Biosorption of chromium by dry algae *Chlorella kessleri*

A Takáčová¹², M Bajuszová¹, J Kohanová³, A Lux³, P Valent¹, A Kokavcová³ and L Takáčová²

¹Department of Environmental Ecology and Landscape Management, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, Mlynská dolina, 842 15 Bratislava, Slovakia
²VÚRUP, a.s., Vlčie hrdlo, 820 02 Bratislava, Slovakia
³Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovičova 6, Mlynská dolina, 842 15 Bratislava, Slovakia

**Abstract.** Decontamination of environment according to traditional methods is not only economically inaccessible but also often highly environmentally harmful. It is necessary to apply methods that are environmentally friendly as possible. These methods include bioremediation, which uses organisms able to fight with high concentrations of pollutants to decontaminate the environment. In this study, we observed the biosorption of chromium from the aquatic environment using dried algae *Chlorella kessleri*. The results of the specific sorption at pH = 4.00 in the chromium model sample had a value of q = 5.9 mg / g, which represented a decrease in the chromium concentration by 74%. The specific sorption in the case of the native chromium sample q after 24 hours reached the highest value (q = 2.74 mg / g) at pH = 4.00. During the experiment, we observed a constant change in the content of photosynthetic pigments in the prepared solutions where it was shown that after 24 h exposure the yield of chlorophyll a was decrease by 95.91% compare to control. In the case of chlorophyll b, the same trend was demonstrated where 91.92% decrease of its content after 24 hours was observed. This type of dried alga has been shown to be a rapid biosorbent, in relatively short time intervals. The results of the study show that in the process of decontamination there was not only the binding of chromium to the cell surface but also its penetration through the cell wall.

1. Introduction

Ecological wastewater treatment is an issue that has been addressed for decades, and new treatment methods capable of removing even the heaviest contaminants are constantly being sought. Heavy metals such as arsenic, copper, cadmium, chromium, nickel, lead and mercury are major pollutants in drinking water that are difficult to remove due to their non-biodegradable and persistent nature (Azimi et al., 2017). Groundwater and soil pollution are becoming a serious problem due to an accumulation in the food chain, which is attracting increased attention (Zhao et al., 2019). Overexposure leads to a higher level of accumulation in human and animal tissues, leading to harmful changes in the body. To reduce the adverse effects of such metals, authorities and environmental organizations are forcing companies worldwide to meet strict standards for maximum permissible limits for heavy metals. The Ministry of the Environment of Slovak Republic set limit values for the metal content in wastewater in the Regulation of the Government of the Slovak Republic of 25th May 2010, which sets requirements for achieving good water status (Collection of Laws No. 269/2010). Biosorption of heavy metals from solutions has proven to be very promising and offers significant advantages, residual biomass of industrial microorganisms is able to efficiently accumulate heavy metals as a biosorbent (Javanbakht et al., 2014). The term biosorbent refers to a biomaterial that is derived from biological material (bacteria,
microscopic fungi, yeast, microscopic algae), plant material or animal material that can bind toxic chemical elements and other inorganic or organic contaminants (Čerňanský, 2011). It is an alternative technology for removing metals from dilute aqueous solutions, based on the ability of dead biomass to bind heavy metals by various mechanisms such as ion exchange, physical and chemical adsorption, and others (Pagnanelli et al., 2003). The best results for efficient and economical implementation of metal biosorption are achieved by biomass containing chitin (seaweed and algae) and microorganisms (fungi and bacteria) (Volesky, 2003). The cell wall of microorganisms represents the primary structure of the cell, interacting with contaminants, which is also related to its protective function for the cell. The structure responsible for the biosorption phenomenon is primarily the cell wall. The cell wall of microorganisms is composed mainly of polysaccharides and glycoproteins (glucans, glucosamine, mannans, chitin, chitosan, alginate). Due to their unique ion exchange and complex properties, these polysaccharides have been used as new biosorbents for the removal of heavy metals, ions from wastewater or precious metal recovery, mining waste or electronic / catalytic waste. These polymeric substances are rich sources of various functional groups that are responsible for binding contaminants. Such groups include carboxyl (-COOH), hydroxyl (-OH), sulfhydryl (-SH), phosphate (-PO$_4$$^{3-}$), amino (-NH$_2$) and others (Adhikari et al., 2008; Saha and Orvig, 2010; Fomina and Gadd, 2014; Dodson et al., 2015; Tran et al., 2015; Gao et al., 2017). Chemical treatment occurs e.g., to remove the carboxyl and phosphate groups of glycoproteins, phospholipids, and polysaccharides from the cell wall, thus creating space for the precipitation of some metals. It also removes surface impurities on the cell wall, ruptures the cytoplasmic membrane and exposes other binding sites, causing an increase in the sorption of metals and semi-metals. The treatment is suitable for increasing the number of such functional groups which bind metal cations. Modification of biomass with acidic agents usually causes a significant decrease in the biosorption of metal cations. However, it is suitable for the biosorption of metal anions and semi-metals. Various types of biomass, including bacteria, have been monitored to identify highly efficient removal of metal contaminants (Tunali et al., 2005; Lodeiro et al., 2005; Hansen et al., 2006; Tunali et al., 2006; Trivedi and Patel, 2007; Bueno et al., 2008). Many studies have confirmed that algae have a high biosorption capacity. High biosorbert capacity is defined by the weight or molar equivalent of the dye adsorbed per unit mass of biomass ($mg\cdot g^{-1}$), while affinity is defined by the ability of the biosorbent to reach equilibrium sorbate concentration even at low equilibrium sorption capacities (Volesky and Naja, 2007, Mokhtar et al., 2017). Aerobic granular sludge (AGS) from bacterial algae has shown high biological absorption capacity and granular stability, suggesting that AGS of bacterial algae can potentially be used as a biomaterial to remove and regenerate Cr (VI) for Cr (VI) -containing wastewater treatment (Yang et al., 2020). When algae are used, polysaccharide alginate (algicin acid) plays a significant role in the biosorption of metals and semi-metals. Alginic acid is a natural polysaccharide of wide interest and is consistent with its use for its excellent water solubility, biofilm formation, biodegradability, and biocompatibility (Guo et al., 2020). The ability of alginate to form gels by ion-exchange reaction with polyvalent metal ions suggests its use as a metal adsorbent. Thus, alginic acid and alginates are likely to be potentially useful adsorbents for removing not only heavy metals but also radionuclides from waste (Mimura et al., 2001; Gok and Aytas, 2009). The main advantage of biosorption is that it can be used directly at the site of contamination without the need for pre-treatment and can be combined with other environmentally friendly desorption processes (Tewaria et al., 2005). In study of Jaafari and Yaghmaeian (2019) proved that Chlorella coloniales is an excellent alternative for removal of heavy metals, maximum efficiencies for the removal of Cr, Cd, Co, Fe, and As were more than 95%. The aim of the work is searching for the better way of decontamination of metal wastewater pollution. Algae showed high efficiency in decontamination process in lot of studies an ex-situ experiments, therefore, they are one of the reasons why we chose them. Dry algae represent good choice of bioabsorbent of metals contamination due to low costs and easy disposal.

2. Experimental

2.1. Biomass treatment
We obtained the biomass of the alga Chlorella kessleri from the Institute of Microbiology of the ASCR, v.v.i., Opatovický Mlyn. The experiments were performed at two pH values (1.83 and 4.00). Dry
biomass was prepared after washing the biomass in deionized water, drying in an oven at 70°C for 24 hours. After drying, it was ground in a powder mortar.

2.2. Preparation of chromium solutions
A native sample obtained by blast furnace slag mineralization and a model sample prepared from a certified standard were used for the chromium experiment.

2.2.1. Aqueous extract from a native slag sample. The native sample (slag) was mechanically pretreated, then homogenized and sieved to a particle size of less than 10 mm. Subsequently, an aqueous extract was prepared using deionized water according to STN EN 12457-4: Characterization of waste, leaching. Verification test for the leaching of granular and waste materials and sludges - Part 4: Single-stage batch test at a liquid to solids ratio of 10 l/kg for materials with a particle size of less than 10 mm.

2.2.2. Native slag sample. The mechanically pretreated, homogenized and quaternated native sample was mineralized with concentrated nitric acid and concentrated hydrochloric acid (CEM Microwave Sample Preparation System). The mineral was then filtered through a filter (Millipore, pore size 0.45 µm). Analysis of metals from solution after pretreatment by mineralization was performed using the ICP AES method. The measured data are recorded in Table 1.

2.2.3. Model sample. The model sample was prepared from a certified K₂CrO₄ standard (Merck) and deionized water with a final chromium concentration of approximately the same as the native sample (400 mg l⁻¹).

2.3. Chromium sorption
The dry algal biomass was weighed and added to the prepared solution to correspond to a biomass concentration of 1 g/l. The volume of the sorption medium was 20 ml, the pH of the solution was adjusted with 30% NaOH solution and 20% H₂SO₄ solution. pH measurement was performed with a pH meter (DENVER Instrument, Model 220, USA). The inlet pH in the biosorption experiments was 1.83 and 4.00 for both types of chromium solutions. Under the measuring conditions, the temperature was in the range of 23 ± 2 °C with constant shaking on a rotary shaker at a frequency of 120 rpm. Samples for biosorption analysis were taken at specified time intervals of 2, 6 and 24 hours. Algal biomass was separated from the solution by membrane filtration (Millipore, pore size 0.45 µm).

2.4. Photosynthetic pigment analysis
The exact concentrations of pigments were determined in the prepared base sample and model samples at different time intervals of 2, 6, 24 and 48 hours after chromium exposure. The biomass was separated from the solution by filtration under reduced pressure using a water pump. Subsequently, the sample was homogenized using a solvent of 95% ethanol according to Lichtenhaler (1987) at the given wavelengths (ch a-664.2 and ch b-648.6) was determined using a spectrophotometer (JENWAY 6405 UV / VISIBLE, UK). The exact concentration of the pigments in the individual samples was calculated by using the formula.

2.5. Microscopic imaging
The model sample was observed under a fluorescence microscope (Axioskop 2 plus, Carl Zeiss) with UV2 filter (Zeiss Filter Set 16 (excitation: BP 485/20 nm, beamsplitter: FT 510 nm, emission: LP 515 nm) and subsequently photo documentation was created using a camera (Olympus DP 72) with an applied ultrazoom mounted on a special attachment of a binocular microscope.

3. Results and discussion
The initial chromium concentration in the case of the native sample (slag) after mineralization was 417 mg kg⁻¹ dry matter. The results in Table 1 the slag showed the contain of other metals in a relatively high concentration.
Table 1. Concentration of metals.

| Type of sample                                      | Co  | Cr  | Mn  | Fe  | Ni  | Zn  | Cd  | Pb  | Cu  |
|----------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Aqueous extract (48 h) mg.l\(^{-1}\)                | <0,02 | <0,01 | <0,01 | 0,02 | <0,02 | 0,013 | <0,002 | <0,03 | <0,01 |
| Sample after mineralization mg.kg\(^{-1}\)          | 4,59 | 417 | 2 509 | 11101 | 44,1 | 30,3 | 1,27 | 3,17 | 21,59 |
| Method detection limit mg.l\(^{-1}\)                 | <0,02 | <0,01 | <0,01 | <0,01 | <0,02 | <0,013 | <0,002 | <0,03 | <0,01 |

The output values obtained after 48 hours of leaching (Table 1) were at the level of the detection limit. It is clear from Table 1 that with the quaternation of the native sample and its subsequent mineralization, the content of contaminants in the aqueous solution of the expression increased, thus exceeding the permitted limit in wastewater (Collection of Laws No. 269/2010).

3.1. Chemical analysis after chromium sorption

The residual concentration of chromium in the obtained filtrate was determined by atomic absorption analysis. The sorption (q) was calculated based on the general relation:

\[
q \ (\text{mg.g}^{-1}) = V \ (c_0 - c_n) / S
\]  

where \(c_0\) [mg.l\(^{-1}\)] and \(c_n\) [mg.l\(^{-1}\)] are the input and residual (equilibrium) concentration of the metal, \(V\) [l] is the volume of the solution and \(S\) [g] is the weight of the biosorbent used.

Table 2. Measured values of specific sorption in the native sample.

| Solution pH | q (mg.g\(^{-1}\)) |
|-------------|-------------------|
|             | Biosorption - 2 hours | Biosorption - 6 hours | Biosorption - 24 hours |
| 1,83        | 0,84              | 1,34              | 2,30               |
| 4,00        | 1,92              | 2,54              | 2,74               |

Table 3. Measured values of specific sorption in the model sample.

| Solution pH | q (mg.g\(^{-1}\)) |
|-------------|-------------------|
|             | Biosorption - 2 hours | Biosorption - 6 hours | Biosorption - 24 hours |
| 1,83        | 2,60              | 2,90              | 3,98               |
| 4,00        | 3,64              | 4,20              | 5,90               |

Comparing the results in Tables 2 and 3, it can be judged that the sorption of chromium in the native sample due to the high concentration of other metals, especially Fe (Table 1), is significantly lower than in the simulated model sample. The specific sorption in the case of the native chromium sample q after 24 hours reached the highest value (q = 2.74 mg.g\(^{-1}\)) at pH = 4.00. In the case of sorption conditions at pH = 1.83, the surface of the biosorbent is disturbed. Similar effects have been observed in work Kuyucak and Volesky (1989), in the study of cobalt biosorption using several species of seaweed, including the brown alga Sargassum natans. According to these authors, the increase in pH could be the result of disruption of the cytoplasmic membrane of algae, thereby increasing the concentration of carbonates in the extracellular environment.

The results of the specific sorption at pH = 4.00 in the chromium model sample had a value of q = 5.9 mg.g\(^{-1}\), which represented a decrease in the chromium concentration by 74%. It is known from the literature that at high chromium concentrations more than 200 mg.l\(^{-1}\) the pH values decrease spontaneously. This fact is an advantage for the sorption method used, which allows to selectively sorb chromium in the presence of other metals.
Cossich et al. (2002) state that heavy metals are predominantly bound from aqueous solution by ion exchange and carboxyl groups contained in algae, their availability being pH dependent. At a pH in the range of 3.5-5.5, these groups form a negatively charged surface and electrostatic interactions between the metal cation and the carboxyl group are responsible for the biosorption of the metal. Yoon et al., (2011) point out that chromium is well reduced from the environment in a wide range of pH ranges, with its reduction possible under both oxic and anoxic conditions, although they showed that at pH 5 the reduction was 1.7 times greater as at pH 7.5.

In a study with seaweed Sargassum sp. the influence of modification of sorption conditions - amount of biomass, pH and temperature on achieving metal-biosorbent equilibrium was studied. The results showed that pH has a significant effect on the biosorption capacity of chromium. The concentration of the biosorbent was not a decisive parameter to increase the rate and capacity of chromium biosorption. The suitability of the selected pH value 4 for chromium sorption, which is reported by the results of sorption of a real sample in the study (Liu et al., 2004). The selectivity of chromium sorption was highest the highest chromium biosorption capacity was reached at 40 ° C and pH 4.0 (a precipitate formed at pH 5.0). The seaweed biomass of Sargassum sp. showed good chromium biosorption capacity from the seaweed Sargassum sp. reached relatively fast at 60 percent. The total biosorption capacity was completed in ten minutes. The results of the experiment Dittert et al., (2014) indicate that the optimal pH for chromium removal by Laminaria digitata algae was 2.5, with a maximum the amount of chromium reduction was 2.1 mmol / g. In one of the most recent studies, glauconite was used as a sorbent, again points out that the reduction of chromium is possible under different pH conditions (Naghipour et al., 2018). The maximum adsorption capacity of Cr (VI) was achieved using the biomass of the alga Spirigrya sp. and at pH = 2.0 it reached 14,7.10³ metal mg.kg⁻¹ dry weight biomass (Gupta et al., 2001). In their work, Netzahuatl-Muñoz et al. (2012) state that the ideal pH for the biosorption of chromium from solution is pH 5, with the optimal pH varying depending on the contact time.

3.2. Determination of pigment content

The exact content of pigments was determined in the prepared basic sample (rehydrated Chlorella kessleri) and model samples using a spectrophotometer. The aim of this assay was to observe changes in the content of pigments based on the time of exposure of algae to high chromium concentration. In table no. 4, changes in content relative to original values to samples exposed to toxicity for 2, 6, 24, and 48 hours can be observed.

| Treatment (hour) | chl a         | chl b         | Decrease of chl a | Decrease of chl b |
|-----------------|---------------|---------------|------------------|------------------|
| Z               | 60,581 ±3,43  | 29,957 ± 2,56 | 61.14%           | 51.68%           |
| 2h              | 23,538 ± 3,06 | 14,475 ± 2,5  | 84.72%           | 72.36%           |
| 6h              | 9,254 ± 0,2   | 8,28 ± 0,54   | 95.91%           | 91.92%           |
| 24h             | 2,475 ± 0,13  | 2,419 ± 0,25  | 96.08%           | 91.85%           |
| 48h             | 2,376 ± 0,27  | 2,44 ± 0,22   |                  |                  |

Values are mean ± SD of 3 measurements.

Based on the obtained values, we found out specific amounts of pigments in individual samples using formulas for content calculating. Our experiment showed an increased both chlorophyll a and b content in samples exposed to chromium. In chart no. 1, we can observe a constant change in pigments depending on the time of exposure. In the case of chlorophyll a, rapid changes in content 61.14% was observed 2 hours after exposure of algae for chromium. After 24 hours of testing, we can observe a decrease in the amount of chlorophyll up to 95.91%. In the case of chlorophyll b, we observe the same trend where after 2 hours exposure there was a change in pigment content by 51.68% and after 24 hours of exposure, by 91.92% compare to control. Chlorophyll in dried algae is still active despite exposure to 70°C temperature. Chlorophyll activity after algae drying is also represented by the results of the study Takáčová et al., (2014) where dried algae Chlorella kessleri consumed 86-89% of added Benzo [a] Pyrene while live algae consumed 75-78%. In the case of our experiment, although chlorophyll leakage
is expected, the reason for this analysis was the visible discoloration of algae after the addition of Cr. Based on the results, we assume that the addition of a toxic substance caused the changes in chlorophyll content. Study Sarka a kol., (2020) shows the difference between extraction of chlorophyll pigments in dry and wet alga Chlorella termophylla where was occured yield extraction to be 2.7fold higher from wet biomass than dry biomass. The effect of toxic heavy metals on live algae was studied in several studies, where a rapid decrease in chlorophyll amount was demonstrated. Study of Kondzior and Butarewicz (2018) has shown phenomenon where after exposure of the alga Chlorella vulgaris to increased concentrations of Cu there was a high decrease in the concentration of chlorophyll and up to 63% for the period of exposure for 2 days. In the experiment, they also used Zn, which caused a decrease in chlorophyll b of up to 100% during an incubation of 7 days. These results indicate a high toxic effect of metals on the functioning of living photosynthetic organisms. The result of the study showed that the increasing concentration of heavy metals leads to a decrease in cell density and intracellular pigment content in the phytoplankton of Nitzschia sp. (Hindarti and Larasati, 2019). Changes in chlorophyll content in dried algae after the addition of acidic methanol extracts were observed in a study by Osório et al., (2020) where they describe a decrease in chlorophyll content in the alga Laminaria ochroleuca. A significant decrease in chlorophyll a was observed even after exposure of the alga Tetraselmis tetrahele to high concentrations of Hg and Cd, but the toxicity showed no effect on cell size (Naorbe and Serano, 2018). The subject of further studies will be the monitoring of gradual changes in the content and decolorization of photosynthetic pigments at different concentrations of Cr and, of course, to determine the main cause of the changes.

3.3. Microscopic imaging

After chromium sorption in the case of dry Chlorella kesleri biomass, there was a change in the intensity of cell staining in the case of microscopic imaging under a bright field and fluorescence microscope. The change was manifested by damaged cell surface (changed of cell walls) in most cells. In the case of recording from a fluorescence microscope, the intensity of photosynthetic pigments decreased after exposure to chromium.

![Figure 1. Changes in cell clusters of inanimate algal culture of Chlorella kesleri after 24 hours - bright field (a,b) and fluorescence microscopy images (c,d) - cell at start of sorption (a, c) - cells after 24 hour of sorption (b, d).](image)

4. Conclusion

Based on the comparison of the measured data in the native sample (slag) and the model sample, it can be stated that the presence of other metals slows down the sorption of chromium from the solution.
can be seen from the results of the specific sorption of chromium \( q = 5.9 \text{ mg.g}^{-1} \) at pH = 4 in the model sample (decrease in chromium concentration 74%), the loss of chromium in the native sample (33%) was lower. For these reasons, it is necessary to choose a suitable type of biomass and ensure sorption conditions. The basic factors influencing the biosorption of metal include - metal concentration, pH, temperature, the presence of cations and anions, ionic strength. During the experiment, we observed a constant change in the content of photosynthetic pigments in the prepared solutions where it was shown that chlorophyll \( a \) content after 24 h exposure shows decrease by 95.91% compare to control. In the case of chlorophyll \( b \), the same trend was demonstrated where its content present 91.92% decline after 24 hours treatment. This species of dried alga has been shown to be a rapid biosorbent, in relatively short time intervals. The results of the study show that in the process of decontamination there was not only the binding of chromium to the cell surface but also its penetration through the cell wall. In conclusion, the metabolism-independent adsorption and absorption of metal ions by cell walls and cell itself is a very rapid process, and relatively high concentrations of metals can be sorbed.

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