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Original Article

Identification of Free Ammino Acids in *Saccharomyces cerevisiae* Living Cells after Heavy Metals Ions Biosorbtion

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Abstract

A lot of microorganisms can sorb heavy metals (Zn, Cu, Pb, Cd, Ni, Cr) from aqueous solutions to varying extent. Biosorption depends on parameters such as pH, metal ion and biomass concentrations, physical or chemical pretreatment of biomass. Yeasts of genera *Saccharomyces* are efficient biosorbents for heavy metal ions. The aim of this study was to identify if the free amino acids present in brewer yeast are involved in metal biosorption due to their capacity to coordinate metal ions. As biosorbent was used brewer's yeast living cells type *Saccharomices cerevisae*. Zinc, lead and copper ions solutions of 1mg/L concentrations were prepared using their salts. The experiments were conducted at pH=5.5, which is the most appropriate for amino acids extraction. The amino acids were identified by HPLC-DAD/-ESI-MS chromatography. The experiments were conducted by mixing metals solution with yeast and shaken at a constant speed of 120 rpm at 20^0C for 120 minute. The samples were centrifugated at 3000 rpm for 15 minutes and the supernantant was analysed for amino acids identification. The HPLC analysis was performed on a Agilent 1200 system equipped with a binary pump delivery system LC-20 AT, a degasser DGU-20 A3, diode array SPD-M20 A, UV–VIS detector (DAD). Amino acids were identified using an EEZ:Faast Kit for free amino acids, The amino acids identified by HPLC method were glycine (GLY), glutamic acid (GLU), leucine (LEU), isoleucine (ILE), ornithine (ORN), lysine (LYS), histidine (HIS), homophenylalanine (HPHE), tyrosine (TYR), glutamine (GLN), alanine (ALA), valine (VAL), triptophan (TRP), phenylalanine (PHE), α-aminobutyric acid (ABA), asparagines (ASN) in control brewer yeast (before biosorption) and their profile differs according with the metal ions types. According with the peaks area there are differences in the presence of the amino acids due to the possible coordination with metal ions.

Keywords: brewer yeast, amino acids, biosorption, heavy metals.

1. Introduction

In recent years the process of accumulation of heavy metal ions by microorganisms was intensively studied. Microorganisms like bacteria, yeast and fungi, as well as algae can accumulate large amounts of heavy metal ions. Biosorption is considered to be a fast physical or chemical process. The biosorption rate depends on the type of the process.

According to literature, biosorption can be divided into two main processes: adsorption of the ions on cell surface and bioaccumulation within the cells [4, 10].

The bioaccumulation of heavy metals is closely connected with their toxicity, microorganism's metabolism and growth. The values of the critical and threshold concentrations depend on the type of the microorganism and the properties of the metal. The metal toxicity is affected also by the form in which they exist in the medium, the amount of cells in the medium and the stage of growth of the cell culture.

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Biosorption is a process with some unique characteristics. It can effectively sequester dissolved metals from very dilute complex solutions with high efficiency. This makes biosorption an ideal candidate for the treatment of high volume low concentration complex waste-waters. The selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells is defined as biosorption. It refers to physicochemical mechanisms of inactive (i.e. non-metabolic) metal uptake by microbial biomass [9]. Metal sequestering by different parts of the cell can occur via various processes: complexation, chelation, coordination, ion exchange, precipitation, reduction [1, 11]. Immobilization may be the result of more than one mechanism, for example, metal complexation may be followed by metal reduction or metal precipitation. These biopolymers, constituents of the cell wall and the other parts of the cell possess functional groups that have a significant potential for metal binding [3].

Metal uptake by non-living cells is mainly a passive biosorption and consists in an adsorption of metal ions to the cells surface by interactions between metal and functional groups displayed on the surface of the cells [6].

Brewer's yeast is made from a one-celled fungus called *Saccharomyces cerevisiae* and is used to make beer. It also can be grown to make nutritional supplements. The yeast biomass has been successfully used as biosorbent for removal of Ag, Au, Cd, Co, Cr, Cu, Ni, Pb, U, Th and Zn from aqueous solution. Yeasts of genera *Saccharomyces, Candida, Pichia* are efficient biosorbents for heavy metal ions. A number of literatures have proved that *S. cerevisiae* can remove toxic metals, recover precious metals and clean radionuclides from aqueous solutions to various extents [7, 8].

2. Material and Method

Saccharomyces cerevisae biomass was supplied as a by-product from industrial ethanol production. Prior use as a biosorbent, the biomass was pretreated in order to remove fine particles and to displace any metals already bound to the sorption sites. The waste biomass as washed with deionized water by stirring followed by centrifugation at 3000 rpm for 20 minutes.

The supernatant was discarded and the pellet was reslurried in deionized water. The procedure was repeated for three times until the supernatant was clear.

Metals solution were prepared using the mixture of their salts $CdCl_2$, $NiCl_2 \cdot 6H_2O$, $HgCl_2$ of analytical reagent grade. The concentrations of metals ions established were 1 mg L^{-1} for Cd^{2+}, Ni^{2+}, Hg^{2+} and were obtained by dissolving the appropriate salts in deionized water.

Experimental procedure:

Metal ion binding experiments were performed by incubation of 10 ml biomass with 10 ml of metals ions-containing solution in 125-ml Erlenmeyer flask on an orbital rotary shaker at 120 rpm for 120 minutes. The experiment was conducted at pH value 5.5 and was established by adjusting it with HCl 0.1M or NaOH 0.1M solutions.

For metal ions biosorption the pH is one of the most important environmental factor. Experiments could not be performed at higher pH value due to the possibility of the metal ions precipitation, especially lead ions.

In order to identify the free amino acids from biomass by HPLC chromatography the samples were centrifuged at 3000 rpm for 15 minutes and the supernatant was analysed.

HPLC analysis was performed on a Agilent 1200 system equipped with a binary pump delivery system LC-20 AT (Prominence), a degasser DGU-20 A3 (Prominence), diode array SPD-M20 A, UV–VIS detector (DAD). Amino acids (100 µl) from control brewer's yeast and samples provided after biosorption of metal ions were identified using an EZ:Faast Kit for free amino acids, provided by Phenomenex (USA). The results are presented in the next table and figures.

3. Results and Discussions

The absorption mechanisms of metals are different and depend on cellular metabolism. The aim of this study was to establish the amino acids from brewer yeast living cells involvement in metal ions biosorption, as it is known that amino and carboxyl groups could be responsible for complexation depending on pH intervals and metal:ligand ratio. In the HPLC chromatograms these involvement could be observed by changing the absorption intensities between amino acids from brewer yeast control and brewer yeast charged with metal ions by biosorption.

The standard solutions of amino acids used for analysis were selected according with the literature data regarding the presence of these compounds in brewer yeast [7]. The main amino acids identified by HPLC method were glycine (Gly), asparagine (ASN), glutamic acid (GLU), leucine (LEU), isoleucine (ILE), ornithine (ORN), lysine (LYS), histidine (HYS), homophenylalanine (HPHE), tyrosine (TYR), glutamine GLN), alanine (ALA), valine (VAL), triptophan (TRP), phenylalanine (PHE), α-aminobutyric acid (ABA).

The separation and identification of amino acids were performed on brewer yeast uncharged

with metal ions (control) and brewer yeast charged with metal ions after their biosorption.

The results are presented in the next table and figures. The profiles of chromatograms are different between control brewer yeast and samples charged with metal ions due to the absorption of zinc, lead and copper ions after incubation.

It is supposed that amino acids from brewer yeast has the capacity to coordinate these metal ions and the metal complexes resulted have different retention times and peaks area than free amino acids as it can be seen in the Table 1.

From the data above it can be presumed that amino acids are involved in metal coordination due to their capacity of metal binding through carboxyl and amino groups. Another explanation regarding the changes in amino acids profiles and content is that that it could be affected by autolysis and fermentation conditions like time, temperature, pH, moisture content [2].

Table 1. Amino acids identified in brewer yeast uncharged (control) and charged with metal ions.

Nr crt	Aa. control m/z	Aa. control area	Aa.+ Zn^{2+} (area)	Aa.+ Pb^{2+} (area)	Aa.+ Cu^{2+} (area)
1	GLN/275	10704200	-	-	-
2	ASN/243	1,11E+08	-	17414728	24690218
3	GLY/204	2,93E+08	34229200	62596676	-
4	ALA/218	4,8E+08	59079340	76963752	2,02E+08
5	ORN/347	6,34E+08	18151634	4,06E+08	2,96E+08
6	PRO/244	8,99E+08	2,16E+08	25244664	5,98E+08
7	HIS/370	3,2E+08	7473274	55393300	-
8	VAL/246	1,29E+08	-	-	-
9	GLU/318	6,34E+08	31089642	-	59059104
10	TRP/333	76783688	-	23041980	25558244
11	LEU/260	3,63E+08	16612316	38825024	87357976
12	PHE/294	2,4E+08	-	47576664	-
13	ILE/260	68063784	6770684	-	25713988
14	ABA/232	23497502	-	-	-
15	HPHE/189	2,83E+08	26532408	94582776	2,33E+08
16	TYR/396	7,83E+08	37071216	1,21E+08	2,67E+08

The metal binding capacity differs from an amino acids to other: GLN, VAL and ABA which are present in uncharged brewer yeast sample disappears in the chromatograms of charged brewer's yeast with all metal ions; ASN, VAL, PHE, ABA and TRP have good affinity for zinc ions. For the lead ions good affinity present GLN, HIS, VAL, ILE and ABA. The next amino acids: GLN, GLY, HIS, VAL, PHE and ABA have affinity for copper ions. The lack of amino acids in charged brewer yeast samples could be attributed to the complexation with metal ions. The others amino acids present different affinity for metal ions according with their molecular structure and the capacity to coordinate the metal ions added [5, 12].

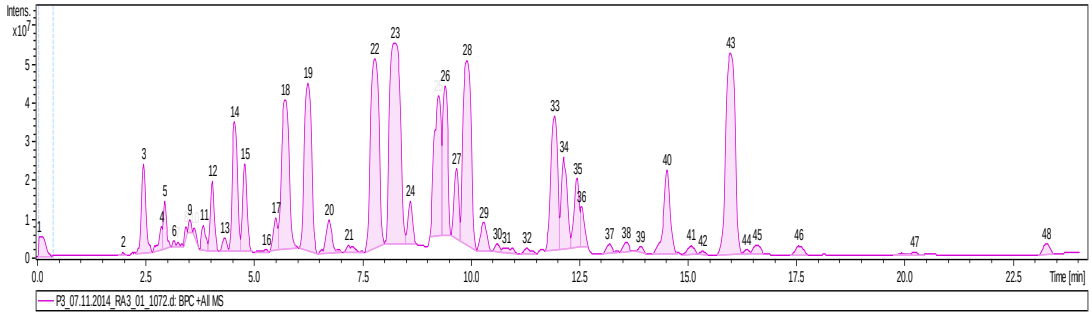

Figure 2. 1. HPLC chromatogram of amino acids extracted from control brewer's yeast

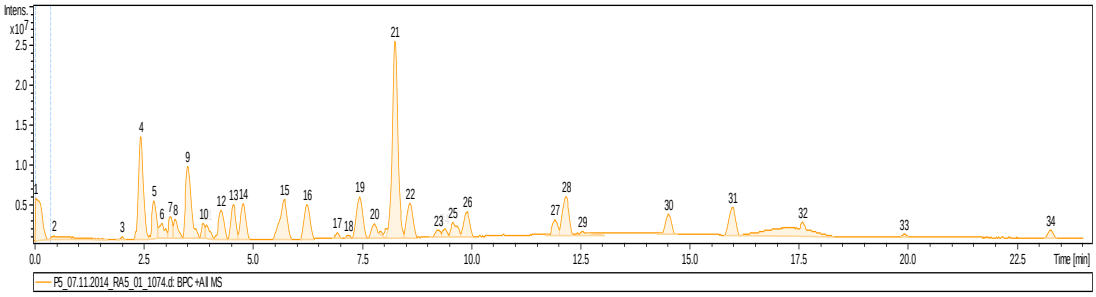

Figure 2. HPLC chromatogram of amino acids extracted from brewer's yeast charged with zinc ions

4. Conclusions

This study represents a first step in elucidation of the absorption mechanism of metal ions from aqueous solution by brewer yeast. Thus, the preliminary results lead to the conclusion that free amino acids, above other constituents from yeast, could be involved in metal biosorption. This process is strongly dependent on experimental conditions, especially on pH level, as it is known that each metal ions have characteristic pH limits for coordination with different organic ligands.

The experiment reveal the presence of 16 free amino acids in brewer's yeast and significant differences in their profile and content after biosorption of zinc, lead and copper, which could be explained by the coordination of these ions by carboxyl and amine groups of the ligands.

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