Identification of free Amino Acids in Brewer's Yeast after Heavy Metals Biosorption

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Abstract. 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 non-living brewer's yeast type *Saccharomices cerevisae* at 0.5% yeast dose. Copper, lead and zinc solution of 1mg/L concentrations were prepared using their salts. The experiments were conducted at pH=6, 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 centrifuged at 2500 rpm for 15 minute and the pellet were analysed for amino acids identification. The amino acids extraction from pellets was performed using HCl 0,05M/deionized water (v/v) 1/1. 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, glutamic acid, leucine, isoleucine, ornithine, lysine, histidine, homophenylalanine, tyrosine, glutamine 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 copper, lead and zinc ions.

Keywords: Brewer yeast, amino acids, biosorption, heavy metals

1 Introduction

Biosorption can be defined as the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells. 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 (Gadd, 1993; Wang, 2006). The selective sequestering of metal soluble species that result in the immobilization of the metals by microbial

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cells is defined as biosorption. It refers to physicochemical mechanisms of inactive (i.e. non-metabolic) metal uptake by microbial biomass (Volesky 2001). Metal sequestering by different parts of the cell can occur via various processes: complexation, chelation, coordination, ion exchange, precipitation, reduction (Wang 2009, Alluri, 2007). 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 (Dakiky, 2002).

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 (Paknikar, 2003).

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 (Podgorskii, 2004; Tálos, 2009)

2 Materials and Methods

Saccharomices cerevisae biomass was supplied as a lyophilized 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 $CuSO_4 \cdot 5H_2O$, $PbNO_3 \cdot 2H_2O$, $ZnSO_4$ of analytical reagent grade. The concentrations of metals ions established were 1 mg/L for Cu^{2+}, Zn^{2+}, Pb^{2+} and were obtained by dissolving the appropriate salts in deionized water.

Experimental procedure:

Metal ion binding experiments were performed by incubation of 25 mg biomass (dry weight) with 50 ml mixture 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=6 and was established by adjusting it with HCl 0.1M or NaOH 0.1M solutions. In order to identify the free amino acids from biomass by HPLC chromatography the samples were centrifuged at 2500 rpm for 15 minutes, the supernatant was discarded and the pellets were analysed.

The free amino acids were extracted from the pellets using two types of solutions: HCl 0,05M/deionized water (v/v) 1/1. 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. In non-living brewer yeast this mechanism is one of physical-chemical interaction between metal ions and some functional groups, especially amino and carboxyl. The aim of this study was to establish the amino acids from brewer yeast 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 (Podgorskii, 2004). 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 at different pH values. 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 copper, zinc and lead after incubation. It is supposed that amino acids from brewer yeast has the capacity to coordinate these metal ions and the 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 (Cabuk, 2005).

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. Cu biosorption (area)	Aa. Pb biosorption (area)	Aa. Zn biosorption (area)
1	GLN/275	10704200	-	-	-
2	ASN/243	1,11E+08	-	17414728	24690218
3	GLY/204	2,93E+08	34229200	62596676	86537960
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	79203976
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	8980013	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 AAA which are present in uncharged brewer yeast sample disappear in the chromatograms of charged brewer's yeast with all metal ions; ASN and TRP have good affinity for copper ions, GLU for lead ions, and PHE for copper and zinc ions. The others amino acids present different affinity for metal ions according with their molecular structure and the capacity to coordinate copper, lead and zinc. According to covalent index Pb(II) ion is classified as a class *b* ion, while Cu(II) and Zn(II) are classified as borderline ion, so the behavior of coordination is not full the same and the binding capacity is different (Iqbal, 2004; Niebeer, 1973).

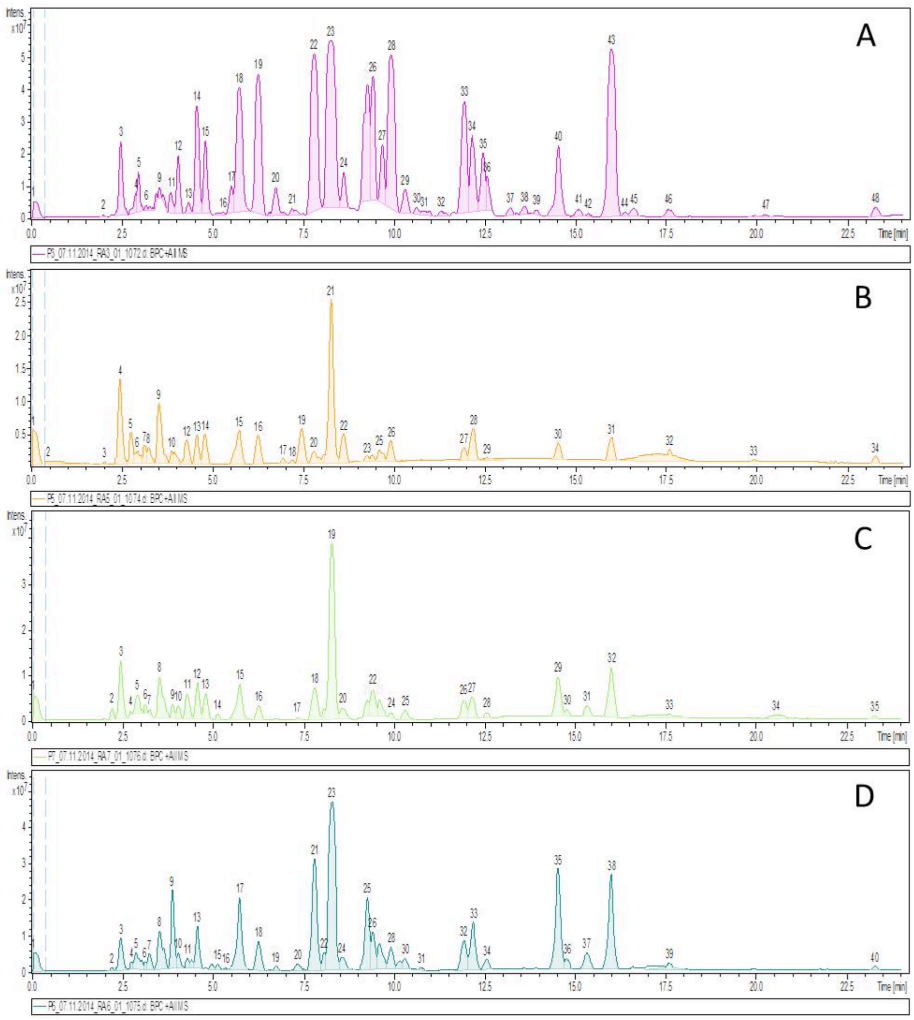

Fig. 1. HPLC chromatogram of amino acids extracted from control brewer's yeast (A), amino acids extracted from brewer's yeast charged with copper ions (B), with lead ions (C), with zinc ions (D).

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

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