using biosorbents with an algae content of 76% (APR=3.2) and additionally the strongly acidic cation exchanger IRA 120 Amberlite. This type of cation exchanger is often used in the metal-processing industry, because it can be used in a wide pH-range without being damaged.

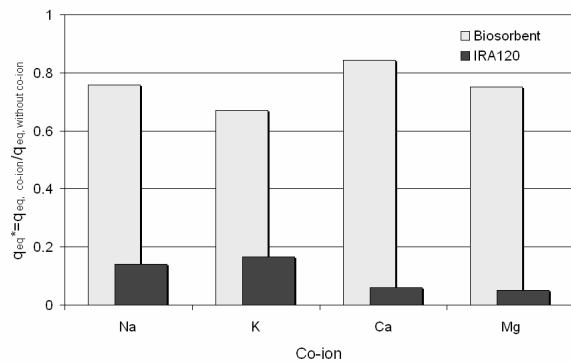


Figure 11. Influence of the co-ions sodium, potassium, magnesium and calcium in the low concentration range ($37 < q_{eq} < 49$ mg Pb²⁺/g) on the lead adsorption isotherm with q_{eq}^* as the normalised equilibrium uptake, $q_{eq, co-ion}$ as the equilibrium uptake in the presence of the co-ions, and $q_{eq, without co-ion}$ as the equilibrium uptake without the presence of the co-ions.

The sorption capacity of the cation exchanger is 2.1 mval/g, which is approximately two times higher than the biosorbent based on SCS immobilization of *L. taylorii*. The first set of experiments was designed to get a low equilibrium concentration in the range of $37 < c_{eq} < 49$ mg Pb²⁺/L. If the co-ions influence the Langmuir parameter b, this should be observed in this concentration range, because the maximum differences of the uptake were expected at low equilibrium concentrations (Figure 11).

Within the applied concentration range, the co-ions caused an uptake reduction between 16-33%, which resulted from the competition of the ions for the binding site of the biosorbents. There were no differences in sorption characteristics caused by mono- and bivalent co-ions.

In case of the cation exchanger IRA 120, the competition of the binding sites caused substantial decrease in the uptake of the heavy metal lead. The presence of sodium and potassium caused the uptake decrease of about 84%. The bivalent ions calcium and magnesium showed even stronger influence. The original loading was reduced to 6% and 5% as compared to the heavy metal uptake when no co-ions were present.

In the second set of experiments we examined the influence of the co-ions on the maximum uptake q_{max} . In contrast to the first set of experiments, here a high equilibrium concentration has been applied ($c_{eq} > 225$ mg Pb²⁺/L). At this concentration the area a maximum sensitivity on the Langmuir parameter q_{max} was expected. Figure 12 shows the influence of the co-ions on q_{max} which was comparable to the observed Langmuir parameter b.

The biosorbent showed a decrease of its maximum uptake of 18 % when the co-ion sodium was present and 7% in the presence of potassium. Calcium and magnesium had no influence on the lead uptake.

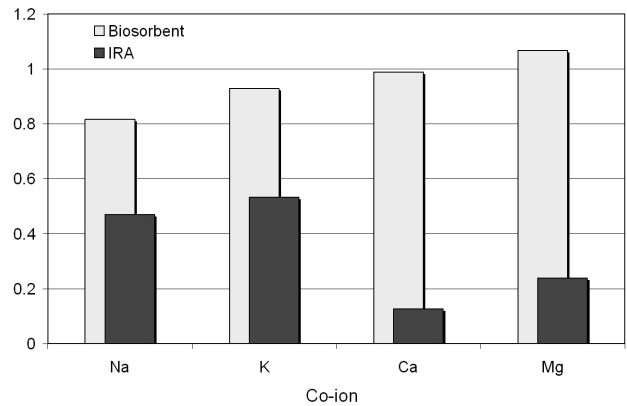


Figure 12. Influence of the co-ions sodium, potassium, magnesium and calcium in the high concentration range ($q_{eq} > 250$ mg Pb²⁺/g) on the of lead adsorption isotherm of biosorbents with q_{eq}^* as the normalised equilibrium uptake, $q_{eq, co-ion}$ as the equilibrium uptake in the presence of the co-ions, and $q_{eq, without co-ion}$ as the equilibrium uptake without the presence of the co-ions.

In contrast to the biosorbent, the co-ions showed a significant competition effect when the cation exchanger IRA 120 was used. In the applied concentration range, where all binding sites were saturated, the monovalent co-ions sodium reduced q_{\max} to 47% and potassium down to 53%. With the bivalent magnesium and calcium the original uptake was decreased to 13% and 27% in comparison to the uptake observed when no co-ions were used.

In general, the cation exchanger showed substantially higher capacity loss caused by the co-ions in comparison to the biosorbent. The influence of calcium and magnesium was more distinctive than that of the monovalent co-ions sodium and potassium.

DISCUSSION

Heavy metal screening

The screening results of the examined algae revealed no relationship between taxonomic class and heavy metal capacity. The sorption ability of the eukaryotic chlorophyceae varied between very low in case of *Dunaliella bioculata* and very high metal uptake in case of *Chlorella salina*. A similar behaviour was observed in case of the prokaryotic cyanophyceae: *L. taylorii* and *Anabena inaequalis* (Figure 3).

There was no correlation between the natural habitat of the algae and their properties of the heavy metal uptake; also the sorption capacity of fresh water algae were not different to those of the marine microalgae. Nevertheless, the preferential sorption of the metal lead was obvious. Sorption capacity of some strains examined in this study was substantially higher (*L. taylorii*, *Scytonema hofmani*, *Chlorella salina*) than in case of a number of bacteria and fungi, described in the literature. Higher adsorption capacities were achieved only by some macro algae species like *Sargassum* and *Ascophyllum* (Veglio and Beolchini 1997; Volesky and Holan 1995).

Optimization of the immobilization procedure

The immobilization process of the microalgae is an essential condition for the operation of a fixed bed column. Such a biosorbent has to fulfil some minimal characteristic demands concerning its mechanical, thermal and chemical stability.

Different methods for biomass immobilization are well known. The immobilization using alginate have the advantage to be economical. The disadvantage is the high mass transport resistance of this immobilization matrix, which leads to a slow adsorption and desorption kinetics and poor chemical stability. Chemically cross-linking agents like glutaraldehyde is connected with blocking of functional groups and therefore results in a decreased adsorption capacity. The use of encapsulation techniques in combination with SCS facilitates obtaining high biomass densities.

Modification of the immobilization procedure (Wilke et al. 2000) enables an algae yield of 76% in the biosorbents. With other immobilization techniques, usually only much lower biomass contents are feasible (Table 2).

Minor loading capacities of biosorbents led to a larger dimensioning of the columns with given flow rates of the waste water. Considering the sorption capacity of the matrix material SCS, the maximum uptake of the unimmobilized algae and the algae to polymer ratio of 3.2, a theoretical uptake of this biosorbent of 1.19 mmol Pb^{2+} /g can be calculated. The experimental uptake of 0.94 mmol Pb^{2+} /g was slightly lower than expected. The reasons for this difference between calculated and experimental Pb^{2+} uptake could be explained by blocking the functional groups in the outer membrane of the biosorbents as well as by the partial destruction of functional groups during the immobilization process. Although the method used for determination of the Langmuir binding constant b allows for larger deviation, it is obvious that the resulting biosorbents showed a stronger affinity to the heavy metal lead as compared to the pure matrix material or the pure biomass (Table 1). At present the reason for this fact cannot be explained. The characterization of the binding mechanism in the future work would help answering this question.

Optimization of the bulk density

There were two factors affecting the bulk density and the biomass density in a fixed bed sorption column: the algae polymer relationship (APR) and the drying process after the immobilization procedure.

While an increase of the APR mainly influenced the mass related uptake, the drying procedure only shifted the volume related uptake maintaining the uptake capacity at the same level. High densities of biosorbents in an adsorption column are favourable in dimensioning the technical process. An increasing bulk density leads to smaller columns and therefore demands lower invest and operational costs in technical applications.

Selective uptake of lead, cadmium, nickel and zinc

The experimental results showed how limited is our ability to predict selective adsorption efficiency on the basis of single adsorption isotherms. While the Langmuir parameter q_{\max} derived from the single isotherms led to the following order of selectivity: $Pb > Zn > Ni > Cd$, the same factor measured with the help of the multimetal isotherms gave: $Pb \gg Ni > Cd > Zn$. It is obvious that predictions of the selectivity can only be based on the results of multimetal isotherms. The reasons for the different selectivity in heavy metal mixtures are diverse. Tobin et al. (1984) demonstrated a linear relationship between the maximum loading and the ion radius of the used heavy metals. These results were not confirmed by this study. Ni^{2+} has a smaller ionic radius (78 pm) as compared to Cd^{2+} (103 pm), but it was bound preferentially by the biosorbents based on immobilized *L. taylorii*.

Further indications can be found for the selective uptake of the heavy metals by the biosorbents regarding the chemical and physical characteristics of the examined heavy metals. Due to the small solubility constants K_{sp} of the product (Lide

Table 2. Biomass dry mass content of biosorbents prepared by different immobilization techniques.

Method of immobilization	Biomass content [%] dry weight	Reference
Ca-Alginate	6–38	Chang et al. 1998; Roehricht et al. 1990
Polyacrylamide	7.4	Chang et al. 1998
Polyhydroxyethyl methacrylate	8–15	Veglio et al. 1999
Polysulfone	50	Blanco et al. 1999
Polyvinylformal	60–88	Brady et al. 1999; Tsezos and Deutschmann 1990
Silica gel	1.25	Robles and Aller 1996

2003), the precipitation concentration for lead hydroxide at a constant pH is reached first (compared with the three other metal hydroxides) and microprecipitation of lead hydroxide takes place on the surface of the algae. This process could explain the occurrence of the comparatively large, white spots on the backscattered radiation pictures of Pb^{2+} -loaded biosorbents. Other dominating binding mechanisms would rather lead to a more consistent allocation of lead instead of such concentrated spots observed on the backscattered radiation pictures (Figure 7).

Influence of calcium, magnesium, sodium and potassium as competing ions in the lead uptake process

The results demonstrated the advantages of the examined biosorbents as compared to the cation exchanger IRA120. The wastewater coming from the galvanic industry has a very low pH value and high heavy metal concentrations. In the pretreatment baths corrosion products are cleaned by mineral acids followed by activation (pickling baths), where large quantities of sulphuric acid, hydrochloric acid, boron hydrofluoric acid and hydrofluoric acid are used. The dissolved heavy metals are mostly removed by neutralization and precipitation of the metal hydroxides using calcium oxyhydrate, soda, dolomite or sodium hydroxide from the liquid phase (Hartinger 1991).

The residual concentration of the competing ions remaining in the liquid phase can be roughly calculated by neutralization stoichiometry. The neutralization of an acidic process solution (pH 0) with calcium hydroxide results in a remaining concentration of approximately 20 g Ca^{2+} /L. Therefore in this study the concentration range of the competing ions of up to 12 g/L has been chosen.

Different binding mechanisms of the lead on the biosorbents (microprecipitation, ion exchange, complexation) caused a substantially lower influence of the co-ions on the lead uptake compared to the cation exchanger IRA 120. While the bivalent cations (Mg^{2+} , Ca^{2+}) have a stronger displacing effect than monovalent cations (Na^+ , K^+) on the cation exchanger, no clear statement is possible in case of the biosorbent. Magnesium and calcium had stronger competition effect with the cation exchanger due to the increased presence of these ions in the binding sites. This effect was observed only in case of zinc and

cadmium. Lead and nickel showed no displacing effect. This fact supports the assumption that the binding mechanism of lead and nickel is a combination of different binding mechanisms, e.g. micro-precipitation, ion-exchange and complexation.

In the literature only few structured investigations can be found, which describe the influence of competing ions on the sorption process of heavy metals. Brauckmann (1997) indicated that calcium disturbs the sorption process to a larger extent than do comparable concentrations of sodium and potassium. Similar results have been published by Nagase et al. (1997). They investigated the influence of calcium and magnesium on the sorption capacity of cadmium using the cyanophyceae *Tolypothrix tenuis*. A calcium solution of 4 mmol/L reduced the cadmium adsorption below 20% as compared to the uptake without co-ions, while magnesium in the same concentration range lowered the cadmium uptake to 50%.

While these results indicate that ion exchange is the major binding mechanism, the publication of Kim et al. (1995) shows results about the biosorbents based on immobilized *Lyngbya taylorii* comparable to those described in this work. The macroalgae *Undaria pinnatifida* showed maximum reduction of only about 19% in the presence of the co-ions sodium, potassium, magnesium and calcium (10 g/L). These results support the assumption that different algae strains, especially those with high binding capacities, exhibit more than one binding mechanism.

Summarizing the results of this study the following conclusions can be drawn:

- No prediction of the selective uptake of different heavy metals is possible based on the adsorption characteristics of single isotherms.
- The binding mechanism plays an important role in the prediction of the selectivity of heavy metal uptake.
- Due to the variety of different binding mechanisms in the process of heavy metal uptake (ion exchange, microprecipitation, complexation), technical applications of biosorbents require investigations regarding the major kind of binding mechanism occurring in each specific case.

REFERENCES

- Aderhold, D., C.J. Williams, R.G.J. Edyvean. 1996. The removal of heavy-metal ions by seaweeds and their derivatives. *Bioresource Technology* 58: 1-6.
- Bedell, G. W., D.W. Darnall. 1990. Immobilization of Nonviable, Biosorbent, Algal Biomass for the Recovery of Metal Ions. In: *Biosorption of Heavy Metals* (ed. B. Volesky), pp. 313-326. CRC Press, Boca Raton, FL.
- Blanco, A., B. Sanz, M.J. Llama, J.L.J. Serra. 1999. Biosorption of heavy metals to immobilised *Phormidium laminosum* biomass. *Journal of Biotechnology* 69: 227-240.
- Brady, J.M., J.M. Tobin; J.-C. Roux. 1999. Continuous fixed bed biosorption of Cu²⁺ ions: application of a simple two parameter mathematical model. *Journal of Chemical Technology & Biotechnology* 74: 71-77.
- Brauckmann, B. 1997. Mikrobielle Extraktion von Schwermetallen aus Industrieabwässern. *Wasser Boden* 49: 55-58.
- Chang, J.-S., J.-C. Huang, C.-C. Chang, T.-J. Tarn. 1998. Removal and recovery of lead fixed-bed biosorption with immobilized bacterial biomass. *Water Science and Technology* 38: 171-178.
- Cho, D.H., E.Y. Kim. 2003. Characterization of Pb²⁺ biosorption from aqueous solution by *Rhodotorula glutinis*. *Bioprocess and Biosystems Engineering* 25: 271-277.
- Chu, K. H., A. Hashim, S.M. Phang, V.B. Samuel. 1997. Biosorption of cadmium by algal biomass: adsorption and desorption characteristics. *Water Science and Technology* 35: 115-122.
- Gower, R.A. 1993. Lead toxicity: current concerns. *Environmental Health Perspective* 100: 177-187.
- Hartinger, L. (ed.). 1991. *Handbuch der Abwasser- und Recycling-technik*. 357p. Carl Hanser Verlag, München Wien.
- Kim, Y. H., Y.J. Yoo, H.Y. Lee. 1995. Characteristics of lead adsorption by *Undaria pinnatifida*. *Biotechnology Letters* 17: 345-350.
- Klimmek, S., H.-J. Stan, A. Wilke, G. Bunke, R. Buchholz. 2001. Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. *Environmental Science & Technology* 35: 4283-4288.
- Lide, D.R. (ed). 2003. *CRC Handbook of Chemistry and Physics*. 120 p. CRC Press, Boca Raton, London, New York, Washington D.C.
- Nagase, H., D. Inthron, Y. Isaji, A. Oda, K. Hirata, K. Miyamoto. 1997. Selective cadmium removal from hard water using NaOH-treated cells of the cyanobacterium *Tolypothrix tenuis*. *Journal of Fermentation and Bioengineering* 84: 151-154.
- Robles, L.C., A.J. Aller. 1996. Immobilized bacterial cells as biosorbents for toxic elements. *Quimica Analytica* 15: 21-31.
- Roehricht, M., P. Weppen, W.-D. Deckwer. 1990. Abtrennung von Schwermetallen aus Abwasserströmen – Biosorption im Vergleich zu herkömmlichen Verfahren. *Chemie Ingenieur Technik* 62: 582-583.
- Sag, Y., M. Nourbakhsh, Z. Aksu, T. Kutsal. 1995. Comparison of Ca-alginate and immobilized *Z. ramigera* as sorbents for copper (II) removal. *Process Biochemistry* 30: 175-181.
- Schlösser, U.G. 1994. SAG – Sammlung von Algenkulturen Universität Göttingen – Catalogue of strains. *Botanica Acta* 3: 111-186.
- Tobin, J. M., D.G. Cooper, R.J. Neufeld. 1984. Uptake of metal ions by *Rhizopus arrhizus* biomass. *Applied Microbiology* 47: 821-824.
- Tsezos, M., A.A.J. Deutschmann. 1990. An investigation of engineering parameters for the use of immobilized biomass particles in biosorption. *Journal of Chemical Technology & Biotechnology* 48: 29-39.
- Tsezos, M., G.L. McCready, J.P. Bell. 1989. The continuous recovery of uranium from biologically leached solutions using immobilized biomass. *Biotechnology and Bioengineering* 34: 10-17.
- Vannela, R., S.K. Verma. 2006. Cu²⁺ removal and recovery by Spi SORB: batch stirred and up-flow packed bed columnar reactor systems. *Bioprocess and Biosystems Engineering* 29: 7-17.
- Veglio, F., F. Beolchini. 1997. Removal of metals by biosorption: A review. *Hydrometallurgy* 44: 301-316.
- Veglio, F., F. Beolchini, M. Boaro, S. Lora, B. Corain, L. Toro. 1999. Poly(hydroxyethyl methacrylate) resins as supports for copper(II) biosorption with *Arthrobacter* sp.: Matrix nanomorphology and sorption performances. *Process Biochemistry* 34: 367-373.
- Volesky, B., Z.R. Holan. 1995. Biosorption of heavy metals. *Biotechnology Progress* 11: 235-250.
- Walther, C. 1999. *Entwicklung und Scale-up eines thermisch sterilisierbaren, extern beleuchteten Photobioreaktors*. Dissertation, Nr. 156, FB 15, D83. Technische Universität Berlin.
- Wilde, E.W., J.R. Benemann. 1993. Bioremoval of heavy metals by the use of microalgae. *Biotechnology Advances* 11: 781-812.
- Wilke, A., G. Bunke, P. Götz, R. Buchholz. 1999. Removal of Lead, Cadmium, Zinc and Nickel by Adsorption on Microalgae. *Progress in Mining and Oil Field Chemistry. Challenges of an Interdisciplinary Science – Akademiai Kiado* 1: 337-344.
- Wilke, A., G. Bunke, P. Götz, R. Buchholz. 2000. Partikuläres Konstrukt mit Biomasse. DE-Patent 199 18 953.6.
- Winter, C., M. Winter, P. Pohl. 1994. Cadmium adsorption by non-living biomass of the semi-macroscopic brown alga, *Ectocarpus siliculosus*, grown in axenic mass culture and localisation of the adsorbed Cd by transmission electron microscopy. *Journal of Applied Phycology* 6: 479-487.