Appl Biochem Biotechnol (2010) 160:1540–1556
DOI 10.1007/s12010-009-8635-7

The New Application of Biosorption Properties of *Enteromorpha prolifera*

Izabela Michalak · Katarzyna Chojnacka

Received: 23 January 2009 / Accepted: 31 March 2009 /
Published online: 30 April 2009

Abstract The main goal of this paper was to elaborate the possibility of industrial application of biosorption properties of *Enteromorpha prolifera* (production of mineral feed additives for livestock). In this study, biosorption process was used in the binding of chromium(III) ions from aqueous solution by the green macroalga. The kinetics of biosorption process was studied in a batch system with respect to the initial pH, temperature, initial metal ion concentration, and initial biomass concentration. *E. prolifera* demonstrated good biosorption properties. The equilibrium biosorption capacity increased with pH and with initial concentration of metal ions. The uptake of chromium(III) ions by the dried alga was affected by the temperature, but in small extent. With increase of the biomass concentration, the decrease of biosorption capacity at equilibrium was observed. The best biosorption conditions were determined as the initial pH 5, temperature 25 °C, the initial chromium(III) ions concentration 400 mg/L, and biosorbent concentration 1.0 g/L. Biosorption capacity at equilibrium reached at these conditions was 100 mg/g. The mechanism of the biosorption of chromium(III) ions by *E. prolifera* was analyzed in equilibrium experiments. Equilibrium data were fitted to Langmuir, Dubinin–Radushkevich, and Freundlich adsorption isotherms. The most suitable model for describing the obtained data was Langmuir model. The experimental results and the analysis of the solution before and after biosorption process suggested ion-exchange mechanism.

Keywords *Enteromorpha prolifera* · Biosorption · Chromium(III) ions · Kinetics · Isotherm · Mineral feed additives

Introduction

Increasing attention toward the application of algae in many branches of industry is caused by the permanent growth of human population, pollution, and overexploitation of land.

I. Michalak (✉) · K. Chojnacka
Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology,
Smoluchowskiego 25, 50-372 Wrocław, Poland
e-mail: izabela.michalak@pwr.wroc.pl

Abundance of macroalgae in marine and freshwater environments could provide energy, raw materials, feed for animals, and food for human [1]. In this paper, a particular attention was paid to an edible green macroalga—*Enteromorpha prolifera*—that is available on the Polish coast almost throughout the year. In the littoral zone of the Gulf of Gdańsk, *Enteromorpha* sp. is currently the dominant group of algae [2]. Generally, in the case of *Enteromorpha*, there is a worldwide distribution (e.g., Japan [3], China [4], Mexico [5], Turkey [6], Hawaiian Islands [7]), with some species tolerating waters as warm as 30 °C and others occurring in Antarctic waters where the maximum temperature is 1.8 °C. In addition to growing well at normal salinity, *Enteromorpha* may also occur in hypersaline lakes (at 52% salinity) or in tide pools, as well as brackish and freshwaters [8].

There are several algal-derived applications that have already been proven to be of value. The first is supplementation of food and feed products. Harvesting of macroalgae for consumption has a long history. For example, the commercial product "aonori" (a mixture of *Ulva pertusa*, *E. prolifera*, and *Monostroma latissum*) is commonly consumed mainly in Japan [3]. In India, *Enteromorpha compressa* is used as an ingredient in the preparation of Pakoda, a common traditional snack food [9]. In the recent years, there is also increasing interest in the application of macroalgae, as a source of polysaccharides in food, cosmetic, and pharmaceutical industries [10]. In edible *E. prolifera*, polysaccharides demonstrate gelling abilities [11]. Also, marine algae are considered as a source of bioactive substances that are useful in the treatment of various diseases: bacterial and viral infections, cancer, and inflammation [12]. Some macroalgae are also consumed by animals (directly from the seashore or as an ingredient of the fodder), but it depends on the taste of seaweeds [13]. A special attention is paid to edible *Enteromorph*a sp. because this macroalga is rich in proteins [5, 9, 14], minerals [4, 5, 15, 16], essential amino acids [5], essential fatty acids [5, 9, 14], fiber [5, 9], and carbohydrates [9, 14]. Moreover, *Enteromorpha* sp. is approved for human and animal consumption by the obligatory law [17, 18]. Nevertheless, the major use of seaweeds on a global scale is found in human nutrition. The total volume of seaweeds used in food is considerably larger than the sum of the industrial applications, when considering both tonnage and value [1]. For many years, several macroalgae have been also used as fertilizers [13, 19]. Seaweeds like *Phymatolithon* spp., *Ecklonia* spp., and *Ascophyllum nodosum* are utilized to produce fertilizers and soil conditioners, especially for the horticultural industry [19].

Another application of macroalgal biomass concerns wastewater treatment processes. The use of algae to treat wastewater has been investigated for over 40 years, with one of the first descriptions of this application being reported by Oswald and Gotaas [20]. In the past several decades, increasing attention was paid to the application of biosorption processes in wastewater treatment [21]. Macroalgae, with natural property of binding metal ions from aqueous solutions, were proved to be very effective and cheap biosorbents [21, 22]. Literature reports that *E. prolifera* was found to be a very good biosorbent for the removal of cationic dyes (Acid Red 337, Acid Blue 324, and Acid Red 274) [23, 24] and Ni(II) ions [6] from wastewaters. Another application of algae in wastewater treatment concerns the usage of a high-rate algal ponds (HRAP). Nowadays, they are tested to treat effluents from an intensive marine cultures (e.g., fish farms), which are characterized by high nutrient concentrations (especially nitrate and phosphate, which are responsible for eutrophication). In HRAP, macroalgae act as a biofilter of dissolved nutrients [25, 26]. Moreover, metal and heavy metal content in such algal tissues at the end of the monitoring period suggested no metal contamination so that macroalgal species could be used in the food industry [25].

In this paper, biosorption process of chromium(III) ions by *E. prolifera* was described. The novel application of this macroalga aimed at combination of nutritional value and its

biosorption properties. This means that the biosorption process will be used not for removal of metal ions from aqueous solutions but for incorporation into the biological material. The biomass of *E. prolifera* enriched with chromium(III) ions could be used as mineral feed additive to supplement livestock diet with the recommended daily intake of this microelement. Chromium is accepted as nutritionally essential for animals and humans. The primary role of chromium in metabolism is to stimulate the action of insulin through its presence in an organometallic molecule called glucose tolerance factor. Chromium is thought to be essential for activating certain enzymes and for stabilization of proteins and nucleic acids [27]. In the recent years, there is an increasing interest in the utilization of chromium(III) in animal nutrition. However, the dietary requirement of livestock for chromium has not been defined yet. Nevertheless, it has been reported that dietary chromium supplementation has a positive effect on growth rate and feed efficiency in growing poultry [28].

Previously, we discussed the application of algae enriched with chromium(III) ions (microalgae: *Chlorella vulgaris* [29], *Spirulina* sp., and macroalga *Pithophora varia* [30]) in animal feeding. Moreover, the feeding experiments on laying hens with macroalgae *E. prolifera* and *Cladophora* sp. enriched with microelements confirmed that microelements were transferred to eggs and supplementation of algae enhanced the color of egg yolks (Michalak et al., in review).

The main goal of this paper was to elaborate a possibility of the application of biosorption properties of *E. prolifera* in fodder industry. The application of the biomass of macroalgae on commercial scale requires identification of the best biosorption conditions such as pH, temperature—T, biomass concentration—C_S, and initial metal ion concentrations—C_0. The preliminary experiments concerned the kinetics and equilibrium of biosorption of chromium(III) ions. This kind of studies is significant, because it provides valuable insights into the process pathways and into the mechanism of biosorption [31].

Materials and Methods

Sorbent Preparation

The alga *E. prolifera* was collected in June 2006 from the Baltic Sea (Niechorze—Poland). It was identified in the Department of Botany and Plant Ecology of the Wrocław University of Environmental and Life Sciences. The collected biomass of alga was washed with tap water several times to remove foreign matter and afterward with deionized water three times. Then, the biomass was dried at 60 °C until the constant mass was reached (to ensure there will be no bioaccumulation process). The biomass of dry alga was grinded and used in biosorption experiments.

Batch Biosorption Experiments

The experiments were performed in 250 mL Erlenmeyer flasks containing 200 mL of chromium(III) ions solution (for kinetic experiments) and 20 mL (for equilibrium experiments) in thermostated water bath shaker at 150 rpm. The solutions of chromium (III) ions were prepared in deionized water (by dissolving appropriate amounts of $Cr(NO_3)_3 \cdot 9H_2O$ (from POCh S.A. Gliwice, Poland)). pH of the solutions was adjusted with 0.1 mol/L solution NaOH/HCl (from POCh S.A. Gliwice, Poland). pH was measured with pH meter Mettler-Toledo (Seven Multi; Greifensee, Switzerland) equipped with an

electrode InLab413 with compensation of temperature. Process parameters for kinetic experiments are as follows: the effect of pH 3–5 (C_0 150 mg/L, C_S 1.0 g/L, T 25 °C), of temperature 25–60 °C (C_0 150 mg/L, C_S 1.0 g/L, pH 5), of C_S 0.5–1.5 g/L (T 25 °C, pH 5, C_0 150 mg/L), and of C_0 50–400 mg/L (T 25 °C, pH 5, C_S 1.0 g/L).

The equilibrium experiments were carried out at the best process conditions (25 °C, pH 5, C_S 1.0 g/L) determined in kinetic experiments. The concentrations of chromium(III) ions in the solutions ranged from 10 to 400 mg/L. The contact time was 4 h (determined from the kinetic experiments). Biosorption capacity (q) was evaluated as the difference between the initial (C_0) and concentration of metal ions in the solution at time t (C_k) divided per the concentration of the biomass in the solution (C_S).

Analytical Methods

Samples of sorbent suspension were taken to determine residual concentration of chromium (III) ions in the solution. Before analysis, samples were immediately filtered through no. 2 paper filter. The concentration of metal ions in the solution was determined directly spectrophotometrically by complexation with thylenediaminetetraacetic acid by Varian Cary 50 Conc. Instrument (Victoria, Australia) [32]. The concentrations used in the preparation of the calibration curve were also analyzed by inductively coupled plasma-optical emission spectrometer (Varian VISTA-MPX ICP-OES, Victoria, Australia) in the Chemical Laboratory of Multielemental Analysis at Wrocław University of Technology accredited by International Laboratory Accreditation Cooperation Mutual Recognition Arrangement and Polish Centre for Accreditation.

Approximately 0.5 g of the natural biomass was digested with 5 mL of concentrated 69% nitric acid (Supra pure grade from Merck, Darmstadt, Germany) in a microwave oven (type Milestone MLS-1200 MEGA, Bergamo, Italy). The solution after mineralization was diluted to 50 mL. The concentrations of metal ions in the samples were determined by ICP-OES method. The samples were analyzed in three repeats (the relative standard deviation of the measurement did not exceed 5%). The presented data are the arithmetic average from three measurements. Uncertainty of measurements was reported. For the preparation of standard solutions (1.0, 10, 50, 100 mg/L), the multielemental standard (100 mg/L Astasol®, Prague, Czech Republic) was used [33].

Results and Discussion

Kinetic Studies

Kinetics of biosorption of metal ions by algae is generally described by pseudo-second-order model (Eq. 1) [6, 23, 24, 31, 34–36].

$$\frac{\mathrm{d}q}{\mathrm{d}t} = k_{2,\mathrm{ad}} \times \left(q_{\mathrm{eq}} - q_t\right)^2 \tag{1}$$

where q_{eq} and q_t are the amounts of adsorbed metal ions on the biosorbent at equilibrium and at time t, respectively (milligrams per gram) and $k_{2,ad}$ is the rate constant of second-order biosorption (grams per milligram minute). Pseudo-second-order rate model is based on the assumption that chemical sorption involves valency forces through sharing or exchange of electrons between the sorbent and sorbate and this is the rate-limiting step [31]. In the conducted experiments, the biomass was employed as a free cell suspension in a

well-agitated batch system. According to Aksu [34], all the cell wall binding sites were made readily available for metal uptake, so in this case, effect of external film diffusion on biosorption rate could be assumed not significant and ignored. For the boundary conditions $t=0$ to t and $q=0$ to q_t, the integrated and linear form of Eq. 1 is expressed as (Eq. 2):

$$\frac{t}{q_t} = \frac{1}{k_{2,\mathrm{ad}} \cdot q_{\mathrm{eq}}^2} + \frac{t}{q_{\mathrm{eq}}} \tag{2}$$

If the pseudo-second-order kinetics is applicable, the plot of t/q_t against t of Eq. 2 should give a linear relationship, from which q_{eq} and $k_{2,ad}$ can be determined from the slope and intercept of the plot. The parameters of pseudo-second-order model are presented in Table 1. The experimental data showed good compliance with the proposed pseudo-second-order model (Fig. 1). The regression coefficients for the linear plots were higher than 0.99.

The kinetic experiments of the biosorption process were conducted to determine the contact time required to reach the sorption equilibrium and to assess the impact of process parameters: pH—Fig. 2, temperature—Fig. 3, C_S—Fig. 4, C_0—Fig. 5—on q_{eq} and $k_{2,ad}$. Biosorption of chromium(III) ions by *E. prolifera* was a relatively quick process with the equilibrium reached after 2 h. The quick kinetics has significant practical importance when considering that on industrial scale [34]. The process would be carried out in continuous mode with short biomass residence time, and thus, the dimensions of the biosorber can be small.

Comparing the biosorption properties of marine *E. prolifera* with freshwater *P. varia* Wille [36], it can be concluded that freshwater macroalga was characterized by higher values of q_{eq} than marine alga in the same experimental conditions. The value of q_{eq} at

Table 1 The comparison of parameters of pseudo-second-order model at different pH (C_0 150 mg/L, T 25 °C, C_S 1.0 g/L), temperatures (C_0 150 mg/L, pH 5, C_S 1.0 g/L), C_S (C_0 150 mg/L, pH 5, T 25 °C), and C_0 (T 25 °C, pH 5, C_S 1.0 g/L).

Parameter	q_{eq} (mg/g)	$k_{2,ad}$ (g /mg·min)	R^2
pH			
3	35.0	0.0986	0.999
4	43.9	0.00516	0.995
5	71.9	0.00265	0.998
Temperature (°C)			
25	71.9	0.00265	0.998
40	84.0	0.00354	0.999
50	74.6	0.00372	0.999
60	78.7	0.101	0.999
C_S (g/L)			
0.5	107.5	0.00131	0.997
1.0	71.9	0.00265	0.998
1.5	60.2	0.00444	0.999
C_0 (mg/L)			
50	41.2	0.00945	0.999
150	71.9	0.00265	0.998
300	93.5	0.00134	0.995
400	100.0	0.00109	0.991

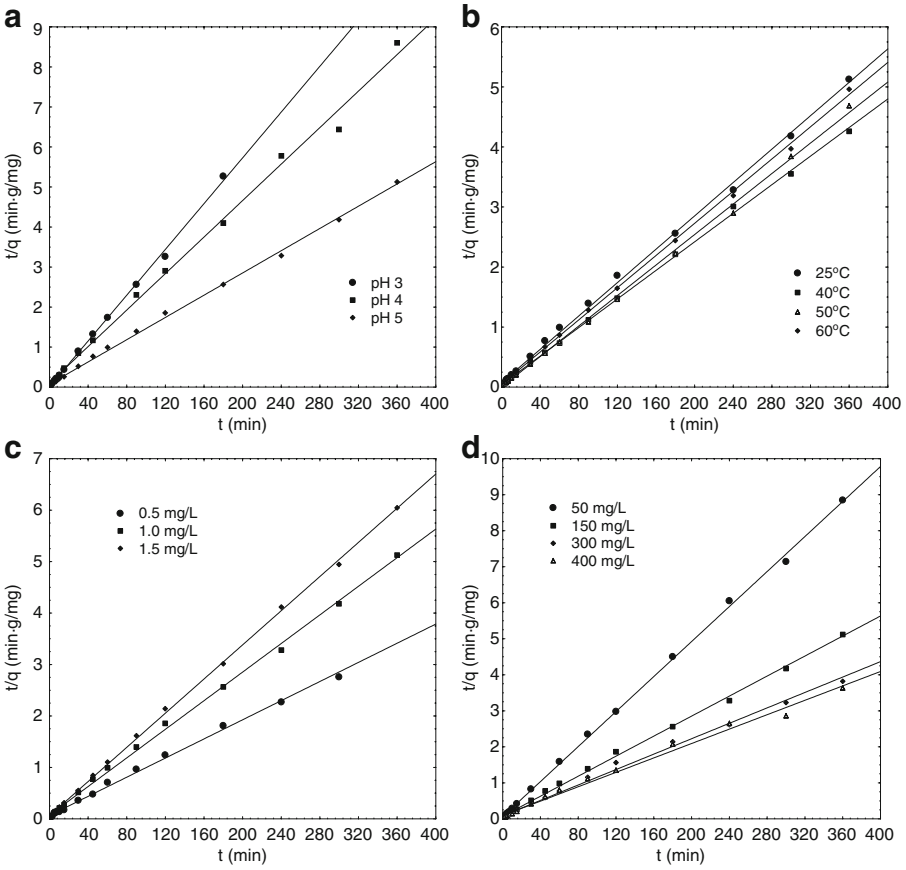

Fig. 1 The pseudo-second-order kinetic model plots at different **a** pH, **b** temperature, **c** C_S, and **d** C_0

different pH was averagely 0.699±0.102 smaller for *E. prolifera* than for *P. varia* Wille, at different temperatures 0.911±0.021, at different C_S 0.695±0.115, and at different C_0 0.907± 0.032. The same tendency was observed for both macroalgae when comparing the value of $k_{2,ad}$ in the same experimental conditions.

Effect of pH

The uptake of chromium(III) ions by *E. prolifera* is a function of solution pH, which influences both cell surface metal binding sites and metal chemistry in aqueous solutions [34]. The effect of initial pH on the equilibrium uptake of chromium(III) ions by the macroalga was analyzed in pH range from 3 to 5 (Table 1). In this range, chromium(III) ions occur as $CrOH^{2+}$, as a result of hydrolysis, according to the generalized expression: $M^{3+}(aq.) + nH_2O = M(OH)^{3-n} + nH^+ \left(\text{where } M^{3+} = Cr^{3+}\right)$. When the initial pH of the solution was adjusted to a value higher than pH 5.5, chromium(III) ions precipitated as $Cr(OH)_3$, because of higher concentration of OH^- ions in the biosorption system [37]. For this reason, the experiments were not conducted above pH 5. As presented in Fig. 2, the biosorption capacity of alga toward chromium(III) ions increased with pH. The highest value of q_{eq} was obtained at pH 5 (71.9 mg/g) and the smallest at pH 3 (35.0 mg/g). At low

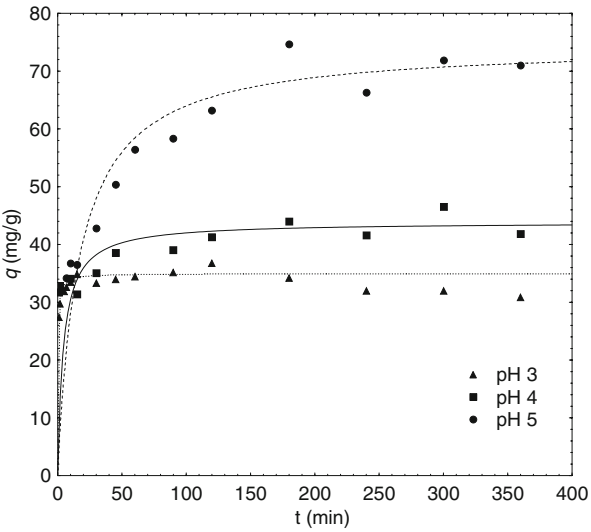

Fig. 2 The effect of pH on biosorption kinetics (C_0 150 mg/L, 25 °C, C_S 1.0 g/L)

pH, there was a high concentration of protons in the solution, which competed with metal ions in forming a bond with the active sites (the functional groups) on the surface of the algae. As the pH increased, more ligands (carboxyl, phosphate, hydroxyl, and amine groups) were exposed and carried negative charges with subsequent attraction of metallic cations and biosorption onto the cell surface [38]. The effect of pH on second-order adsorption rate constant was inverse to the effect on q_{eq}—the higher pH, the smaller $k_{2,ad}$. Similar tendency was observed also for freshwater macroalga *P. varia* Wille [36]. In the literature, the effect of pH on $k_{2,ad}$ is rarely studied. Most papers concern the investigation of the influence of C_S, C_0, and temperature on biosorption kinetics. Some authors report that with the increase of pH, biosorption rate constant increases (i.e., during biosorption of Cu(II) by powdered waste sludge [39]). In the case of biosorption of Cr(III) ions by *Enteromorpha* sp., $k_{2,ad}$ decreased with the increase of pH. This could be explained by the fact that chromium(III) cations in aqueous solution form various complexes. In the range of pH 1–5, chromium cations are present as Cr^{3+} and $CrOH^{2+}$. The first form dominates at pH 3.0. At pH 3.55, the concentrations of Cr^{3+} and $CrOH^{2+}$ in the aqueous phase are identical. At higher pH, $CrOH^{2+}$ is the dominant species (at pH 4.0 ~ 74%) [35]. Tobin et al. indicated that actual radii of complexes with charge <3 are significantly larger than that for a simple chromium(III) ion [40]. Therefore, as the consequence of the size and geometry of complexes of chromium cations, the biosorption rate constant decreased with increase of pH.

Effect of Temperature

To investigate the effect of temperature, the biosorption of chromium(III) ions onto dried biomass of *E. prolifera* was studied at different temperatures—25 °C, 40 °C, 50 °C, and 60 °C (Table 1). The uptake of chromium(III) ions was affected by the temperature, but this effect was not strong—Fig. 3 (from 71.9 mg/g at 25 °C to 84.0 mg/g at 40 °C). This shows that physical adsorption is not rather the dominating mechanism of chromium(III) ions biosorption by *E. prolifera*. With the increase of temperature, the value of $k_{2,ad}$ also increased. This phenomenon could be due to higher frequency of interactions (i.e., high

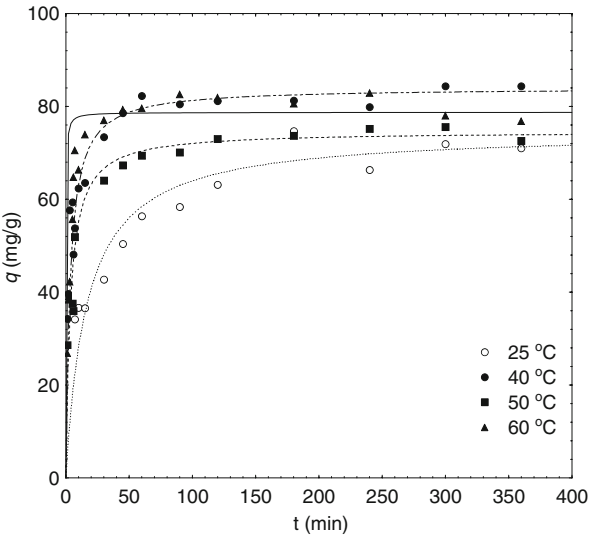

Fig. 3 The effect of temperature on biosorption kinetics (C_0 150 mg/L, pH 5, C_S 1.0 g/L)

energy levels) among the chromium(III) ions and the adsorbent particles at high temperatures [39].

Effect of Biomass Concentration

The influence of biosorbent concentration on the biosorption of chromium(III) ions by *E. prolifera* was investigated at different biomass concentrations—0.5, 1.0, and 1.5 g/L. In the Fig. 4, typical biosorption kinetic curves showed the effect of biomass concentration. With increase of C_S, q_{eq} decreased. As presented in Table 1, the values of $k_{2,ad}$ increased with increase of the biomass concentration. This could be since the increasing biomass concentration in the solution increases the surface area for sorption and hence the rate of

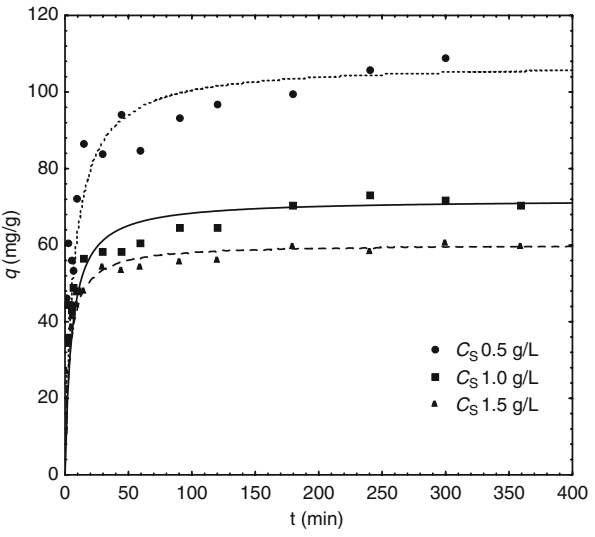

Fig. 4 The effect of C_S on biosorption kinetics (C_0 150 mg/L, 25 °C, pH 5)

chromium(III) sorption increases when the initial metal ion concentration remains constant. Considering economical aspects, it is beneficial to conduct biosorption process at higher biomass concentrations, because with increase of C_S, the amount of bound ions from the solution also increased—for C_S 0.5 g/L–53.7 mg of chromium(III) ions were bound from 1 L of the solution, for C_S 1.0 g/L–71.9 mg/L, and for C_S 1.5 g/L–90.3 mg/L.

Effect of Initial Concentration of Chromium(III) Ions

The effect of initial chromium(III) ions concentration on the biosorption capacity of *E. prolifera* was studied under conditions (pH 5, 25 °C, C_S 1.0 g/L), which seemed to be the most appropriate taking into account the value of q_{eq} and economical aspects. Figure 5 shows the influence of C_0 on kinetics of the process. It was found that with the increase of C_0, q_{eq} increased and $k_{2,ad}$ decreased (Table 1). The same tendency was obtained for the biosorption of Ni(II) ions by *E. prolifera* at identical experimental conditions (pH 5, 25 °C, C_S 1.0 g/L) [6] and for the adsorption of dye—Acid Red 274 (AR 274)—on *E. prolifera* [24]. Increasing concentration of the solute in the solution could reduce the diffusion of solute in the boundary layer and to enhance the diffusion in the solid [41]. At higher concentrations (C_0>300 mg/L), the available sites for biosorption became fewer and the saturation of the sorption sites was observed.

Equilibrium biosorption capacity of *E. prolifera* toward chromium(III) ions determined from pseudo-second-order model under examined experimental conditions increased in the following order: pH 3<4<5, temperature 25<50<60<40 °C, C_S 1.5<1.0<0.5 g/L, and C_0 50<150<300<400 mg/L. Since the future aim of our work is to bring the biosorption process from the laboratory to industrial scale, it was necessary to choose the best process conditions for the manufacture of mineral feed additives with microelements. The best pH was 5, temperature 40 °C, at which q_{eq} was 17% higher than q_{eq} at 25 °C, and C_S 0.5 g/L; however, the biomass bound only 53.7 mg of chromium(III) ions from 1 L (for C_S 1.0 g/L biomass bound 34% more ions) and C_0-q_{eq} for 400 mg/L was only 7% higher than for 300 mg/L. Taking into account the obtained results, for further experiments, the following conditions were chosen: pH 5, 25 °C, C_S 1.0 g/L, and C_0 300 mg/L.

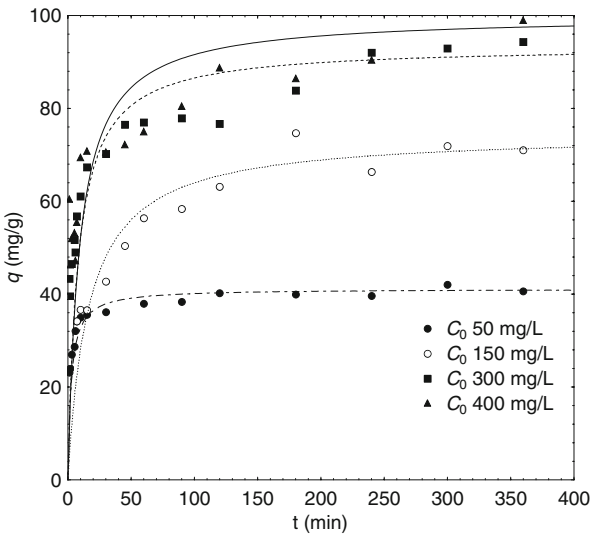

Fig. 5 The effect of C_0 on biosorption kinetics (25 °C, pH 5, C_S 1.0 g/L)

Equilibrium Modeling

The sorption isotherm represents the relationship between the amount adsorbed by a unit mass of solid sorbent and the amount of solute remaining in the solution at equilibrium. The results of biosorption experiment of chromium(III) ions at different initial concentrations of metal ions (ranging from 10 to 300 mg/L) were described by three isotherms: Langmuir and Freundlich (the most commonly used) and additionally by Dubinin–Radushkevich (D–R). The aim was not only to describe the experimental data but also to determine parameters of these models, which will be helpful in the characteristics and identification of the mechanism of biosorption process. The mean free energy determined from D–R will allow additionally distinguishing between the physical and chemical biosorption. The equilibrium experiment was conducted at the best process conditions determined from kinetic experiments (25 °C, pH 5, C_S 1.0 g/L). The isotherm appeared to follow the Langmuir model more closely (R^2 0.983) than the Dubinin–Radushkevich (R^2 0.964) and Freundlich model (R^2 0.958).

The model parameters of Langmuir equation [42] were determined by nonlinear regression (Mathematica v. 3.0.; Eq. 3).

$$q_{eq} = \frac{q_{max} b C_{eq}}{1 + b C_{eq}} \tag{3}$$

The maximum chromium(III) ions biosorption capacity (q_{max}) was found to be 85.8 mg chromium(III) ions per gram of dry mass of biomass (or 4.95 meq/g—milliequivalent is the molar unit, which considers the valency of an ion). High b value, which is the Langmuir adsorption constant (b 0.0344 L/mg), indicated high affinity of biomass for binding of chromium(III) ions. Similar results were obtained for biosorption of Ni(II) by *E. prolifera*, where q_{max} was equal 58.8 mg/g (2.00 meq/g) and b was 0.0754 L/mg (25 °C, pH 5, C_S 1.0 g/L) [6]. The maximum biosorption capacity for chromium(III) ions was 2.5 times higher than for Ni(II) ions, which could result from the valency of cations. The essential characteristics of the Langmuir equation can be also expressed in terms of a dimensionless separation factor, R_L, defied as (Eq. 4):

$$R_L = 1/(1 + bC_0) \tag{4}$$

where C_0 is the highest initial solute concentration and b the Langmuir adsorption constant. The R_L value implies the adsorption to be unfavorable (R_L>1), linear (R_L=1), favorable (0< R_L<1), or irreversible (R_L=0) [43]. In the case of biosorption of chromium(III) ions by *E. prolifera*, R_L was 0.0883, which indicated favorable adsorption.

The Freundlich isotherm model [44] is another commonly used equation to describe the sorption of metal ions on solid sorbents (Eq. 5).

$$q_{eq} = kC_{eq}^{1/n} \tag{5}$$

The values of Freundlich parameters (k—adsorption capacity and n—adsorption intensity) were obtained from the linear correlation (Eq. 6) between the values of ln q_{eq} and ln C_{eq}.

$$\ln\ q_{eq} = \ln\ k + 1/n\ \ln\ C_{eq} \tag{6}$$

These parameters can be used to predict the affinity between the sorbate and sorbent. High values of n (2.86) and k (12.7) indicated high adsorptive efficiency and suggested favorable uptake of chromium(III) ions by algal biomass at pH 5. The value of n, which is greater

than unity, indicated also favorable adsorption of chromium(III) ions by *E. prolifera*, what is in accordance with obtained R_L value, implying also favorable adsorption.

Another less commonly used model is the Dubinin–Radushkevich isotherm, which is based on Polany's adsorption potential theory and Dubinin's minipore filling theory [45]. This model was applied to distinguish between the physical and chemical biosorption of chromium(III) ions by *E. prolifera*. The linear form of the D–R model is given by Eq. 7:

$$\ln\ q_{eq} = \ln q_{max} - \beta\varepsilon^2 \tag{7}$$

where q_{eq} is adsorbed metal ion quantity per gram of biosorbent at equilibrium (mole per gram), q_{max} is the sorption capacity of biosorbent per unit weight (mole per gram), β is a constant related to the mean free energy of biosorption per mole of the biosorbate (square mole per square kilojoule) and ε is Polanyi potential, which is equal to $RT\ \ln(1+1/C_{eq})$, where R (kilojoule per mole kelvin) is the gas constant and T (kelvin) is the absolute temperature. Hence, by plotting $\ln\ q_{eq}$ versus ε^2, it is possible to generate the value of q_{max} from the intercept and the value of β from the slope. The value of q_{max} evaluated from D–R model was 162 mg/g and was almost two times higher than the value evaluated from Langmuir model. Figure 6 shows the D–R isotherm for chromium(III) ions biosorption by *E. prolifera*.

The constant β gives information about the mean free energy E (kilojoule per mole) of biosorption per mole of the biosorbate when it is transferred to the surface of the solid from infinity in the solution and can be calculated using the relationship (Eq. 8) [46]:

$$E = 1/(2\beta)^{0.5} \tag{8}$$

This parameter gives information about the type of biosorption mechanism as chemical ion-exchange or physical biosorption. The magnitude of E between 8 and 16 kJ/mol corresponds to a chemical ion-exchange process [47] while the value of $E<8$ kJ/mol represents a physical nature of the process [48]. In the case of chromium(III) ions biosorption by *E. prolifera*, the value of the mean free energy was found to be higher than 8 kJ/mol (11.3 kJ/mol) for 25 °C and this indicated that the biosorption mechanism may be

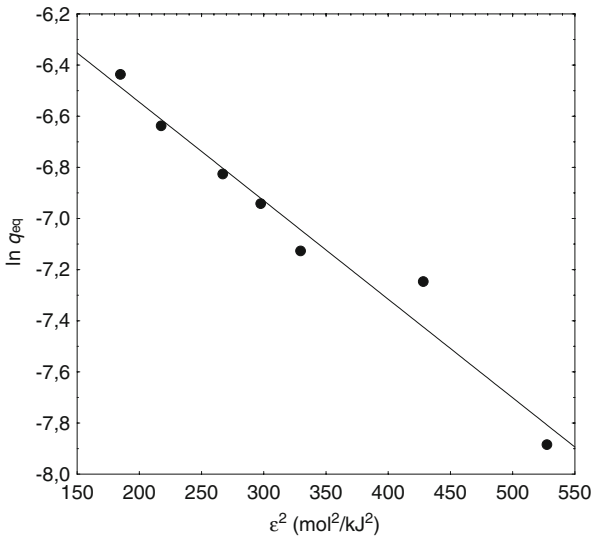

Fig. 6 Dubinin–Radushkevich plot for the biosorption of chromium(III) ions onto *E. prolifera* at 25 °C

a chemical ion-exchange. Langmuir, Freundlich, and Dubinin–Radushkevich isotherms for the biosorption of chromium(III) ions by *E. prolifera* are presented in Fig. 7.

In order to identify the mechanism of the biosorption process, the multielemental analysis of the natural and enriched with chromium(III) ions biomass (Fig. 8a) and the solution (Fig. 8b) before and after biosorption process (25 °C, pH 5, C_S 1.0 g/L, C_0 300 mg/L) was performed. On the basis of the solution composition, it was calculated that *E. prolifera* bound 84 mg/g of chromium(III) ions, which confirmed the value obtained from Langmuir equation (q_{max} 86 mg/g) in another experiment.

During biosorption of chromium(III) ions, *E. prolifera* released from the biomass not only microelement ions (e.g., B(III) ions—50%, Zn(II) ions—56%, and Mn(II) ions—74%) but also mainly alkali (Na(I), K(I)) and alkaline earth metal (Mg(II), Ca(II)) ions. The content of K(I) ions in the biomass decreased after biosorption process 30 times, Mg(II) ions 26 times, Ca(II) ions six times, and Na(I) ions four times. This indicated that chromium(III) ions were exchanged with light metal ions, which are naturally bound with the functional groups on the surface of algal cell wall and were detected by multielemental analysis of the biomass (Fig. 8a). In the solution after biosorption process, significantly higher concentrations of K(I), Mg(II), and Ca(II) ions were observed (Na(I) ions were not taken into consideration, because their high concentration in the solution before (132 mg/L) and after biosorption process (139 mg/L) was attributed to 0.1 M NaOH, which was used to adjust initial pH). The comparison of the composition of biomass before and after biosorption was in conformity with the comparison of the solution composition before and after process. This confirmed that cations were eluted from the biomass to the solution.

On the basis of the conducted experiments, it could be concluded that macroalga *E. prolifera* showed very good biosorption properties. In the biosorption process of chromium (III) ions under the best experimental conditions, the examined alga was able to bind 85.8 mg/g, which means that the concentration of chromium(III) ions in the biomass after the process increased 45,159 times, when compared with the concentration in the natural biomass (0.0019 mg/g). This implies that *E. prolifera* has the potential to be applied in fodder industry as a biological carrier of microelement cations, which are essential to animals. Moreover, the composition of macroalga meets the requirements of Polish and

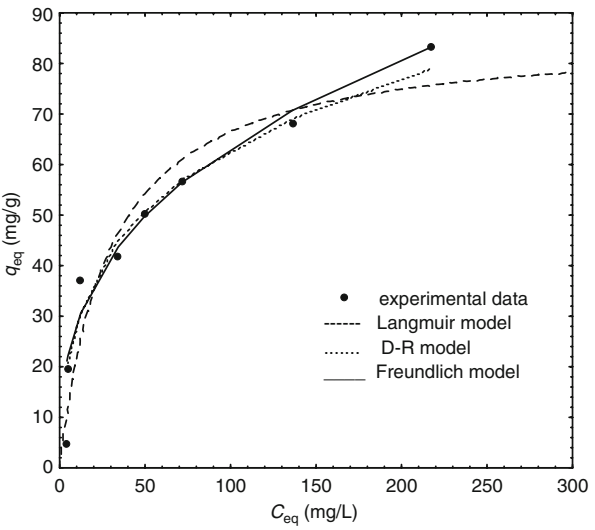

Fig. 7 Langmuir, D–R, and Freundlich isotherms for the biosorption of chromium(III) ions (25 °C, pH 5, C_S 1.0 g/L)

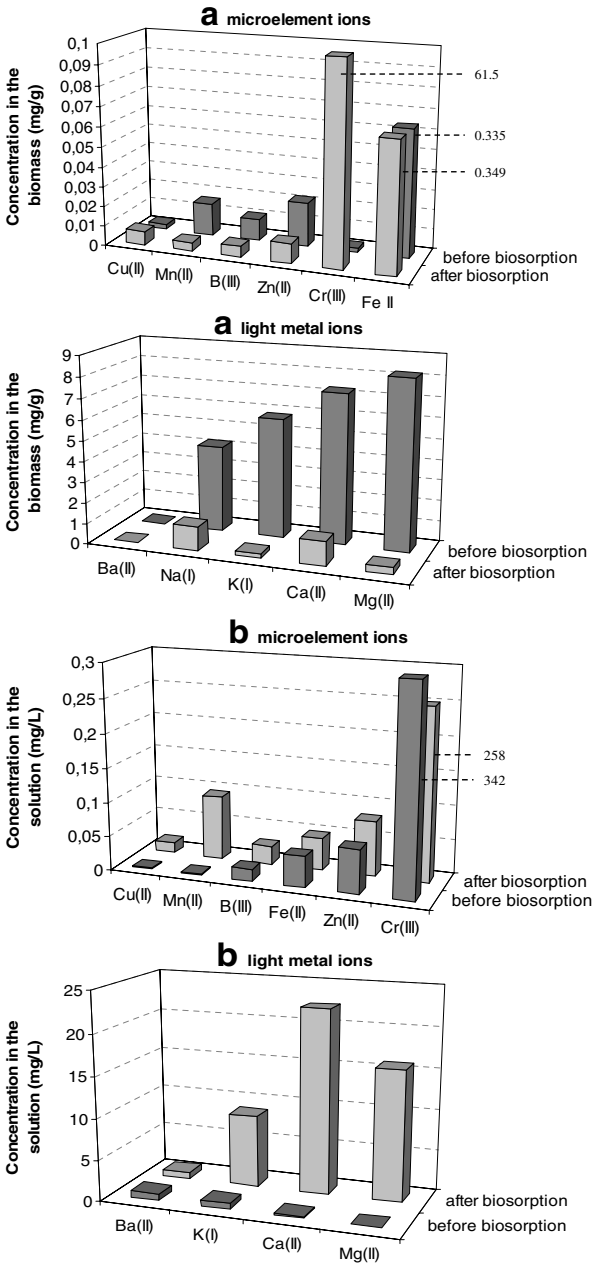

Fig. 8 The mineral composition of the natural and enriched with chromium(III) ions biomass (**a**) and the solution (**b**) before and after biosorption process

European Directive concerning the level of toxic metals in fodder materials [49, 50]. The multielemental analysis of *E. prolifera* revealed the presence of toxic elements (e.g., Pb, Cd, Ni) in the biomass, but their content was below the toxic limits (As 2–40 mg/kg, Pb 5–40 mg/kg, and Cd 0.5–10 mg/kg). In Table 2, the composition of obtained chromium(III) feed additive is presented. Literature reports that chromium(III) should be supplemented in the livestock feed. However, these findings are new and have not been considered in

Table 2 The composition of obtained chromium(III) feed additive.

Element		Concentration (mg/g)
Microelements	Cr(III)	61.5±9.2
	Fe(II)	0.349±0.052
	Zn(II)	0.00991±0.00248
	Cu(II)	0.00687±0.00172
	B(III)	0.00540±0.00140
	Mn(II)	0.00421±0.00105
Light metals	Ca(II)	1.15±0.17
	Na(I)	1.15±0.17
	Mg(II)	0.320±0.048
	K(I)	0.193±0.029
	Ba(II)	0.0268±0.0067
Toxic metals	As(III)	LLD<0.0915
	Pb(II)	0.0002±0.00004
	Cd(II)	0.0002±0.00004
	Ni(II)	0.0009±0.0002

LLD below detection limit (mg/L)

official feeding standards yet. It is suggested that quantity of chromium(III) in the feed should be 10 mg/kg [51]. Assuming that *E. prolifera* would be a feed additive that covers 100% of the total amount of chromium(III) in feed for livestock, 1 kg of the feed should be supplemented with 0.163 g of the biomass enriched with chromium(III). Concluding, macroalgae enriched with microelements during biosorption process would carry a biologically bound concentrated form of microelements to the livestock diet.

Conclusion

Information on the kinetics of metal ions uptake is required for the selection of the best operational conditions for full-scale and continuous batch metal binding processes. Biosorption of chromium(III) ions by *E. prolifera* was influenced by the experimental parameters such as pH, temperature, initial metal ion, and biomass concentrations. The kinetics of chromium(III) ions biosorption by the algal biomass was described by pseudo-second-order model and the biosorption equilibrium by the Langmuir, Dubinin–Radushkevich, and Freundlich adsorption models. The highest regression coefficient was obtained in the case of using Langmuir adsorption model.

E. prolifera demonstrated good biosorption properties. Macroalga appeared to be effective in binding of chromium(III) ions from aqueous solutions. The best biosorption conditions were determined as pH 5, 25 °C, C_0 400 mg/L, C_S1.0 g/L, and for these conditions, q_{eq} was 100 mg/g. These data were necessary to conduct enrichment of macroalgal biomass with microelements essential for animals (Cu(II), Zn(II), Mn(II), Co (II)) in single-metal system, which is planned in the future.

On the basis of the equilibrium data, it could be concluded that metal ions exchange mechanism of biosorption is predominant. Mean free energy (11.3 kJ/mol), determined on the basis of D–R isotherm, indicated chemical ion exchange. Also, slight influence of

temperature on the biosorption kinetics excluded physical adsorption. Moreover, biosorption of chromium(III) ions by *E. prolifera* was a favorable process ($R_L<1$ and $n>1$) and Langmuir parameter b and Freundlich n and k indicated high affinity of biomass for binding of chromium(III) ions.

The main objective of this paper was to draw attention to the enormous and unused potential of macroalgae. Good biosorption properties of *E. prolifera*, confirmed in this paper, should find an application in industrial processes (e.g., in wastewater treatment or in animal feeding). The examined macroalga having relatively high content of microelements in the natural biomass can be potentially used as mineral feed supplement for livestock. Moreover, in this paper, it was shown that there is a possibility to greatly increase the concentration of desired microelements in the biomass by using biosorption process.

Nomenclature

C_{eq}	Residual metal ion concentration at equilibrium (mg/L)
C_0	Initial metal ion concentration (mg/L)
C_k	Final metal ion concentration (mg/L)
C_S	Alga concentration (g/L)
q	Adsorbed metal ion quantity per gram of alga at any time (mg/g)
q_{eq}	Adsorbed metal ion quantity per gram of alga at equilibrium (mg/g)
q_{max}	Maximum biosorption capacity (mg/g)
$k_{2,ad}$	Second-order adsorption rate constant (g/mg min)
b	Langmuir adsorption constant (L/mg)
n	Freundlich adsorption constant—adsorption intensity
k	Freundlich adsorption constant—adsorption capacity
R	The gas constant (kJ/mol K)
T	The absolute temperature (K)
β	Constant related to the mean free energy of biosorption per mole of the biosorbate (mol^2/kJ^2)
ε	The Polanyi potential
E	The mean free energy (kJ/mol)
R^2	Coefficient of determination

Acknowledgments This research was financially supported by Ministry of Science and Higher Education (No. R05 014 01). We would also like to thank Professor Jan Matuła, Dr. Dorota Richter, and Dr. Mirosława Pietryka from the Department of Botany and Plant Ecology of the Wrocław University of Environmental and Life Sciences for identification of the alga.

References

1. Jensen, A. (1993). *Hydrobiologia, 260/261*, 15–23. doi:10.1007/BF00048998.
2. Pliński, M., Florczyk, I., & Galińska, M. (1988). Kieler Meeresforschungen. *Sonderheft, 6*, 265–271.
3. Nisizawa, K., Noda, H., Kikuchi, R., & Watanabe, T. (1987). *Hydrobiologia, 151/152*, 5–29. doi:10.1007/BF00046102.
4. Hou, X., & Yan, X. (1998). *The Science of the Total Environment, 222*, 141–156. doi:10.1016/S0048-9697(98)00299-X.
5. Aguilera-Morales, M., Casas-Valdez, M., Carrillo-Domínguez, S., González-Acosta, B., & Pérez-Gil, F. (2005). *Journal of Food Composition and Analysis, 18*, 79–88. doi:10.1016/j.jfca.2003.12.012.

6. Özer, A., Gürbüz, G., Çalimli, A., & Körbahti, B. K. (2008). *Journal of Hazardous Materials, 152*, 778–788. doi:10.1016/j.jhazmat.2007.07.088.
7. McDermid, K. J., & Stuercke, B. (2003). *Journal of Applied Phycology, 15*, 513–524. doi:10.1023/B:JAPH.0000004345.31686.7f.
8. Poole, L. J., & Raven, J. A. (1997). *Progress in Phycological Research, 17*, 1–148.
9. Mamatha, B. S., Namitha, K. K., Senthil, A., Smitha, J., & Ravishankar, G. A. (2007). *Food Chemistry, 101*, 1707–1713. doi:10.1016/j.foodchem.2006.04.032.
10. Bimalendu, R. (2006). *Carbohydrate Polymers, 66*, 408–416. doi:10.1016/j.carbpol.2006.03.027.
11. Lahaye, M., Ray, B., Baumberger, S., Quemener, B., & Axelos, M. A. V. (1996). *Hydrobiologia, 326/327*, 473–480. doi:10.1007/BF00047848.
12. Venkata Raman, B., Rao, D. N., & Radhakrishnan, T. M. (2004). *Indian Journal of Clinical Biochemistry, 19*, 105–109. doi:10.1007/BF02872402.
13. Podbielkowski, Z. (1978). *Glony.* Warsaw: Wydawnictwo Szkolne i Pedagogiczne.
14. Haroon, A. M., Szaniawska, A., Normant, M., & Janas, U. (2000). *Oceanologia, 42*, 19–28.
15. Szefer, P., & Skwarzec, B. (1988). *Oceanologia, 25*, 87–98.
16. Żbikowski, R., Szefer, P., & Latała, A. (2006). *Environmental Pollution, 143*, 435–448. doi:10.1016/j.envpol.2005.12.007.
17. Mabeau, S., & Fleurence, J. (1993). *Trends in Food Science & Technology, 4*, 103–107. doi:10.1016/0924-2244(93)90091-N.
18. Directive of Polish Minister of Agriculture and Rural Development. (2003). Act on resources of animal feeding. Journal of Laws, No. 29, Item 243.
19. McHugh, D. J. (2003). *FAO Fisheries Technical Paper, 441*, 1–105.
20. Oswald, W. J., & Gotaas, H. B. (1957). *Transactions of the American Society of Civil Engineers, 122*, 73–105.
21. Volesky, B. (1990). Biosorption and biosorbents. In B. Volesky (Ed.), *Biosorption of heavy metals*. Boca Raton: CRC.
22. Pessôa de França, F., Pinto Padilha, F., & da Costa, A. C. A. (2006). *Applied Biochemistry and Biotechnology, 128*, 23–32. doi:10.1385/ABAB:128:1:023.
23. Özer, A., Akkaya, G., & Turabik, M. (2005). *Chemical Engineering Journal, 112*, 181–190. doi:10.1016/j.cej.2005.07.007.
24. Özer, A., Akkaya, G., & Turabik, M. (2005). *Journal of Hazardous Materials B, 126*, 119–127. doi:10.1016/j.jhazmat.2005.06.018.
25. Hernández, I., Fernández-Engo, M. A., Pérez-Lloréns, J. L., & Vergara, J. J. (2005). *Journal of Applied Phycology, 17*, 557–567. doi:10.1007/s10811-005-9006-6.
26. Deviller, G., Aliaume, C., Nava, M. A. F., Casellas, C., & Blancheton, J. P. (2004). *Aquaculture (Amsterdam, Netherlands), 235*, 331–344. doi:10.1016/j.aquaculture.2004.01.023.
27. Vincent, J. B. (2001). *Polyhedron, 20*, 1–26. doi:10.1016/S0277-5387(00)00624-0.
28. N.R.C. (1997). *The role of chromium in animal nutrition*. Washington, DC: National Academy.
29. Chojnacka, K. (2007). *World Journal of Microbiology & Biotechnology, 23*, 1139–1147. doi:10.1007/s11274-006-9344-9.
30. Michalak, I., Zielińska, A., Chojnacka, K., & Matuła, J. (2007). *American Journal of Agricultural and Biological Science, 2*, 284–290.
31. Ho, Y. S., & McKay, G. (1999). *Process Biochemistry, 34*, 451–465. doi:10.1016/S0032-9592(98)00112-5.
32. Yongnian, N., Shouhui, C., & Serge, K. (2002). *Analytica Chimica Acta, 46*, 305–316.
33. Chojnacka, K. (2006). *Talanta, 70*, 966–972. doi:10.1016/j.talanta.2006.05.063.
34. Aksu, Z. (2001). *Separation and Purification Technology, 21*, 285–294. doi:10.1016/S1383-5866(00)00212-4.
35. Kumar, K. V., Sivanesan, S., & Ramamurthi, V. (2005). *Process Biochemistry, 40*, 2865–2872. doi:10.1016/j.procbio.2005.01.007.
36. Michalak, I., Chojnacka, K., Richter, D., Pietryka, M., & Matuła, J. (2006). Na pograniczu chemii i biologii. *WN UAM in Poznan, 16*, 107–116. in Polish.
37. Yun, Y. S., Park, D., Park, J. M., & Volesky, B. (2001). *Environmental Science & Technology, 35*, 4353–4358. doi:10.1021/es010866k.
38. Dönmez, G. Ç., Aksu, Z., Öztürk, A., & Kutsal, T. (1999). *Process Biochemistry, 34*, 885–892. doi:10.1016/S0032-9592(99)00005-9.
39. Yunus Pamukoglu, M., & Kargi, F. (2007). *Enzyme and Microbial Technology, 42*, 76–82. doi:10.1016/j.enzmictec.2007.08.004.
40. Tobin, J. M., Cooper, D. G., & Neufeld, R. J. (1984). *Applied and Environmental Microbiology, 47*, 821–824.
41. Kannan, N., & Sundaram, M. M. (2001). *Dyes Pigm, 51*, 25–40. doi:10.1016/S0143-7208(01)00056-0.
42. Langmuir, I. (1918). *Journal of the American Chemical Society, 40*, 1361–1403. doi:10.1021/ja02242a004.

43. McKay, G., Blair, H. S., & Gardner, J. R. (1984). *Journal of Applied Polymer Science, 29*, 1499–1514. doi:10.1002/app. 1984.070290504.
44. Freundlich, H. (1907). *Zeitschrift für Physikalische Chemie, 57*, 385–470.
45. Dubinin, M. M., & Raduskhevich, L. V. (1947). *Proceedings of the Academy of Sciences of the USSR. Physical Chemistry Section, 55*, 327–329.
46. Hasany, S. M., & Chaudhary, M. H. (1996). *Applied Radiation and Isotopes, 47*, 467–471. doi:10.1016/0969-8043(95)00310-X.
47. Helfferich, F. (1962). *Ion exchange*. New York: McGraw-Hill.
48. Onyango, M. S., Kojima, Y., Aoyi, O., Bernardo, E. C., & Matsuda, H. (2004). *Journal of Colloid and Interface Science, 279*, 341–350. doi:10.1016/j.jcis.2004.06.038.
49. Directive of Polish Minister of Agriculture and Rural Development. (2004). with further changes (2005). Acceptable amounts of undesirable substances in fodders. Journal of Laws, No. 162, Item 1704 and Journal of Laws, No. 151, Item 1267.
50. Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed, L 285/33.
51. Jamroz, D., Podkówka, W., & Chachułowa, J. (2004). *Żywienie zwierząt i paszoznawstwo*. Warsaw: PWN.