

Article

# Use of the Coenobial Green Algae *Pseudopediastrum boryanum* (Chlorophyceae) to Remove Hexavalent Chromium from Contaminated Aquatic Ecosystems and Industrial Wastewaters

Małgorzata Sutkowy and Grzegorz Kłosowski \*

Department of Biotechnology, Kazimierz Wielki University, 85-671 Bydgoszcz, Poland; sutkowy@ukw.edu.pl

\* Correspondence: klosowski@ukw.edu.pl; Tel.: +48-52-325-9220

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**Abstract:** The idea of using microorganisms, especially microalgae, as biosorbents of heavy metals deserves particular attention due to their natural biosorbent properties and the relatively simple and inexpensive methods of obtaining their biomass. The cosmopolitan microscopic green alga of the genus *Pseudopediastrum* is an example of an organism with the desired biosorption properties. The aim of the study was to assess the potential use the dry biomass of *Ps. boryanum* var. *longicorne* in the process of biosorption of chromium(VI) (Cr(VI)) ions from aqueous solutions. Biomass of microalgae was cultivated in the photobioreactor conditions (L-S2T2 medium, light intensity of 4000 lx, photoperiod 12L:12D). The biomass obtained was used for the biosorption of Cr(VI) ions from aqueous solution. The effect of pH (2–6), biosorbent concentration (0.5–2 g/L) and initial chromium concentration (10–100 mg/L) was examined. The highest removal of Cr(VI) ions (70%) was observed at pH 2, initial chromium concentration of 10 mg/L and a biomass concentration of 2 g/L. At this chromium concentration, the sorption capacity of the microalga was the lowest. The results indicated that the biomass of the *Ps. boryanum* is suitable for the development of efficient biosorbent for the removal of Cr(VI) from wastewater.

**Keywords:** green algae; *Pseudopediastrum boryanum*; biosorbent; wastewater; chromium(VI)

## 1. Introduction

The development of civilization and industrialization lead to the introduction of significant quantities of various pollutants into natural waters. Among harmful substances that end up in waters, heavy metals deserve special attention. They are a significant threat for humans, animals and for entire ecosystems due to their toxicity, and mutagenic and carcinogenic effects [1]. High harmfulness of heavy metals and their migration and accumulation in subsequent links of the food chain call for new and efficient methods of their removal. Effective wastewater treatment is the basic process in protection of water, which is one of the most valuable natural resources.

Conventional methods of removing heavy metal ions from the environment include chemical precipitation and filtration, ion exchange, coagulation/flocculation, adsorption, reverse osmosis and electrochemical processes [2,3]. These methods are not suitable for purification of large amounts of water with low concentration of undesirable components. Their application under these conditions is economically unjustified and leads to secondary contamination. Proecological technologies based on biosorption or bioaccumulation [3–5] seem to be an alternative to these processes. These technologies are perfectly suited for the treatment of waters and sewage with low concentrations of pollutants (even below 100 mg/L), as they are cheap, efficient and also enable multiple regeneration of biomass,

combined with metal recovery [6–8]. Reuse of heavy metals also enables more responsible management of available natural resources.

Binding of ions by biomass occurs as a result of biosorption on the surface of the biosorbent, bioaccumulation inside cells and chemical transformation of ions as a result of metabolic activity. Nonliving biomass is responsible only for biosorption, while living organisms are also capable of bioaccumulation [3,4]. In the biosorption process, the dead biomass of microorganisms is usually used, because it does not require nutrients or specific conditions to sustain vital functions. An additional limitation for the live biomass use is the concentration of metal, which must be below the toxicity level for the cell. In addition, dead biomass can be subjected to physical and chemical modifications that increase its sorption capacity and ensure greater efficiency in removing metals [4,5].

The use of biosorbents in technologies for water and wastewater treatment requires a series of preliminary studies leading to determination of the biomass sorption properties, which would allow the selection of guidelines for the further design of technical installations. The first task in biosorption technology is to choose suitable biosorbents with high sorption capacity from the available and cheap biomaterials. The sorption capacity is affected by the surface area and the chemical structure (composition) of the biosorbent cell wall, which in turn determine the number and availability of active sites binding metal ions. The biosorption efficiency depends not only on the properties of the biosorbent, but also on the conditions of the process, that is, pH and temperature of the solution, concentration and type of metal (sorbate), the presence of other ions in the solution, and the size and dose of biosorbent [3–5]. Skillful control of these factors enables the biosorption process to be carried out under optimal conditions to achieve its maximum efficiency.

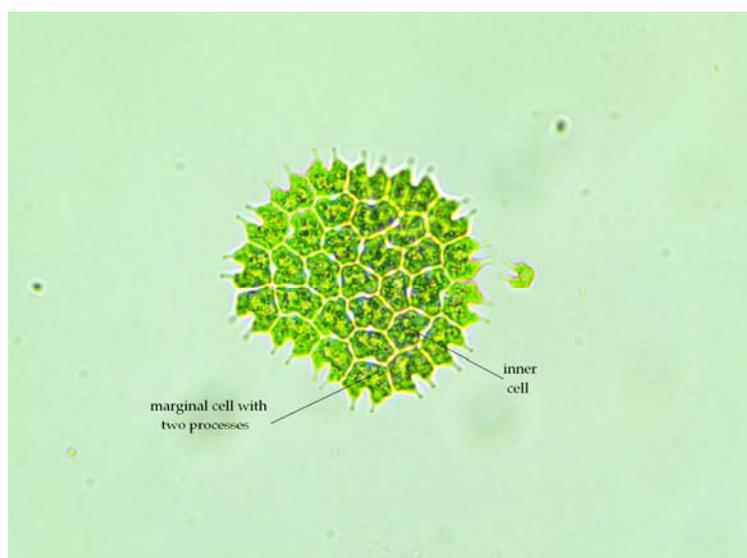
Currently, much attention is devoted to research on the methods of obtaining biomass of microalgae, including coenobial green algae of the genus *Pediastrum* Meyen *sensu lato* (Sphaeropleales, Hydrodictyaceae), for their use in processes related to wastewater treatment and bioremediation. *Pediastrum* is one of the most abundant algae in freshwater reservoirs. It has a cosmopolitan distribution, but only four of its species occur all over the world: *P. boryanum*, *P. duplex*, *P. tetras* and *P. simplex* [9]. The structure of the coenobium of these organisms, consisting of many cells ( $2^n$  cells, usually 8, 16, 32) arranged in one plane [10], making them an exceptionally well-developed absorbent surface in relation to their volume. The cell wall of these algae contains sporopollenin, which is resistant to enzymes and strong chemical reagents used, for example, when preparing fossil samples for palaeoecological analyses. For this reason, these algae are good bioindicators in the reconstruction of processes occurring in water reservoirs [9]. Sporopollenin, due to its ability to absorb UV-B rays (280–315 nm) and bind heavy metals, found its practical application in the production of sun-protecting cosmetics, but also as a metal chelator in ion exchangers used in sewage treatment plants [11].

The specific structure of the coenobium, rapid biomass growth and widespread occurrence of *Pseudopediastrum boryanum* (Turpin) E. Hegewald [12] in freshwater reservoirs were the reasons for using this algae for pretreatment of sewage containing heavy metals. When choosing a variety of *Ps. boryanum*, the main criterion that has been addressed was its distribution. *Pseudopediastrum boryanum* var. *longicornis* Reinsch was selected for the study. This variety dominates in all climate zones under various environmental conditions [13], in contrast to thermophilic var. *brevicornis*, or var. *boryanum*, which prefers clean and slightly alkaline waters. We examined the sorption capacity of the dried biomass *Ps. boryanum* var. *longicornis* for chromium(VI) ions. This metal is considered highly toxic and its compounds in the +6 oxidation state are classified as carcinogenic to humans in accordance with U.S. Environmental Protection Agency and International Agency for Research on Cancer [1]. In our previous studies, we developed process that enabled significant acceleration of the growth rate of green algae biomass. The parameters included the selection of a suitable culture medium, light intensity, daily photoperiod and supplementation of the medium with carbon dioxide. Using the biomass obtained in this way, we analyzed the effect of the most important parameters of the biosorption process, that is, pH, initial concentration of chromium(VI) (Cr(VI)) and biosorbent, on the efficiency of metal removal and sorption capacity of microalgae.

## 2. Materials and Methods

### 2.1. Preparation of Biosorbents

A monoclonal strain of the microscopic alga *Pseudopediastrum boryanum* (Turpin) E. Hegewald var. *longicorne* Reinsch (Chlorophyceae, Sphaeropleales, Hydrodictyaceae) marked as 03.080210 in the Laboratory of Algae Cultures of the Department of Phycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (Poland), was the subject of the present study (Figure 1).



**Figure 1.** 32-cell coenobium of *Pseudopediastrum boryanum* var. *longicorne* under a light microscope Nikon with 400× magnification.

The biomass of *Ps. boryanum* used for biosorption studies was obtained in a photobioreactor culture using optimal conditions identified at previous studies. L-S2T2 medium (pH  $7.0 \pm 0.1$ ) [14] was used, at  $22.0 \pm 2.0$  °C, light intensity of 4000 lx with a cycle of light (L)/dark (D) phase at a daily rhythm of 12L:12D [15]. During the light phase, air enriched with CO<sub>2</sub> at a concentration of 6% (v/v) was dosed into the culture system [16]. The biomass obtained of microalgae was separated from the medium by centrifugation at 4500 rpm for 15 min (Sorvall RC-5B Centrifuge, Kendro Laboratory Products, Asheville, NC, USA) and decanting. The biomass was then rinsed three times by centrifugation (4500 rpm, 15 min) with sterile deionized water. This treatment allows the removal of ions that can affect the course and efficiency of biosorption. The biomass of *Ps. boryanum* was dried at 60 °C, and then used to analyze the biosorption process.

### 2.2. Biosorption Studies

The experiment was carried out in 0.5 L Erlenmeyer flasks containing dry microalgae biomass (0.5–2.0 g/L), to which 250 mL of the solution with the selected Cr(VI) concentration (10; 30; 50; 80; 100 mg/L) and pH (2.0; 3.0; 4.0; 5.0; 6.0) was added. pH was determined with the use of a pH meter (Mettler Toledo MA 235, Switzerland). In general, chromium(III) compounds are amphoteric, so we analyzed the effect of pH 2–6 on the biosorption of an aqueous solution of Cr(VI) ions. In acidic solutions, two main forms of chromium(VI) prevail: chromate (CrO<sub>4</sub><sup>2-</sup>) and dichromate (Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup>), i.e., forms of chromium(VI) ion, which are common in wastewater from tanneries, dye houses and electroplating plants. Concentrations of chromium(VI) used in our research are based on the literature reports. Authors of those papers agree that classical methods for removal of heavy metals from industrial wastewater can generate secondary pollution and are too expensive. But most importantly, these methods are inefficient in treatment of wastewater, in which the initial concentration of metal

does not exceed 100 mg/L [6–8]. So we examined the concentrations of 10–100 mg/L, because for this range the biosorption process is considered an effective, economically justified and environmentally friendly method.

The whole system was mixed on a shaker (Pol-Eko LS 500, Wodzislaw Slaski, Poland) with a constant mixing speed of 250 rpm at  $22.0 \pm 2.0$  °C for 180 min. After biosorption, 10 mL of solution was withdrawn, the biosorbent was separated from the solution on the filter (Whatman, particle retention: 11  $\mu$ m), and then the concentration of Cr(VI) in the filtrate was determined.

We divided the biosorption into stages for which we determined the influence of process parameters (pH, biosorbent concentration, initial concentration of Cr(VI)) on the percentage of chromium removal from solution and the sorption capacity of *Ps. boryanum*. The effect of pH was analyzed at chromium concentration of 10 mg/L, the effect of biosorbent concentration was examined at pH 2.0 and chromium concentration of 10 mg/L. The effect of chromium concentration was tested at biosorbent concentration of 1 g/L and pH 2.0.

The experiment was repeated three times.

### 2.3. Hexavalent Chromium [Cr(VI)] Solutions

The solution with the appropriate concentration of Cr(VI) was prepared by dissolving in deionized water an appropriate amount of a certified chromate standard solution at a concentration of 1000 mg/L (Merck, Darmstadt, Germany), referenced to higher order standards (reference to SRM from NIST). Initial concentrations of Cr(VI) prepared from stock solution varied between 10 and 100 mg/L. The solutions were freshly prepared before the experiment.

### 2.4. Determination of the Cr(VI) Content in the Solutions

The concentration of chromium(VI) in the analyzed solutions was determined by spectrophotometric method using the Spectroquant<sup>®</sup> test (1.14758.0001 Cr, Merck, Germany). The analysis was carried out immediately after sampling. In this method, chromium(VI) is reduced using diphenylcarbazide in a solution containing a small amount of hydrogen fluoride. Unhydrated chromium(III) is an intermediate product of this reaction, which forms a red-purple complex with diphenylcarbazone (the oxidation product of diphenylcarbazide). The complex was determined on a UV-Vis spectrophotometer (Pharo 300, Merck, Germany).

### 2.5. Data Evaluation

The percentage of hexavalent chromium removal was calculated as follows [17]:

$$\text{Percentage of Cr(VI) removal} = \frac{C_0 - C}{C_0} \cdot 100 \quad (1)$$

where:

$C_0$ —is the initial concentration of chromium in the solution, mg/L.

$C$ —is concentration of chromium in the solution after biosorption, mg/L.

The sorption capacity ( $q$ ) determining the amount of chromium(VI) adsorbed in the dry matter of the biosorbent compared to the current concentration in aqueous solution was calculated as follows [18]:

$$q = \frac{(C_0 - C) \cdot V}{m} \quad (2)$$

where:

$C_0$ —is the initial concentration of chromium in the solution, mg/L.

$C$ — is concentration of chromium in the solution after biosorption, mg/L.

$V$ —is volume of solution that was in contact with the biosorbent, L.

$m$ —is the dry mass of the biosorbent that has been introduced into the solution, g.

## 2.6. Statistical Analysis

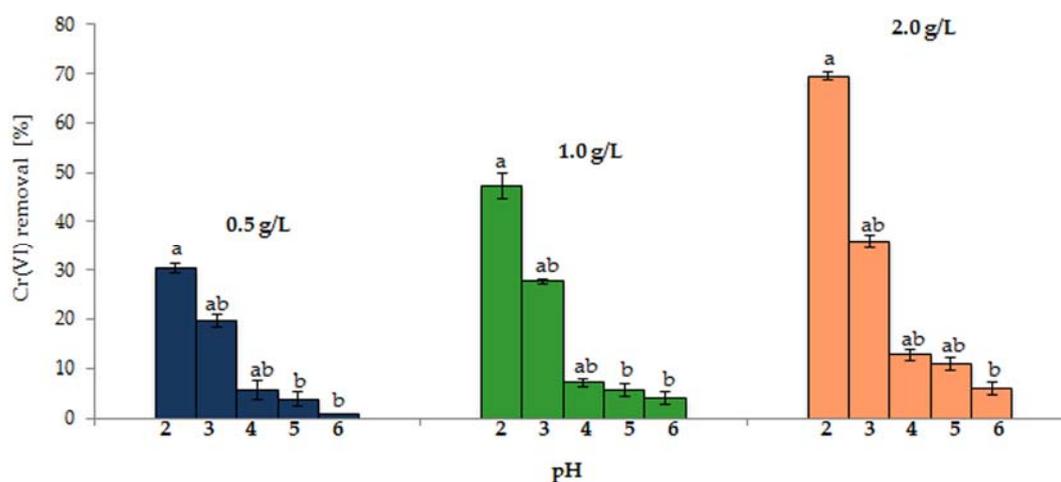
The results obtained were subjected to statistical analysis using a post-hoc: Tukey's honest significant difference (HSD) test of a one-way analysis of variance (ANOVA). In the post-hoc analysis interpretation, all the assumptions of the ANOVA test were considered (equal sample sizes, Levene variance homogeneity test, Shapiro-Wilk normality test). However, if they were not fulfilled, then such results were analyzed using Kruskal-Wallis test. The results are presented in the form of arithmetic mean  $\pm$  standard deviation. Differences between means at  $p < 0.05$  were considered statistically significant.

## 3. Results and Discussion

### 3.1. Effect of Initial Solution pH

pH is a parameter that has a significant impact on biosorption efficiency. The acidity of the solution affects both the chemical activity of the compounds in the solution and the degree of dissociation of functional groups located on the surface of the biosorbent [3–5].

In this work we analyzed the effect of pH in the 2.0–6.0 range on biosorption of 10 mg/L Cr(VI) using biomass of *Ps. boryanum* with increasing concentration: 0.5, 1.0 and 2.0 g/L (Figure 2). For all concentrations of the biosorbent, the highest efficiency of removing the metal from the solution was observed at pH 2.0–3.0. At pH greater than 4.0, the biosorption efficiency dropped significantly. The highest percentage of Cr(VI) removal (for biomass concentration of 2.0 g/L) was observed at pH 2.0 and the lowest one was at pH 6.0. Statistical analysis revealed that for the analyzed biomass concentrations (0.5–2.0 g/L) the efficiency of chromium elimination from the solution at pH 2.0 was statistically higher than that at pH 5.0 (except for biomass concentration of 2.0 g/L) and pH 6.0, but no differences were observed ( $p > 0.05$ ) in relation to pH 3.0 and 4.0 (Figure 2).



**Figure 2.** Influence of pH on the percentage of chromium(VI) removal from the solution by microalga *Pseudopediastrum boryanum* var. *longicorne*. Biosorption conditions: initial metal concentration 10 mg/L, biosorbent concentration 0.5, 1.0 and 2.0 g/L, temperature:  $22.0 \pm 1.0$  °C. Presence of the same letter index indicates the lack of statistically significant difference ( $p > 0.05$ ).

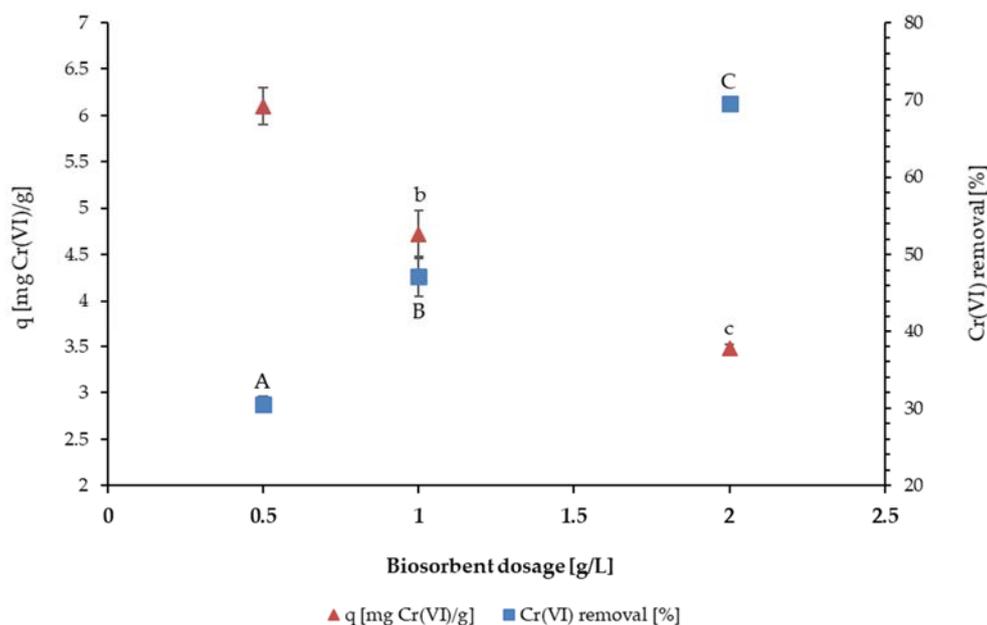
It was observed that, with the decreasing pH, the percentage of chromium removal increased. Thus, the reduction of pH increased the efficiency of Cr(VI) biosorption using the dead biomass of *Ps. boryanum*. This phenomenon is accounted for by the increase in both the protonation of the biomass function groups and the degree of dissociation of chromium ions. The functional groups are then

positively charged, so they can strongly bind electrostatically with negatively charged chromium ions ( $\text{HCrO}_4^-$ ,  $\text{Cr}_2\text{O}_7^{2-}$ ,  $\text{Cr}_4\text{O}_{13}^{2-}$ ) [18–20]. When the pH of the solution gradually increases, the functional groups are deprotonated. The resultant surface charge on the microalgae cell wall becomes negative and the biosorption decreases [18].

Our observations regarding the pH influence coincide with the results presented by other authors who studied the sorption properties of other microalgae. Ozer et al. [18] analyzed the biosorption of Cr(VI) by free and immobilized biomass of *Ps. boryanum* in the pH range 2.0–8.0. The highest degree of Cr(VI) removal, 47–97%, was obtained at pH 2.0 for the immobilized form (3 forms of immobilization of microalgae were used), while for the free form it was 20%. The best results for the chromium(VI) biosorption at pH 2 were also reported for many other microalgae, for example: *Sargassum muticum* [17], *Chlorella vulgaris*, *Scenedesmus obliquus*, *Synechocystis* sp. [21], *Sargassum* sp. [22], *Spirogyra* sp. [23], *Dunaliella* sp. [24] or *Oedogonium hatei* [25]. The initial concentration of chromium(VI) and biosorbent used in the cited work were much higher, which surely affected the sorption capacity. Moreover, Ozer et al. cultivated the biomass of microalgae under different conditions than ours, which could also influence the sorption capacity [6]. An important element distinguishing our work was also the determination of optimal biomass culture conditions for *Ps. boryanum* that we carried out in our earlier studies, which was also used in this work [14–16].

### 3.2. Effect of Algae Concentration

The effect of the *Ps. boryanum* dry matter concentration on chromium(VI) biosorption was analyzed at a metal concentration of 10 mg/L and pH 2.0. The results are shown in Figure 3. The percentage of chromium(VI) removal increased from 30% to 70% when the biosorbent concentration was increased from 0.5 g/L to 2.0 g/L. Ozer et al. [18] also investigated the effect of *Ps. boryanum* dry matter concentration on the biosorption of chromium(VI) ions, but for a higher initial metal concentration (100 mg/L). The biomass concentrations used by them were also much higher than those used in our studies. The authors reported an increase in the percentage of chromium removal from 21% to 51% when the biosorbent concentration was increased from 2 g/L to 10 g/L.



**Figure 3.** Effect of *Pseudopediastrum boryanum* var. *longicorne* concentration on chromium(VI) biosorption. Process conditions: initial metal concentration 10 mg/L; pH 2.0; temperature  $22.0 \pm 1.0$  °C. Difference in letter index indicates a statistically significant difference ( $p < 0.05$ ).

Based on the test results obtained it can be concluded that the increasing biomass concentration strongly increases the percentage of metal removal, but the sorption capacity of the microalgae itself decreases ( $q$ ). The highest value of  $q$  was obtained for the lowest biomass concentration in the solution (0.5 g/L). This sorption capacity was twice as large as the sorption capacity obtained for a four times higher concentration of biosorbent (2.0 g/L). Statistical analysis of the percentage of chromium(VI) removal and sorbent capacity of microalgae revealed significant differences ( $p < 0.05$ ) between all tested concentrations of *Ps. boryanum* (Figure 3).

The percentage of chromium removal is highly dependent on the initial concentration of both the metal and the biosorbent. The increase in biomass concentration enhances the metal binding, which results from the increased sorption surface ensuring a greater number of binding sites. At the same time the sorption capacity decreased because of a less use of functional groups [17,25].

### 3.3. Effect of Initial Cr(VI) Concentration

Another factor affecting the effectiveness of biosorption is the sorbate concentration. The initial metal concentration generates a significant driving force to overcome the resistance occurring between the water and solid phases [17,24].

Table 1 shows the effect of the initial concentration of chromium(VI) at pH 2 and the microalga dry matter concentration of 1.0 g/L on the efficiency of metal removal and sorption capacity. It was observed that with the increase of the initial metal concentration, the sorption capacity increased, and the percentage of metal removal decreased. Among the initial concentrations analyzed, a statistically significant higher percentage of metal removal was observed for the lowest initial concentration of Cr(VI) of 10 mg/L. At this concentration, a significantly lower sorption capacity of the *Ps. boryanum* biomass was obtained. There were no statistically significant differences in the metal removal efficiency either between initial Cr(VI) concentration of 30 and 50 mg/L, or 80 and 100 mg/L. We found no significant differences ( $p > 0.05$ ) in the sorption capacity of microalgae between initial concentration Cr(VI) 50 and 80 mg/L (Table 1).

**Table 1.** Sorption capacity of *Pseudopediastrum boryanum* var. *longicorne* ( $q$ ) and the percentage of chromium(VI) removal from the solution depending on its initial concentration ( $C_0$ ). Biosorption conditions: biosorbent concentration 1.0 g/L; pH 2.0; temperature  $22.0 \pm 1.0$  °C.

$C_0$ (mg/L)	Cr(VI) Removal (%)	$q$ (mg/g)
10	$47.70 \pm 2.44$ <sup>a</sup>	$4.89 \pm 0.01$ <sup>A</sup>
30	$26.90 \pm 1.74$ <sup>b</sup>	$8.07 \pm 0.52$ <sup>B</sup>
50	$25.10 \pm 1.64$ <sup>b</sup>	$12.57 \pm 0.82$ <sup>C</sup>
80	$16.24 \pm 0.47$ <sup>c</sup>	$13.25 \pm 0.38$ <sup>D</sup>
100	$16.00 \pm 0.97$ <sup>c</sup>	$16.68 \pm 0.97$ <sup>D</sup>

Presence of the same letter index for the analyzed Cr(VI) concentrations at the percentage of metal removal (a, b, c) and the sorption capacity (A, B, C) indicates the lack of statistically significant difference ( $p > 0.05$ ).

The amount of bound sorbate per unit weight of the biosorbent increased with the increase of the initial metal concentration. The observed decrease in the percentage of Cr(VI) removal may result from the deteriorating sorption capacity of biomass at increasing metal concentrations, as the “saturation” of active sites increases. Similar results were presented by Bermúdez et al., Gupta et al. and Yu et al. [17,25,26]. Yu et al. [26] observed that at low initial metal concentrations, the biosorption does not actually depend on the initial sorbate concentration, because the availability of active sites on the biosorbent surface was sufficient. At higher metal concentrations, the availability of sites on the biosorbent surface decreases and then the effectiveness of biosorption depends on initial metal concentration.

#### 4. Conclusions

We demonstrated the possibility of effective use of dead biomass of *Pseudopediastrum boryanum* var. *longicorne* for the biosorption of chromium(VI) ions in aqueous solutions. The highest percentage of metal removal (70%) was obtained for the initial Cr(VI) concentration of 10 mg/L and the biomass concentration of 2.0 g/L. At this chromium concentration, however, the sorption capacity of microalgae was the lowest (3.5 mg/g).

With the increase in the initial concentration of Cr(VI), the sorption capacity increased and the percentage of chromium removal decreased.

It would be advisable to extend the research on the suitability of algae for removal of heavy metals from the aqueous environment by analytical methods. These methods would allow us to broaden the current state of knowledge on chromium(VI) binding mechanisms by the biomass of microalgae (including *Ps. boryanum*). We agree with other authors that in order to explain difficult issues related to the biosorption process one should consider the application of new research methods, in particular FTIR (Fourier Transform Infrared Spectroscopy), SEM (Scanning Electron Microscopy) and X-ray based Photoelectron Spectroscopy (XPS). This would give an insight into the process mechanism and the properties of the biosorbent, such as its specific surface, porosity or pore size and distribution [22,27].

**Author Contributions:** M.S. and G.K. (scientific supervisor at all stages of research and preparation of the manuscript) designed, performed the experiments, contributed to data analysis and paper writing.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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