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The application of multielemental analysis in the elaboration of technology of mineral feed additives based on *Lemna minor* biomass

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Abstract

Supplementation of microelements (Mn, Zn, Cu, Cr) to livestock diet is of particular concern. There are various mineral feed additives available on the market. The most frequently used are inorganic feed additives, characterized with low bioavailability and high toxicity. Also, organic feed additives are used, including amino acids chelates, in which bioavailability was greatly improved and toxicity reduced. The problem is high price of these products. Therefore, there is the need to search for new biological mineral feed additives with designed composition, that would be characterized with high bioavailability, low toxicity, low cost and that would also possess a nutritional value. Such a possibility offers biological materials. It was found that biomaterials have metal-binding capabilities. Metal ions may be bound to biomass via either biosorption or bioaccumulation process.

When elaborating technology of the production of feed additives, containing simultaneously various elements, it is necessary to use the tool of multielemental analysis in order to simultaneously analyze the content of Mn, Zn, Cu, Cr. In the present work, ICP-OES multielemental analysis was used to investigate the process of production of mineral feed additives based on the biomass of aquatic plant *Lemna minor*. The effectiveness of the processes of biosorption and bioaccumulation of microelements by an aquatic plant *L. minor* was studied. The mechanism of the process as well as equilibrium was investigated with the use of multielemental analysis by ICP-OES Vista-MPX instrument from Varian (Australia).

Keywords: Biosorption; Bioaccumulation; *Lemna minor*; Microelements; Mineral feed additives

1. Introduction

Supplementation of microelements to natural feeds of livestock has recently become particularly significant. Microelements are supplied in the form of mineral feed additives, the most commonly in the form of inorganic salts. This form is however characterized with low bioavailability to animals and therefore microelements are of transit character. Also, inorganic salts ingested by animals may cause adverse health effects. Therefore, there is the need to elaborate such mineral feed additives that would supply minerals in non-toxic form and the mostly bioavailable to animals. This can be achieved when the minerals are supplied in the form possibly similar to the natural. It would be also advantageous if these products would have a nutritional value. Recently, amino acid chelates have been used in supplementation of livestock diet. The problem of bioavailability and toxicity has been greatly reduced, however these products are still expensive (the cost is 10 times higher than inorganic salts). The problem of high cost could be overcome by binding microelements to readily available biomass of, i.e. aquatic plants [1]. It is well known that various types of biomaterials possess metal-binding properties. This topic has been widely discussed in the literature [2–5]. These capabilities however are employed in industrial wastewater treatment processes in which biomass is mainly used to remove heavy metal ions from solutions. In bioremoval methods, two distinctive processes can be distinguished: biosorption (the process performed by not-living biomass) and bioaccumulation (the process carried out by living cells). The same processes, although at different process conditions may be used to bind metals. The aim would be not to remove cations, but to incorporate them into biological material. Therefore, in bioremoval processes the aim would be to

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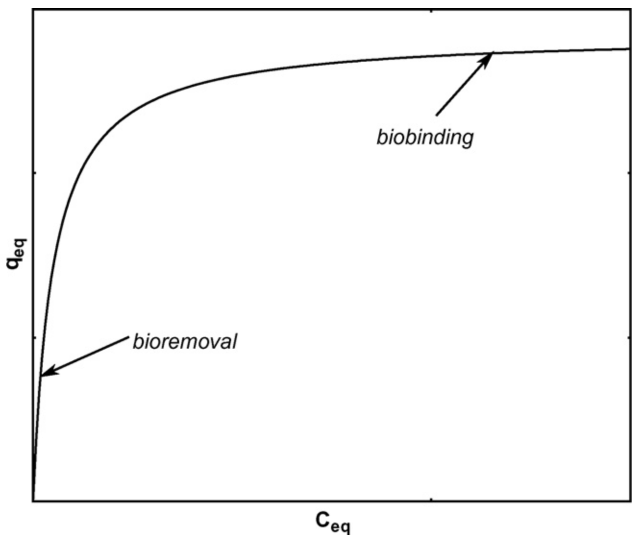

Fig. 1. Differences in bioremoval and biobinding equilibrium.

reach possibly low equilibrium metal ions concentration and in ‘biobinding’ processes the aim would be to reach possibly high equilibrium metal-binding capacity (Fig. 1).

In the present work, microelements, important in livestock feeding were bound to the biomass of commonly worldwide available freshwater floating aquatic plant *Lemna minor* (duckweed) [6]. *L. minor* was found to be cold tolerant, it grows rapidly and is easy to harvest. The plant is used as a valuable feed [7]. Since *L. minor* is an organism that can grow in highly polluted environments, it was applied in wastewater treatment processes to remove either nutrients from municipal wastewaters (so-called constructed wetlands) [8–11] or to bind heavy metals from industrial effluents, since it possesses a capability to concentrate metal ions from solutions, several fold (1000–100,000 times) [12–16]. The produced biomass was used either as feed for poultry [8,17,18], fish [19] and cattle (as soybean substitute) [6] or as fertilizer [20,21]. It was found that the supplementation of *L. minor* to hens diet resulted in increased yolk pigmentation [17,22]. Also, *L. minor* can be considered as rich in protein (30 mass%), minerals and vitamins animals feed [8,17] and poor in fiber that was considered advantageous [9].

The processes of biobinding in real systems occur in the presence of various ions. Also, in the case of technology of biological mineral feed additives, binding of cations of feeding significance (Mn, Cu, Zn, Cr) could be considered as multi-ion biosorption system. To study this process, simultaneous multielemental analysis is indispensable. Such a possibility offer ICP techniques, commonly used in biosorption experiments, in particular in studies on multi-metal systems [23–27].

The aim of the present work was to investigate the applicability of an aquatic plant *L. minor* in the production of mineral feed additives in the processes of biosorption and bioaccumulation. The experiments were performed in a multi-metal system, in the presence of minerals of feeding significance: Mn, Cu, Zn, Cr. Inductively coupled plasma optical emission spectroscopy (instrument Vista-MPX, Varian, Australia) was used in this study.

2. Materials and methods

2.1. Characterization of an organism

L. minor (duckweed) was used as research material in the experiments on biosorption and bioaccumulation of microelements from aqueous solutions. *L. minor* was obtained from a drainage ditch in Wrocław (Poland). The plant was washed and rinsed with demineralized water three times for 10 min.

The multielemental analysis of biomass was performed with the use of ICP-OES technique. Before the analysis, the samples were digested in the microwave system. The biomass underwent also macroelemental analysis (CHNS) with the use of Eurovector EA 3000 (Milan, Italy) instrument. Cation-exchange capacity was determined with potentiometric titration according to the procedure described previously [28].

2.2. Biosorption experiments

In biosorption experiments, dry biomass was used to assure that cells were not alive. The biomass was dried at 60 °C for 5 days and exposed to microelements solution for 2 h (kinetic experiments showed that the equilibrium was reached after few minutes) at various initial concentrations of microelements in the ratio (mm^{-1}) Cr:Mn:Zn:Cu (1:1:1:0.1). The following inorganic salts were used to prepare the solutions: $Cr(NO_3)_3 \cdot 9H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ (from POCH, Gliwice). The proportions and the salts used, were in conformance with animal feeding standards [29,30]. Biosorption tests were performed in water bath shaker at ambient temperature and pH 5. The initial concentration of microelements solution (C_0) was determined by ICP-OES technique. After biosorption was finished, the suspension was filtered through no. 2 paper filters and was analyzed for the content of microelements with ICP-OES (C_{eq}).

2.3. Bioaccumulation experiments

In bioaccumulation experiments living cells were used. Fresh *L. minor* was used in these tests. Two types of bioaccumulation experiments were performed: with the use of non-growing cells (in the solution of microelements in demineralized water) and with the use of growing cells (in the solution of microelements in boiled tap water). The contact time was also 2 h. The aim was to compare the efficiency of three processes: biosorption, bioaccumulation without and with growth. Bioaccumulation tests were performed in water bath shaker at ambient temperature, with natural lighting (light intensity ca. 400 lx). The solution (before and after bioaccumulation) was analyzed for the content of microelements with ICP-OES method.

2.4. Analytical methods

Multielemental analysis was performed with the use of ICP-OES measurements by Vista MPX instrument with charge coupled device (CCD) simultaneous detection systems (Varian Inc., Victoria, Australia). Demineralized water was prepared using