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Bioaccumulation of Cr(III) ions by Blue-Green alga *Spirulina* sp. Part I. A Comparison with Biosorption

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Abstract: The paper presents the results of studies on kinetics and equilibrium of bioaccumulation process of Cr(III) ions by blue-green alga *Spirulina* sp. Bioaccumulation was described as the process that consists of two stages, passive (identical with biosorption) and active (accumulation inside the cells). The passive stage (similarly as biosorption itself) was found to be quick process and the subsequent active stage (accumulation) was much slower and required metabolic activity of cells. The overall equilibrium of bioaccumulation process was reached after ca. 30 hours. The efficiency of bioaccumulation was compared with biosorption performance of cells grown under the same conditions. Two applications of bioaccumulation were discussed: metal ions removal from wastewater and metal ions binding to the biomass to produce biological feed supplement with microelements. The experimental results showed that bioaccumulation capacity was greater than biosorption capacity. Wastewater treated by the process of bioaccumulation contained lower residual metal ions concentration due to equilibrium shift of external (biosorptive) metal ions binding capacity towards lower values, resulting in lower residual metal ions concentration and thus higher treatment efficiencies. If bioaccumulation was considered as the method of biomass enrichment to produce mineral feed supplements for livestock, bioaccumulation enabled to bind more microelements, not only to the surface of cell wall, but also to accumulate inside the cells.

Key words: bioaccumulation, biosorption, *Spirulina* sp., chromium(III), wastewater treatment, mineral feed supplements

INTRODUCTION

Biosorption and bioaccumulation processes involve metal ions binding by either non-living (biosorption) or living (bioaccumulation) biomass. These techniques can be used in either bioremoval or biobinding methods. The first finds an application in wastewater treatment, the second, in the production of biological mineral feed supplements.

It is impossible to degrade heavy metals present in the environment. The only way to remove these contaminants from waste waters and polluted waters is to concentrate and transfer into the form that does not undergo biological cycles. Of particular concern is chromium – considered as heavy metal and pollutant, but on the other hand also as microelement - the element also used extensively in a wide range of industrial applications, such as metallurgical processes, electroplating, tanning, stainless steel, hard-alloy steel and stainless steel foam manufacture. It is also used as catalyst and coating material[1]. The industrial application of chromium caused that chromium-containing industrial effluents were discharged into the environment. Trivalent chromium is also considered as microelement significant for the proper growth of livestock.

Recently, increased interest in the applications of microorganisms for metal ions sequestration from solutions has been observed. Biological methods might become soon a very interesting alternative to traditional methods[2]. Biological methods of heavy metal ions removal may be soon applied in practice for the treatment of large-volume low-contamination level effluents that contain also other pollutants (organic or inorganic). The use of renewable biomass enables to repeatedly concentrate metal ions. The advantage of biological processes is that there is no need to use aggressive, concentrated chemicals. The sludge formed in this process (biomass bound with metal ions) could be utilized, i.e. via elution or biomass incineration.

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Metal ions binding to the biomass offers a possibility to elaborate a technology of production of biological feed supplements in which the biomass would be a carrier of bioavailable form of microelements in livestock diet. Binding microelements to the biomass provides an opportunity of supplementation of microelemenets in the form the mostly similar to naturally occurring in conventional feeds. Supplementation of livestock diet with microelements in the form of mineral salts was recently showed to be of very low efficiency. Microelements from inorganic salts were showed to possess low bioavailability and were found to be of transit character in the organism of animals.

When discussing metal ions binding to the biomass, it is necessary to distinguish between, metabolically passive biosorption and metabolically active bioaccumulation. Biosorption is the process in which biomass is simply used as adsorbent[3-4]. Bioaccumulation process involves using growing biomass in metal ions removal or binding[5-8]. Ions are partly bound on the cellular surface, and partly are transferred into the cellular interior. The latter process requires metabolic activity and expenditures of additional energy[9].

Bioaccumulation process is defined as the transfer of organic or inorganic pollutants into the interior of living cells[10]. In this process, also nutrients can be removed from treated effluents (nitrates, phosphates, sulfates, organic and inorganic carbon compounds)[11]. For instance, artificial ponds containing photosynthetic microorganisms can be used to treat mining leachates with the use of controlled eutrophication[12]. Cultivated biomass can be used as the biosorbent in another stage of treatment. The subsequent and widely applied example of bioaccumulation is activated sludge wastewater treatment process[11,13-14]. If the biomass, to which microelements were bound, was used as feed supplement, it can also be a valuable source of other nutrients: protein, unsaturated fatty acids, colorants etc.

The significant limitation of bioaccumulation in wastewater treatment is that an effluent serves simultaneously as the growth medium, and thus should provide growth nutrients to the cells[13, 15]. Serious disadvantage is low process rate that results from the need for the construction of large installations for continuous bioaccumulation wastewater treatment plants with high residence times. The advantage of bioaccumulation is that there is a possibility to reduce the concentration of metal cations to a very low level, mainly due to the complex nature of the process, that consists of biosorption itself and of the subsequent accumulation inside the cells[11], simultaneously with the removal of other contaminants (as potential nutrients).

Since in bioaccumulation process, simultaneous growth of bioaccumulator occurs[15], the application of growing cultures in metal ions binding, enables to simplify the configuration of an installation. Therefore, it was possible to eliminate a separate biomass cultivation unit (biomass culture, harvesting, drying, processing and storage)[15,16].

Among metal-ions sequestering organisms, microalgae were found to effectively bind metal ions from solutions[17]. As it was shown previously, mixotrophic[18] blue-green alga *Spirulina* sp. was a very good biosorbent of metal ions, Cr(III), Cd(II) and Cu(II) from model solutions[19]. It can be also used in the treatment of copper smelter and refinery effluents, containing low level of contaminants[20]. In order to precisely asses the process parameters, it is necessary to carry out laboratory studies on the process mechanism, kinetics and equilibrium.

Literature data concerning bioaccumulation of metal ions by microorganisms are not comprehensive. They rarely report results of systematic studies. The majority of works is of semi-quantitative character and discuss only initial and final concentration of metal ions, not showing data on the process kinetics or equilibrium[21-22]. There is also the scarcity of information on metal cations bioaccumulation by a given strain, for instance, there is the lack of literature studies on chromium(III) ions bioaccumulation by *Spirulina* sp. Substantially, there is the need to precisely define the terms, since the mechanism of bioaccumulation includes stages, the first of which is identical with the biosorption process.

The aim of the present work was to examine kinetics and equilibrium of bioaccumulation of Cr(III) ions by growing blue-green alga *Spirulina* sp. in the process of bioaccumulation to investigate its potential applicability in wastewater treatment, as well as in the production of biological mineral feed supplements. The obtained results were compared with the biosorption process itself. Also, nomenclature of processes that contribute to bioaccumulation was discussed.

MATERIALS AND METHODS

Microorganism and growth conditions: *Spirulina* sp., a blue-green alga, obtained from Sigma (USA) was used in this study. *Spirulina* sp. was grown in a photobioreactor (working volume 1 L) at 35 ^{o}C in Zarrouk liquid medium[23] enriched with glucose, containing (g/L), $NaNO_3$, 2.50; K_2HPO_4, 0.50; $NaHCO_3$, 10.00; NaCl, 1.00; MgSO4·7 H2O, 0.2;

CaCl2·2 H_2O, 0.02; $FeSO_4$·7 H_2O; 0.01 glucose, 2.50; and chromium(III) ions, 0-25.6 mg/L under illumination (32.7 W/m^2) with photoperiod of 12 hours light and 12 hours dark. Sampling (10 mL) was performed over a period of ca. 100 hours.

Bioaccumulation experiments: A rectangular algal photobioreactor was constructed especially for the cultivation of microalgae, with uniform, artificial lighting on the front and back of the vessel. The reactor consisted of two glass sides (that enabled artificial illumination) and two brass sides (that enabled thermostating). Eight 4 W white fluorescent lamps (Starlicht) were used for illumination with four lamps located vertically on each side of the reactor. The total volume of the photobioreactor was 2.0 L with height of 25.0 cm, width of 5.25 cm and breadth 15.0 cm. The working volume was 1.0 L with depth of 12.8 cm. The light transmission distance of 5.25 cm from front to back was selected in order to shorten the light penetration distance. The light intensity at the center was 6 % less than at the front and back of the photobioreactor. Therefore, it was assumed that the light was spread uniformly throughout the vessel.

Microalgal cultures in the presence of Cr(III) ions were initiated with ca. 0.25 g of *Spirulina* sp. cells in the form of lyophilisate that was used to inoculate the medium (1 L), and grown at the average light intensity 32.7 W/m^2, C_{0Me}=0-25.6 mg/L 35 °C. The culture was aerated with sterilized air (0.45 µm filter) at a constant flow rate 4.0 ml/s.

Analytical methods: All samples were filtered immediately through prewashed and preweighed paper filters (No 2), and cell dry weights were determined after drying filters at 80 °C until the samples reached the constant weight. Cell concentration was determined with a UV-160A UV-visible recording spectrophotometer (Shimadzu) at a wavelength of 560 nm (the measurement independent on pigment concentration). Light intensity was measured with PU 550 Metro Blansko luxmeter. The concentration of chromium(III) ions in the samples was determined with Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES PU 7000 from Philips, Cambridge, United Kingdom) connected with ultrasonic nebulizer CETAC (USA). The samples were analyzed in three repeats (the relative standard deviation of the measurement did not differ from acceptable for Certified Reference Materials).

RESULTS AND DISCUSSION

Biosorption versus bioaccumulation process: The first step of bioaccumulation process is biosorption (passive stage, $q^{external}$, eq. (1)). In the subsequent step, metal ions are transferred into the cells interior (active stage, $q^{internal}$, eq. (2)).

$$q^{bios} = q^{external} = \frac{C_0 - C_{eq}}{X} \quad (1)$$

$$q^{bioacc} = q^{external} + q^{internal} = \frac{C_0 - C_{eq}}{X} \quad (2)$$

If only the passive stage occurs (biosorption process), equilibrium between $q^{external}$ and C_{eq} is reached (Fig. 1a). When the active stage begins, metal ions are transferred from the outside into the inside of cells, and thus external metal ions binding capacity ($q^{external}$) decreases and internal metal ions binding capacity ($q^{internal}$) increases. External capacity ($q^{external}$) additionally decreases as the result of microbial growth. Decrease of $q^{external}$ results in the shift of biosorption equilibrium towards lower C_{eq} values. Thus, the efficiency of metal ions binding in bioaccumulation process was higher than in biosorption, in particular if $q^{external} << q_{max}^{external}$ (Fig. 1b).

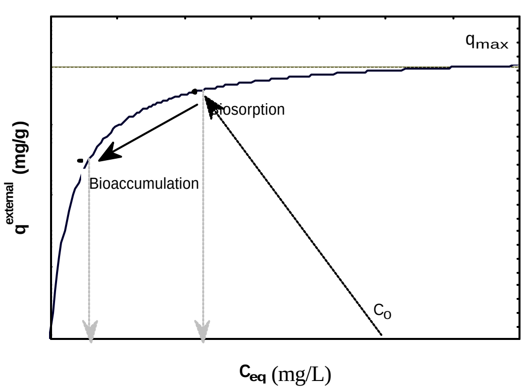
Bioaccumulation kinetics: Studies on kinetics of chromium(III) ions binding enabled to determine the mostly fundamental parameters necessary for the proper design of bioaccumulation unit. Also, the information on the mechanism of the process was obtained.

Kinetic data on chromium ions binding (at different initial concentration of Cr(III)) are shown in Fig. 2. During the process of bioaccumulation, microbial growth occurred and this was the main difference between widely discussed biosorption process. The final concentration after bioaccumulation (C^{bioacc}_{eq}) of metal ions was lower than the equilibrium concentration of ions after biosorption process (C^{bios}_{eq}), similarly as metal ions binding capacity. At biomass concentration 0.25 g/L and initial concentration of Cr(III) ions 25.6 mg/L, after biosorption process, C^{bios}_{eq}=18 mg/L, q^{bios}=31 mg/g[9] and after bioaccumulation C^{bioacc}_{eq}=0.65 mg/L, q^{bioacc}=101 mg/g. Equilibrium concentration of Cr(III) ions in the solution in bioaccumulation process was reached after ca. 30 h (while in biosorption, after few minutes). The highest quantity of metal ions was removed during the first 7 hours of the process (ca. 90 %), in the passive stage. The passive stage was much quicker than the active stage (Chojnacka, 2003). In bioaccumulation process, the equilibrium shifted towards higher metal binding capacity that resulted from cellular growth and surface regeneration, due to metal ions transport into the cells interior.

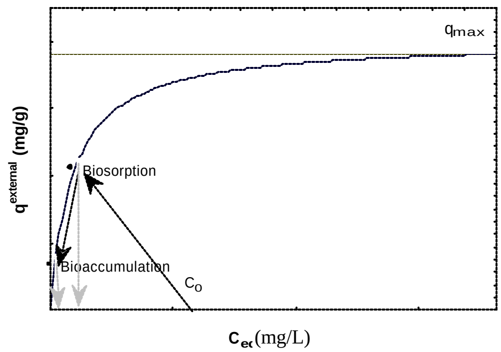
The dependence of bioaccumulation capacity (q^{bioacc}, mg of Cr(III) ions bound by 1 g of initial mass of cells) in time is shown in Fig. 3. At higher initial concentration of metal ions, higher values of bioaccumulation capacity were reached. The advantage was that metal ions were bound with the biomass stably.

Bioaccumulation equilibrium: Literature reports that accumulation of metal ions depends on external concentration of metal ions in the solution until their concentration leads to toxic effects, and thus to decreased performance of bioaccumulation (Wong and Tan, 1998). In the studied range of initial Cr(III) ions concentration (below 25.6 mg/L) this phenomenon was not observed. Literature reports that the maximum concentration of Cr(III) ions tolerated by microalga *Scenedesmus acutus* was 15 mg/L and *Chlorella vulgaris* 45 mg/L[24].

Discussion on the dependence of equilibrium metal ions concentrations in the solution and initial concentration of these ions (Fig. 4) (when considering permissible Polish limits for Cr(III) ions in discharged waters and wastewaters on the level 0.5 mg/L[25]), enabled to assess the initial concentration of chromium ions and for given process conditions in the solution (at given cells concentration), at which permissible concentrations by the Polish law would be reached. This value was evaluated as 18 mg/L. In the case of higher initial concentration, it will be necessary to apply preliminary treatment (for instance with the use of traditional methods or biosorption process), mixing with effluents containing lower level of these pollutants or to increase the biomass concentration.



a)



b)

Fig. 1: Equilibrium shift towards lower values of C_{eq} in bioaccumulation process when comparing with biosorption; (a) $q^{external} \gg q_{max}^{external}$, (b) $q^{external} << q_{max}^{external}$

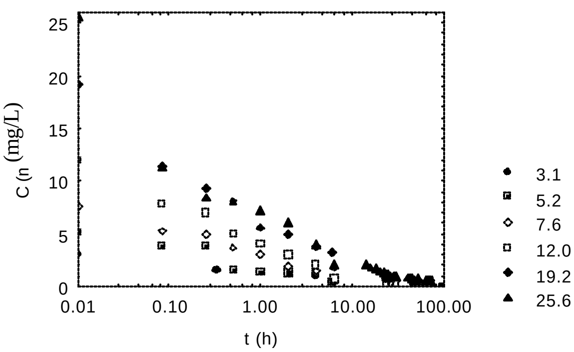


Fig. 2: Kinetics of Cr(III) ions binding in the process of bioaccumulation at different initial concentration of metal ions in the solution (3.1-25.6 mg/L).

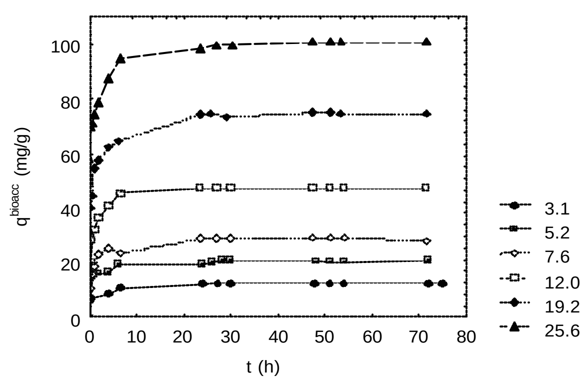


Fig. 3: Bioaccumulation capacity (calculated per initial concentration of the biomass) of Cr(III) ions in the process of bioaccumulation at different initial concentration of metal ions.

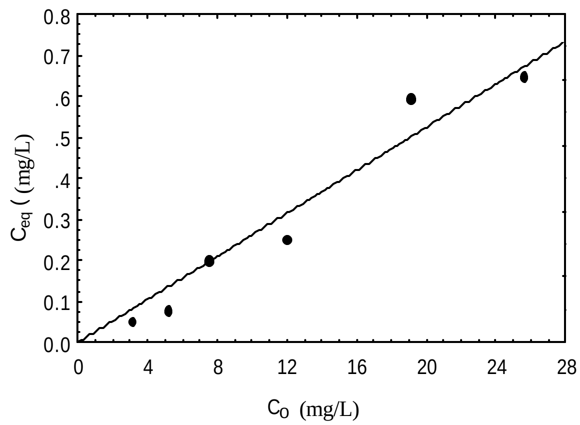


Fig. 4: The dependence of equilibrium concentration of metal ions in the solution (Ceq) and the initial concentration (C0) of metal ions, at a given process conditions.

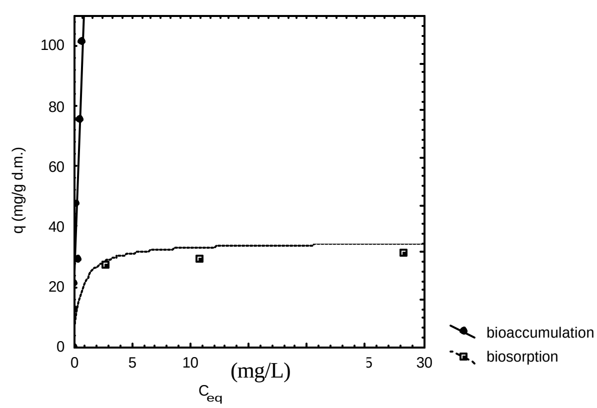


Fig. 5: A comparison of bioaccumulation and biosorption isotherms for Cr(III) ions by the biomass of Spirulina sp.

In the studied range of Cr(III) ions concentration, it was not possible to determine a complete bioaccumulation isotherm, because it was unattainable to saturate the biomass with metal ions (the dependence q_{bioacc}=f(C_{eq}) was a steep line) due to limitations resulting from metal ions toxicity to cells and limited solubility of Cr(III) ions in the growth medium due to the presence of anions that form insoluble salts at higher concentration of Cr(III) ions. It was found that bioaccumulation capacity was always greater than biosorption capacity of cells cultivated at the same process conditions (in mixotrophic mode) (Fig. 5).

CONCLUSIONS

In the present study it was found that bioaccumulation consisted of two constituent processes,

– passive – identical with biosorption; metal ions were bound to metal-binding sites present on the cells surface; evaluated by determination of external metal ions binding capacity ($q^{external}$);
– active – specific accumulation; metal ions are transferred into the cellular interior; evaluated by determination of internal binding capacity ($q^{internal}$);

Biosorption, as well as the passive stage of bioaccumulation are rapid processes, associated with the biomass surface (ion-exchange)[26]. In the case of biosorption, the process reaches equilibrium at this stage, however in the case of bioaccumulation, the subsequent stage begins. Due to the cellular activity, if the composition of the solution fulfills the criteria of minimal growth medium and exerts no toxic effect to cells, metal ions are furtherly transferred from cells surface into the interior, resulting in decrease of external metal ions binding capacity, that results in the equilibrium shift towards lower concentration of metal ions in the solution, thus yielding greater metal ions binding capacity. Therefore, although equilibrium in bioaccumulation was reached after significantly longer time than in biosorption (30 hours, 5 minutes, respectively), the efficiency of bioaccumulation was significantly higher than in biosorption, since biosorption was the first stage (passive) of bioaccumulation. This resulted in much higher bioaccumulation than biosorption capacity. At the initial concentration of metal ions < 18 mg/L and the initial biomass concentration 0.25 g/L, in the bioaccumulation process, concentration of chromium(III) ions can decrease below the permissible level of 0.5 mg/L. Therefore, it was concluded that bioaccumulation was the perspective method, which could be used as the final stage of treatment of industrial effluents polluted with heavy metal ions, if traditional methods are not capable to remove contaminants below the level required by the law. Bioaccumulation can be also employed in biomass enrichment with microelements, to produce highly concentrated form of these constituents bound with the biomass of an edible microalga *Spirulina* sp.

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