PHYTOEXTRACTION FOR GREEN TECHNOLOGIES: EVALUATION OF THE ABILITY OF CHLORELLA TO ABSORB SIGMA AND CADMIUM IN SEPARATE AND COMBINED PRESENCE

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Abstract

The article is devoted to the study of bioaccumulation of cadmium and lead ions by the unicellular alga Chlorella vulgaris to assess the potential of this species for phytoextraction of metals from wastewater. The algae were grown in Bold's medium supplemented with lead and cadmium nitrates at concentrations of 0.2 g/L, 0.1 g/L, and 0.01 g/L. Experiments were conducted with both individual and combined metal additions . It was shown that at the highest concentrations, metal accumulation occurs at a low rate, due to the drastic suppression of plant life processes by toxic metals and the inability to accumulate due to the cessation of division and growth. At lower concentrations, intense metal accumulation is observed, which is significantly enhanced by the simultaneous presence of both metals in the nutrient solution.

Keywords : heavy metals, bioaccumulation , chlorella, biotechnology, phytoextraction

I. Introduction

Heavy metals are among the most common and dangerous pollutants : they are stable in the environment and can migrate through food chains, are not decomposed by microflora, and can interact with organic and mineral substances. [13] The danger of metal pollution is primarily due to their ability to form stable bonds with sulfhydryl groups of proteins, causing their irreversible denaturation. Lead, copper, zinc, cadmium, and other metals exhibit this effect. [12]

It is generally accepted that the main source of heavy metal pollution is the mining and processing industry, but a more detailed assessment reveals that in many regions the main source of heavy metal ions in the water of natural reservoirs are natural sources - ore deposits, volcanic activity. [3,15] The transfer of metal ions from other regions with sediments can also be of great importance. [9]

One of the most toxic metals is mercury. The danger of this element is due to its high cumulative properties . Organomercury compounds are particularly toxic. [8,7]

In terms of their importance to plants, heavy metals are usually divided into essential (participating in physiological processes and required in certain doses) and non-essential (having no physiological significance). [9] The accumulation of the latter generally occurs at a lower rate.

Metal accumulation is not always associated with the transport of ions into the cell through the cytoplasmic membrane. Often, large quantities of ions can be firmly retained by the cell wall and surface membrane apparatus, but for intensive metal extraction from wastewater, plant growth processes must occur. [2]

Lead in large quantities causes a sharp inhibition of growth and development, a slowdown in photosynthesis, energy metabolism, water absorption, cell division and differentiation. [9]

Copper ions are necessary in small doses, but at excessive concentrations they cause the oxidation of vitamin C and disruption of the transmembrane transfer of protons and electrons, and suppress the synthesis of chlorophyll. [8,11]

Cadmium is also highly toxic, its salts belong to hazard class I , it was previously considered a rare metal, but recently more and more data has appeared about its presence both in natural ecosystems and in industrial waste. [15]

At the same time, cations of many metals are necessary for plants in small doses, since they

- are coenzymes of many enzymes
- are part of some secondary substances
- participate in the work of electron transport chains (copper, iron)
- participate in the formation of the unique spatial structure of proteins ("zinc fingers")
- participate in the photolysis of water during photosynthesis (manganese)
- participate in nitrogen metabolism (molybdenum, manganese, iron). [10]

The fact that plants need metals explains their high capacity for metal accumulation and, at the same time, their high resistance (compared to animals) to the accumulation of large amounts of metals in tissues. [9] Plants have more reliable and diverse mechanisms for detoxifying metal ions. Thanks to this ability, we can use plants to extract metal ions from wastewater and heavily polluted natural bodies of water.

Essential metals are generally taken up by plants more intensively than non-essential metals , but some algae show the opposite pattern; in particular, cadmium can accumulate more intensively than lead.[1]

Algae have a high absorption capacity and can accumulate not only essential metals, which makes them promising for the phytoextraction of metals from wastewater. [6]

However, the process of accumulation by algae is often difficult to predict, in particular, when comparing the content of metals in different types of algae over the years, the most significant differences were observed in the same species in different years, while interspecific differences within one year were smaller. [5]

When several heavy metals are present in water at significant concentrations, the accumulation results are difficult to predict. [4] In particular, for Cystoseira , it has been shown that there is a negative correlation between lead and mercury levels, but a positive correlation between lead levels and cadmium and chromium.

II . Methods

Chlorella culture was used for the study. vulgaris), a single-celled green alga that reproduces only asexually.[14]

Bold's medium supplemented with lead and cadmium nitrates at varying doses in a KS-200 climate controller at a constant temperature of 25 ˚C and 24-hour full illumination for 7 days. 2-liter plastic containers were used for cultivation. After cultivation, the algal mass was separated in an Eppendorf centrifuge. MiniSpin at 10,000 rpm for 5 minutes. Chlorella was grown on Bold's medium without added lead and cadmium nitrates as a control. Nutrient solutions were prepared with distilled water.

The resulting algal mass was ached by heating in quartz beakers with the addition of concentrated nitric acid and hydrogen peroxide. The resulting zone was dissolved in 9 ml of bidistilled water and 1 ml of concentrated formic acid. The resulting aliquot was used to measure cadmium and lead concentrations on a TA - lab inversion potentiometric analyzer . Formic acid was used as the background electrolyte.

III . Results

The results of measuring the content of lead and cadmium in the algal mass are shown in Table 1.

Table 1. Mass concentration of lead and cadmium in algal mass when present separately and together in the environment.

Lead content in algal mass in the isolated presence of lead in the environment				
Concentration of the corresponding metal in the environment				
0.2 g / l	0.1 g / l	0.01 g / l	control	notes
Lead content in algal mass in the isolated presence of lead in the environment, in mg/kg dry mass				
318.20 ± 18.30	378.05 ± 29.52	470.20 ± 51.20	95.50 ± 8.50	
Cadmium content in algal mass in the isolated presence of cadmium in the environment, in mg/kg dry mass				
209.5 ± 15.30	765.18 ± 63.20	-	84.00 ± 6.20	In an environment with a cadmium concentration of 0.01, measurements were not carried out.
The content of cadmium and lead in algal mass in the presence of both				
Lead content				
44.30 ± 3.50	365.50 ± 27.20	2767.50 ± 192.20	95.50 ± 8.50	
Cadmium content				
68.00 ± 5.20	437.00 ± 3.20	3356.50 ± 272.20	84.00 ± 6.20	

The study results showed that at high concentrations, metal accumulation occurs less intensely than at lower concentrations. This is most likely due to the fact that at high concentrations, membrane transport proteins are rapidly damaged, many proteins are denatured, and cell viability is reduced to the point that cells almost cease dividing, suppressing all vital processes. This hypothesis is indirectly supported by the fact that the green color density of the medium after cultivation was significantly lower at high concentrations, and separation of the algal mass was difficult. Only prolonged centrifugation at 10,000 rpm produced a noticeable sediment of the algal mass; other centrifugation modes yielded no results .

The mass concentrations of lead and cadmium in the algal mass are comparable in magnitude, that is, it is impossible to assert that there is a competitive absorption of these two ions by chlorella cells.

The detection of cadmium and lead in the control is apparently due to the presence of traces of these metals in the reagents used to prepare pure Bold's medium .

The extremely high concentrations of lead and cadmium in the algal mass are noteworthy, even when they are present together in a solution with the lowest concentration. The metal content is significantly higher than in solutions with the same concentration when each metal is present separately. Thus, a certain synergistic effect occurs: if the concentration of one metal is sufficiently high, the accumulation of the other metal is enhanced.

When comparing the absolute concentrations of the two metals, opposite differences are observed: when present in isolation, lead content at a concentration of 0.2 g/L is significantly higher than cadmium, while at a concentration of 0.1 g/L, the opposite is true. When the two ions are present together in the medium, the mass concentrations of the metals in the algal mass are close to each other.

In the control, that is, with the simultaneous presence of metals in trace quantities, the mass concentrations of lead and cadmium in the algal mass are closest to each other.

Overall, the metal content of the algae mass is high enough to be used as a raw material for the production of additional metals, which would not only ensure wastewater treatment but also make the production cycle more complete and efficient.

IV . Discussion

At first glance, the potential for using chlorella in wastewater treatment appears to be quite broad. High mass concentrations of metals, especially when present together, fall within the hyperaccumulation range , allowing the algal mass to be used for additional product production. However, in real-world production settings, the presence of lead in the discharge is not always accompanied by equally significant concentrations of cadmium. Certainly, if both metals are present in the wastewater, phytoextraction using chlorella could be the optimal solution. However, if they are present in the discharge alone, this seems impractical. Artificially adding a second metal to enhance accumulation can lead to secondary pollution of the wastewater.

The concentrations chosen for the study are quite high and are relatively rarely detected under real-world production conditions. Most often, lead and cadmium concentrations are only a few milligrams per liter of wastewater. The patterns observed in our experiment suggest that, at low metal concentrations in the discharge, phytoextraction of metals using chlorella would be most effective and safe, while physical and chemical methods (sedimentation, flotation) are more appropriate for higher concentrations. If metal concentrations in the discharge are sufficiently high, pre-dilution of the wastewater may be appropriate for treatment purposes.

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