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Biosorption of Cr(III) by Microalgae and Macroalgae: Equilibrium of the Process

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Abstract: Equilibrium experiments of chromium(III) biosorption by microalga (*Spirulina* sp.) and macroalga (*Pithophora varia*) were investigated in this paper. Influence of the following process parameters was studied: pH, biomass concentration and temperature. The equilibrium of the process was described with Langmuir equation. The model parameters were determined at various process conditions. The best operation conditions for the process were selected: pH 5, T 20^{o}C, C_S 1g.l^{-1} for *Pithophora varia* and the same for *Spirulina* sp. Under these conditions, the maximum biosorption capacity 60.6 mg.g^{-1} and 34.6 mg.g^{-1}, and affinity constant 0.182 l.mg^{-1} and 0.0553 l.mg^{-1} were reached for *Pithophora varia* and *Spirulina* sp., respectively. It was found that *Pithophora varia* was more efficient in biosorption of Cr(III) ions than *Spirulina* sp. Biosorptive properties of microalgal and macroalgal biomass can be employed either in the production of biological feed supplements for livestock or in wastewater treatment processes.

Key words: biosorption, Cr(III) ions, macroalga *Pithophora varia*, microalga *Spirulina* sp., mineral feed supplements, wastewater treatment

INTRODUCTION

Chromium is considered as a toxic metal and on the other hand as microelement. Hexavalent chromium is known to be more toxic than trivalent chromium. Extensive use of chromium, e.g. in electroplating, tanning, and as biocide in cooling water of plants, resulted in discharges of chromium-containing effluents[1]. The concentration of chromium in effluents is regulated by environmental law. Chromium is also essential trace element for human and animals. Nowadays, efforts are made to develop high-value foods enriched with chromium(III) to prevent diseases, such as diabetes. Also, in the recent years, there is an increasing interest in the utilization of chromium(III) in animal feed. However, the dietary requirement of livestock for chromium has not been defined yet. It is suggested, that the quantity in feeds should be 10 mg.kg^{-1}[2]. It has been reported that chromium(III) supplementation to diets of animals has a positive effect on poultry production yield[3].

Literature reports, that chromium is supplemented to animal feeds mainly as mineral salts ($CrCl_3$)[4], chelates (Cr-picolinate)[3,5], or as chromium enriched brewery yeasts[4,6-9]. There is a considerable difference in the availability of microelements from various sources[10]. It is considered that organic form of trace minerals is more available and more similar to forms that occur in an organism[11]. The bioavailability of chromium(III) from organic forms is 10 times higher than from inorganic sources[9]. However, the main disadvantage is high price[10]. In the last years, a number of organic chromium preparations were made commercially available that claimed to protect animals from stress associated with environmental and management conditions of intense livestock farming. Since these organic chromium supplements are often highly concentrated, it is possible to accidentally overdose during feed preparation[4].

In the recent years, enriched yeasts have been proposed as a source of chromium. Their application is limited due to the possibility of causing fermentation processes in digestive tract and disease blastomycosis in the case of their excess in fodders[12]. Beside this, yeasts are characterized by low biosorption capacity. The maximum biosorption capacity for chromium(III) ions obtained from Langmuir equation was reported as 13.3 mg.g^{-1} [13].

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In the literature it is suggested, that biological materials, such as aquatic plants (*Lemna minor*)[14] or algae[15-17] can be used as mineral feed additives for animals. This kind of feed additives would be characterized with high bioavailability, low toxicity, low cost and high nutritional value[14]. Moreover, European and Polish feed law allows supplementation of algae to livestock diet. According to Directive of Polish Minister of Agriculture and Rural Development, algae can be used as a source of minerals and proteins [18].

Natural feeds of plant origin are known to be deficient in chromium. However, they possess a unique property of binding minerals from aqueous solutions in the biosorption process. This is a well known equilibrium separation process, which is mainly used in removal from wastewaters heavy metal ions. Equilibrium data, commonly known as adsorption isotherms, provide information on metal binding capacity of the adsorbent[19]. In the literature, there are some reports evaluating biosorption of chromium(III) and (VI) by marine macroalgae (*Ecklonia* sp.[20], *Sargassum*[21]), microalgae (*Spirulina* sp.[22], *Spirulina platensis*[23], *Dunaliella* sp.[24], *Chlorella miniata*[25]) and freshwater algae (*Spirogyra* sp.)[26].

In the present work, chromium(III) was bound by the biomass of a representative of microalgae (*Spirulina* sp.) and macroalgae (*Pithophora varia*). In the literature, there is significantly more information about biosorptive prosperities of *Spirulina* sp. than *Pithophora varia*. *Pithophora* sp. - a member of *Cladophorales* is a filamentous green alga that forms free-floating mats in many littoral algal communities. This macroalga is tolerant to high temperature, grows rapidly in the summer in shallow ponds and lakes, where temperatures regularly exceed 30 °C, and is able to use low light intensities[27-28]. In the literature, it is reported that *Pithophora* sp. could be applied as the biosorbent to remove malachite green, a cationic dye from wastewater[29] as well as in livestock feeding[30]. The second alga - *Spirulina* sp. is better known. *Spirulina* sp. (*Arthrospira*) is a cyanobacterium (blue-green alga) that has a long history of use as food and feed[31]. *Spirulina* sp. have been already proposed to be an alternative protein supplementing source as single cell protein (SCP)[32]. Nowadays, with good results is used as food supplement and food coloring agent[33,34].

Currently, there are many companies producing *Spirulina* sp. in different countries in the world to the amount of 3000 tons a year [35]. A lot of surveys were carried out considering immune actions, antioxidant antiviral, anticancer and cholesterol reduction effects of *Spirulina* sp.[35]. In the literature, there are information on biotransformations in which not efficient inorganic forms of microelements are transformed into organic forms by binding with proteins, lipids and polysaccharides and other cell components, which are more bioavailable[11]. One of many examples of modified organisms is *Spirulina* sp., which was transformed into Se-supplementable alga in biacumulation process[36,37]. Biomass of *Spirulina* sp. as a carrier of microelements was also used to obtain selenium- and iodine- containing pharmaceuticals [38]. High-value *Spirulina* enriched with chromium(III) was also obtained by other authors[22,23].

In this paper, utilization of microalga (*Spirulina* sp.) and macroalga (*Pithophora varia*) as chromium(III) feed additives was proposed. Mentioned algae could be used as a carrier of highly bioavailable chromium(III) in fodders. The experiments on biosorption of Cr(III) ions by a macroalga and microalga were carried out. Sorption of chromium(III) by algae was investigated as a function of temperature (20–60 °C), initial pH (3–5) and initial sorbent concentration (0.5-1.5 gl^{-1}). The aim of these experiments was to determine the best operation conditions. The equilibrium of the biosorption process was described with Langmuir model.

MATERIALS AND METHODS

Sorbent preparation: The alga *Pithophora varia* Wille was collected from the Botanical Garden in Wroclaw. It was identified in the Department of Botany and Plant Ecology of the Agricultural University of Wroclaw. The collected biomass of alga was washed with tap water several times to remove foreign matter and afterwards 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. The second alga - *Spirulina* sp. (lyophilized cells) is commercially available and was obtained from SIGMA (USA).

Batch sorption experiments: The experiments were performed in Erlenmeyer flasks containing 20 ml of chromium(III) solution in thermostated water bath shaker at 150 rpm. The solutions of chromium(III) ions were prepared in deionized water (by dissolving appropriate amounts of $Cr(NO_3)\cdot 9H_2O$ (from POCh S.A. Gliwice). pH of the solutions was adjusted with 0.1 $mol\,l^{-1}$ solution NaOH/HCl (from POCh S.A. Gliwice)). pH measurements were conducted with pH-meter Mettler-Toledo (Seven Multi) equipped with an electrode InLab413 with compensation of temperature.

In equilibrium experiments the contact time was 5 hours for *Pithophora varia* and 2 hours for *Spirulina* sp., as determined previously in kinetic experiments[39].

Samples of sorbent suspension were taken to determine residual concentration of chromium(III) ions in the solution. Before the analysis, samples were filtered through medium paper filter. The concentration of metal ions in the solution was determined directly spectrophotometrically by complexation with EDTA by Varian Cary 50 Conc. Instrument[40].

RESULTS AND DISCUSSION

Biosorptive capacity is influenced by many factors including: properties of metal ions (radius of ion, valence, etc.) in aqueous solution, biosorption conditions (such as pH, temperature, contact time, the presence of other ions in the solution, initial concentration of metal ions and the biomass)[40] and algal species. To evaluate the best conditions for biosorption process, it was necessary to conduct equilibrium experiments. Equilibrium data are helpful to design adsorption system, which will be used to enrich algae with essential microelements (e.g. chromium) in larger scale. Langmuir equation was used to model equilibrium between metal ions adsorbed to the biomass and metal ions in the solution at a given temperature. This model is simple and gives a good description of experimental behavior in a wide range of operating conditions[19]. The main advantage of this model is the possibility of evaluation of q_{max} - maximum possible quantity of metal ions adsorbed per gram of adsorbent and b - constant related with the affinity of binding sites for the metal ions. The model parameters of Langmuir equation for biosorption of chromium(III) by *Pithophora varia* and *Spirulina* sp. were determined by nonlinear regression (*Mathematica v. 3.0*) and are presented in Table 1.

The $q_{\max}$ obtained for *Pithophora varia* (evaluated from Langmuir equation) was higher than the value of $q_{\max}$ reported in the literature for other algae: e.g. $q_{\max}$ for *Chlorella miniata* was 41.12 mg.g^{-1} (pH 4.5, 5 gl^{-1})[25], for *Ecklonia* sp. was 34.1 mg.g^{-1} (pH 4, 25 °C, 5 g.l^{-1})[20], for *Chlorella vulgaris* was 30.2 mg.g^{-1} (pH 5, 25 °C, 1 g.l^{-1})[41] and *Sargassum* seaweed bound up to 40 mg.g^{-1} of chromium(III) (pH 4, 4 g.l^{-1})[21], but three times lower than $q_{\max}$ obtained for lyophilizate of *Spirulina* sp. – 185 mg.g^{-1} (pH 7, 35 °C, 1 g.l^{-1})[22].

Effect of concentration of algal biomass on biosorption process: The value of q_{max} obtained at 0.5 g.l^{-1} (69.3 mg.g^{-1}, maximum uptake, Table 1) for *Pithophora varia* appears to be higher in comparison with the uptake obtained at the same biomass concentration for *Spirulina* sp. (64.5 mg.g^{-1}). But in the range of biomass concentration 1-1.5 g.l^{-1} maximum metal uptake obtained for *Spirulina* sp. was two times lower. Such a strong effect of biomass concentration for *Pithophora varia* was not observed (Figure 1). Lower concentration of biomass improved the performance of biosorption. As seen from figure 2, the strong effect of biomass concentration was observed for *Spirulina* sp. particularly in range the 0.5-1.0 g.l^{-1}.

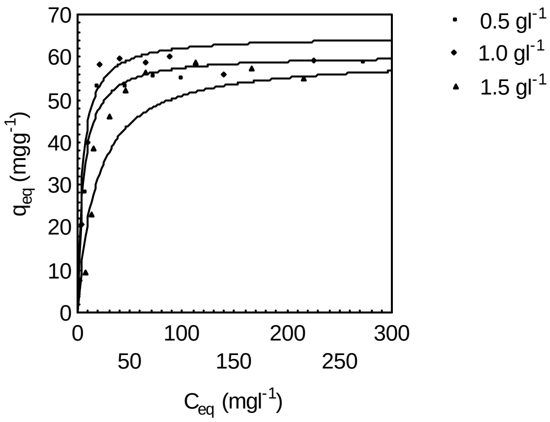

Fig. 1: Isotherms carried out at various sorbent concentrations at 25 °C, pH 5 (*Pithophora varia*)

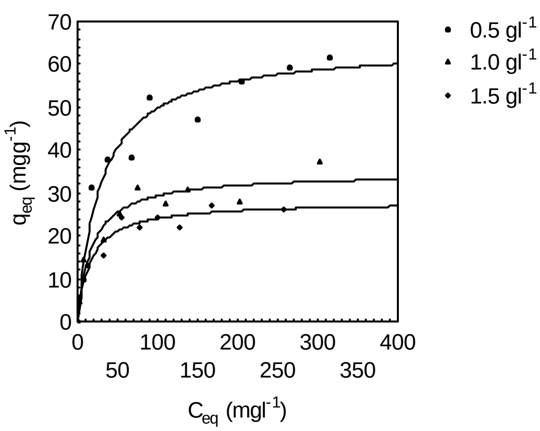

Fig. 2: Isotherms carried out at various pH at 25 °C, pH 5 (*Spirulina* sp.)

Effect of pH on biosorption process: The effect of pH on the biosorption capacity of chromium(III) of biomass of *Pithophora varia* is shown in figure 3, and for *Spirulina* sp. in figure 4. In both cases, it was shown that with increase of pH from 3 to 5, biosorption capacity increased. For *Pithophora varia*, the highest q_{max} was reached at pH 5 (60.6 mg.g^{-1}). At the same pH, the maximum metal uptake for *Spirulina* sp. was also the highest (34.6 mg.g^{-1}). Significantly lower uptakes of

Table 1: Model parameters for Langmuir isotherm for biosorption of Cr(III) by *Pithophora varia* and *Spirulina* sp, [1] pH 5, C_S=g.1^{-1}, [2] T 25 °C, C_S=g.1^{-1}, [3] pH 5, T 25 °C

Parameter	*Pithophora varia*			*Spirulina* sp.		
	q_{max} (mgg^{-1})	b (lmg^{-1})	R^2	q_{max} (mgg^{-1})	b (lmg^{-1})	R^2
[1]Temperature (°C)						
25	60.6	0.182	0.988	34.6	0.0553	0.987
40	85.9	0.0511	0.958	41.4	0.1216	0.984
50	88.6	0.0711	0.957	37.4	0.2123	0.994
60	79.9	0.0933	0.925	45.2	0.1058	0.996
[2]pH						
3	*	*	*	4.77	0.278	0.991
4	*	*	*	16.1	0.0395	0.992
5	60.6	0.182	0.988	34.6	0.0553	0.987
[3]C_S (gl^{-1})						
0.5	65.0	0.215	0.988	64.5	0.0338	0.990
1.0	60.6	0.182	0.988	34.6	0.0553	0.987
1.5	60.0	0.0564	0.984	28.0	0.0601	0.992

*equilibrium dependence did not follow pattern of Langmuir equation

chromium(III) were obtained at pH 3. At pH 3 for *Pithophora varia*, especially at higher concentration of chromium, these ions were rather released than bound by the biomass. It can be explained with the natural presence of chromium in the biomass (*Pithophora varia*) (13 mg.kg^{-1}) obtained from ICP-OES analysis. In the case of *Spirulina* sp., the natural concentration of chromium in the biomass was three times lower (4.6 mg.kg^{-1}). This could be the reason for the elution of Cr(III) ions only from the biomass of *P. varia* at pH 3. At pH 3 and 4 *Pithophora varia* did not follow Langmuir equation. It was impossible to determine Langmuir parameters because at lower pH less binding sites were available for metal ions what is the reason that *plateau* was not reached, because of equilibrium shift.

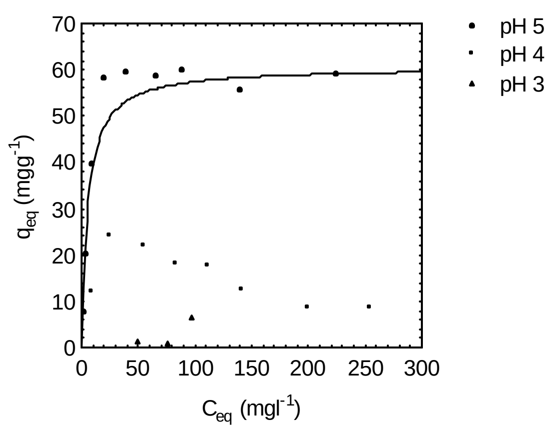

Fig. 3: Isotherms carried out at various pH at 25 °C, C_S 1g.l^{-1} (*Pithophora varia*)

The equilibrium binding capacity decreased with pH decrease. The effect of pH on chromium(III) uptake showed that the best results were obtained at pH 5. pH strongly influenced protonation of metal binding sites exposed by cell surface. If pH increased, more ligands such as carboxyl, phosphate, imidazole and amino group would become deprotonated and thus available of positively charged metal cations [42].

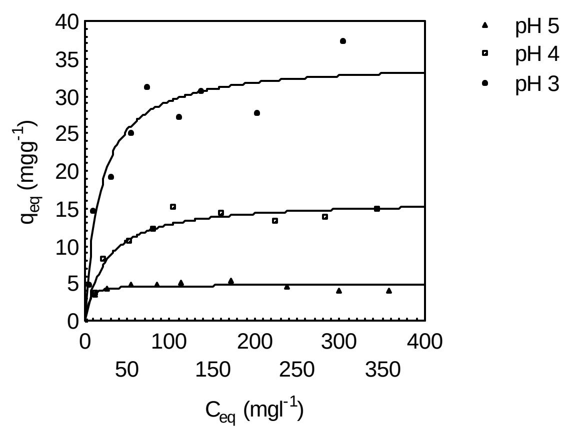

Fig. 4: Isotherms carried out at various pH at 25 °C, C_S 1g.l^{-1} (*Spirulina* sp.)

Effect of temperature on biosorption process: According to Fig. 4 and Fig. 5, effect of temperature on chromium(III) uptake was not so strong when compared with the effect of pH. In the case of *Pithophora varia*, the maximum biosorption capacity increased with increasing temperature up to 50 °C. At 60 °C, decrease of q_{max} was observed. This decrease at higher temperature may be due to the limitation of accessibility of metal ions to metal binding sites, as a consequence of biomass denaturation. The highest q_{max} was obtained at 50 °C (88.6 mg.g^{-1}), and the lowest at temperature 25 °C (60.6 mg.g^{-1}). But, on the other hand, the highest value of b was obtained at 25 °C (0.182 l.mg^{-1}) which showed strong binding of chromium(III) to the dry biomass of *Pithophora varia* at this temperature.

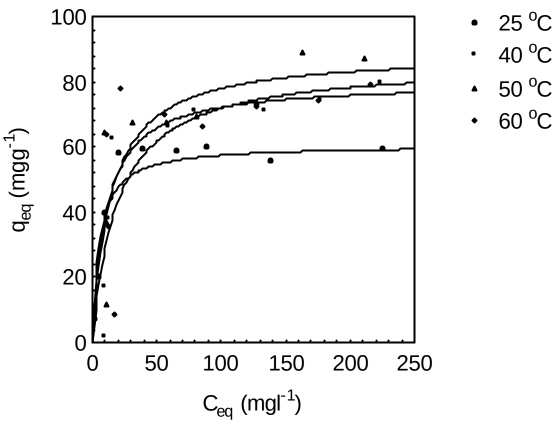

Fig. 5: Isotherms carried out at various temperatures at pH 5, C_S 1g.l^{-1} (*Pithophora varia*)

According to Fig. 6 the maximum metal uptake for *Spirulina* sp. imperceptibly increased with temperature increase.

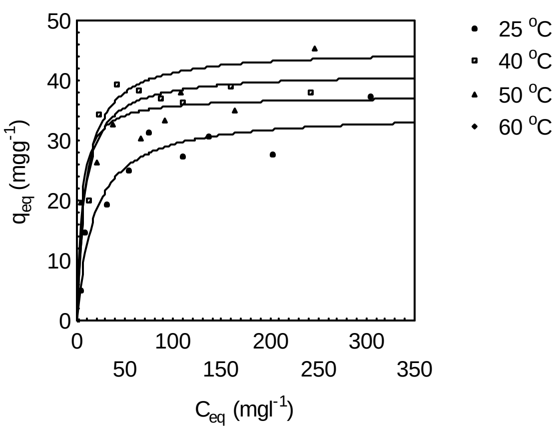

Fig. 6: Isotherms carried out at various temperatures at pH 5, C_S 1g.l^{-1} (*Spirulina* sp.)

CONCLUSIONS

This work has demonstrated the possibility of utilization of the biomass of *Spirulina* sp. and *Pithophora varia* as chromium enriched feed additive. Taking into consideration values of model parameters of Langmuir equation, *Pithophora varia* showed better biosorption properties. Maximum metal uptakes for *Pithophora varia* evaluated from Langmuir equation were two times higher than obtained for *Spirulina* sp. The best maximum metal uptake for both, microalga and macroalga was obtained at biomass concentration C_s 0.5 g.l^{-1} (q_{max}=64.5 mg.g^{-1}) and at temperature 50 °C (q_{max}=88.6 mg.g^{-1}), respectively. These data showed that initial pH strongly affected the uptake capacity of both biosorbents, more than temperature. In the case of *Spirulina* sp., the biomass concentration had also significant impact.

To supply suggested requirement quantity of chromium (10 mg.kg^{-1} of fodder), 0.15 g of *Spirulina* sp. and 0.11g of *Pithophora varia* should be added to 1 kg of fodder.

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