# Studies on Surface Properties of Yeast Cells During Heavy Metal Biosorption

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**Abstract**

Biosorption of heavy metals by microbial biomass is an innovative and alternative technology for removal of these pollutants. It is particularly promising for the treatment of large volumes of effluents with low concentration of pollutants. The mechanism of metal biosorption is complicated and requires further elucidation. Metal ions most likely bind onto the cell surface by complexation, coordination, ion exchange, adsorption (electrostatic interactions, van der Waals forces), microprecipitation or some combination of these processes. Both properties of metal solution, environmental conditions and the type of biomaterials (microorganism genus, species or even strain), influence the mechanism of metal biosorption and consequently metal adsorption capacity, affinity and specificity. The cell surface properties determine the metal-microorganism interactions to a large extent. In this work the relationship between surface properties of yeast cells and the removal efficiency of lead, cadmium and copper by various strains was investigated. Surface charge and hydrophobicity before and after biosorption were monitored. Cell surface charge and hydrophobicity were determined using dye retention and solvent partition assays [1, 2]. There were differences in the surface charge and relative hydrophobicity among different yeast strains. The higher adsorption capacity for more negatively charged yeast cells was observed. Biosorption of heavy metals resulted in modifications to the surface charge and hydrophobicity of yeast cells. Changes in the surface charge and hydrophobicity of biomass after binding of heavy metals did not depend on the nature of the metal, initial metal concentration or solution pH

## Introduction

The biosorption efficiency of heavy metals by microbial biomass is mainly connected with a various structure of microorganism cell wall. The cell wall structure determines the nature of the interactions between the organisms and metals. The cell wall of different microorganisms, species or even strains vary considerably in its overall composition, which leads to varying adsorption capacity, affinity and specificity. Cell wall phosphates and carboxyl groups have been reported to be the major determinant of yeast cell surface charge. Yeast cell walls are negatively charged and the ability of yeast cells to bind heavy metal cations is likely due to electrostatic interactions. Cell surface hydrophobicity may also affect biosorption capacity, facilitating hydrophobic bonds.

In this work yeast surface properties in relation to cadmium, lead and copper binding by yeast was studied.

## Materials and Methods

*Saccharomyces pastorianus* W34/78 (Hefebank Weihenstephan) and *Saccharomyces cerevisiae* (LOCK 0271) were cultivated from pure culture in wort broth (Merck) for 48h at temperature 25°C. Then biomass was harvested by centrifugation (3000×g, 5min.) and washed twice with deionised distilled water. Waste yeast biomass was provided by a local brewery. Metal ions solutions of cadmium, lead and copper were made by dissolving analytical grade $Pb(NO_3)_2$, $Cd(NO_3)_2{\times}4H_2O$ and $CuSO_4$ in deionised distilled water.

Biosorption experiments were performed with shaking at 150 rpm at constant temperature of 10°C for 30 min using 100 mL of 10 mg $L^{-1}$ $Cd^{2+}$ and $Pb^{2+}$ solutions and 2 g of wet biomass, which corresponded to 0.42 g of dry biomass. pH vaue of the solution was adjusted to 6.0 or 5.0 with 0.1 mol $L^{-1}$ $HNO_3$ or NaOH. Following the cadmium, lead and copper biosorption biomaterials were separated by centrifugation (3000×g, 3 min.) and the concentration of residual $Cd^{2+}$, $Pb^{2+}$, $Cu^{2+}$ ions in the supernatant was determined by atomic absorption spectrometry (AAS, GBC 932 Plus). All experiments were conducted in triplicate and the mean values were used in data analysis.

Surface charge and hydrophobicity before and after biosorption were monitored. Negative cell surface charge was measured by the degree of adsorption of Alcian Blue dye. Alcian Blue is a phthalocyanine complex that has four positively charged sites in the molecule and is adsorbed by negatively charged cell surfaces, especially the mannosylphosphate moiety (Fukudome et al., 2003). The degree of this dye's adsorption reflects the magnitude of the cell surface's negative charge. Yeast cells at a concentration $5\times10^7$ $mL^{-1}$ were washed twice in phosphate buffer (pH = 7.0) and harvested by centrifugation at 1430×g for 5 min at 4°C. Then yeast was suspended in 0.02 mol $L^{-1}$ sodium acetate buffer (pH = 4.0) and washed twice with the same buffer. Yeast was incubated with 1.8mL of Alcian Blue tetrakis-chloride solution (50mg L-1 in the buffer for 30 min at 25°C. After centrifugation at 20,000×g for 10 min at 20°C the supernatant was decanted and its absorbance (A) was measured at 615 nm. The Alcian Blue retention ratio (ABR) was calculated according to the following formula:

$$ABR=(A_{ABsolution}-A_{supernatant}) \times 100 / A_{ABsolution}$$

The ABR was expressed as the mean of three experiments.

The relative hydrophobicity was estimated by solvent partition assays (Powell et al., 2003). Yeast cells were washed twice in phosphate buffer (pH = 7.0) and diluted to a concentration of $5\times10^7$ cells $mL^{-1}$. 20mL of suspension was mixed with 5 mL of xylene, transferred to a separatory funnel, shaken for 30 s and allowed to stay for 30 min. When two phases were completely separated, yeast cells in aqueous layer were calculated in the Thoma counter. The relative hydrophobicity was expressed as the ratio of the yeast cell number in the aqueous phase after emulsification ($N_e$) to the yeast cell number in the aqueous phase before emulsification (N).

$$RH= (1- N_e/N) \times 100$$

**Results and Discussion**

There were differences in the surface charge and hydrophobicity among the employed yeast biomass (Tables 1-4). The negative surface charge was the highest for *S. cerevisiae* and the lowest for waste yeast. On the contrary the relative hydrophobicity of *S. cerevisiae* was the lowest. There was a strong correlation between the negative surface charge of yeast cells and the relative hydrophobicity ($R^2$=0,999).

The relative hydrophobicity of yeast showed mainly the presence of hydrophilic groups at the surface. Hydrophilic molecules are generally polar or charged while hydrophobic are non-polar (Laurent et al., 2009). A lower relative hydrophobicity and a higher negative surface charge can be related with a better availability of polar/charged groups such as carboxyls, mannosylphosphates at the yeast cell surface. It is likely that the biggest number of negative and/or polar sites at the yeast surface results in the higher number of active sites for fixation of heavy metals. And in fact the metal biosorption effectiveness was affected by the relative hydrophobicity and the surface charge of biomass (Tables 1-5). There was a relation between the relative hydrophobicity, the surface charge of yeast biomass and the capability for sorption of cadmium, lead and copper. The findings showed that the negative surface charge and the relative hydrophobicity of biosorbent could play a role in sorption of heavy metals.

**Table 1.** The negative cell surface charge of different biomass measured after biosorption of $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$ from solutions at 0.05 g $L^{-1}$ and 0.1 g $L^{-1}$ concentration at pH = 5.

| Biomass | Negative surface charge [%] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | After sorption of $Cu^{2+}$ | | After sorption of $Cd^{2+}$ | | After sorption of $Pb^{2+}$ | |
| | | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ |
| *S. cerevisiae* | 85.3±1.7 | 77.1±2.4 | 64.7±1.4 | 74.1±4.7 | 71.3±1.3 | 78.7±4.8 | 75.2±8.3 |
| *S. pastorianus* | 66.0±3.2 | 56.9±4.1 | 75.0±1.8 | 61.9±5.9 | 64.0±1.8 | 52.6±4.8 | 64.4±1.8 |
| Waste yeast | 55.3±2.1 | 61.9±1.4 | 64.6±2.6 | 60.6±2.4 | 72.9±1.0 | 61.1±1.2 | 68.6±1.6 |

**Table 2.** The negative cell surface charge of different biomass measured after biosorption of $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$ from solutions at 0.05 g $L^{-1}$ and 0.1 g $L^{-1}$ concentration at pH = 6.

| Biomass | Negative surface charge [%] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | After sorption of $Cu^{2+}$ | | After sorption of $Cd^{2+}$ | | After sorption of $Pb^{2+}$ | |
| | | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ |
| *S. cerevisiae* | 85.3±1.7 | 78.4±1.8 | 78.0±2.2 | 81.5±1.9 | 78.4±1.0 | 79.6±2.7 | 82.0±3.5 |
| *S. pastorianus* | 66.0±5.2 | 52.8±1.7 | 51.1±4.3 | 58.6±3.2 | 35.7±8.1 | 55.9±1.5 | 51.1±1.4 |
| Waste yeast | 55.3±2.1 | 69.7±2.6 | 72.6±1.7 | 59.6±0.5 | 60.0±3.0 | 71.1±2.6 | 63.0±2.7 |

**Table 3.** The relative hydrophobicity of different biomass measured after biosorption of $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$ from solutions at 0.05 g $L^{-1}$ and 0.1 g $L^{-1}$ concentration at pH = 5.

| Biomass | Hydrophobicity [%] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | After sorption of $Cu^{2+}$ | | After sorption of $Cd^{2+}$ | | After sorption of $Pb^{2+}$ | |
| | | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ |
| *S. cerevisiae* | 8.6±2.1 | 11.3±4.6 | 9.9±5.5 | 13.7±1.6 | 19.7±3.2 | 24.5±3.1 | 19.8±1.7 |
| *S. pastorianus* | 21.3±4.4 | 35.2±4.4 | 26.6±1.3 | 23.2±1.3 | 22.6±2.3 | 30.6±0.1 | 21.7±3.9 |
| Waste yeast | 28.9±2.5 | 21.8±1.6 | 35.9±3.5 | 9.8±2.5 | 8.7±3.5 | 10.8±3.1 | 29.7±3.1 |

**Table 4.** The relative hydrophobicity of different biomass measured after biosorption of $Cu^{2+}$, $Cd^{2+}$, $Pb^{2+}$ from solutions at 0.05 g $L^{-1}$ and 0.1 g $L^{-1}$ concentration at pH = 6.

| Biomass | Hydrophobicity [%] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | After sorption of $Cu^{2+}$ | | After sorption of $Cd^{2+}$ | | After sorption of $Pb^{2+}$ | |
| | | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ | Conc. 0.05g $L^{-1}$ | Conc. 0.1g $L^{-1}$ |
| *S. cerevisiae* | 8.6±2.1 | 14.2±2.2 | 9.9±1.1 | 9.9±2,6 | 10.1±2.0 | 11.7±2.8 | 12.5±0.4 |
| *S. pastorianus* | 21.3±4.4 | 18.6±3.1 | 15.9±1.2 | 20.5±2.9 | 25.4±3.5 | 21.3±1.8 | 11.4±6.7 |
| Waste yeast | 28.9±2.5 | 20.4±2.5 | 9.2±0.7 | 13.5±2.8 | 14.7±4.0 | 20.7±0.7 | 20.9±4.1 |

By now the relation between the heavy metal sorption effectiveness and the surface charge and relative hydrophobicity of biosorbent was only shown for activated sludge. In Laurent et al. study (2009) the increase of the negative surface charge and decrease of the relative hydrophobicity of sludge due to sonication caused the increase of the availability of fixation sites of metal ions at the surface of the flocs and consequently the increase of the cadmium and copper adsorptive capacity.

Hydrophobic properties of biosorbents were investigated when organic compounds were bound. Hydrophobic interactions have been described as an important mechanism in the sorption of natural organic matter onto organic coated minerals or organic particles in soil. A comparative study on the biosorption capacity of phenol and chlorophenols by acclimated residential

biomass at different pH values showed that the adsorption amount of phenol and chlorophenols was greatly correlated to their hydrophobicity (Antizar-Ladislao and Galil, 2004).

**Table 5.** The heavy metal uptake capacities of different biomass

| Biomass | Initial metal conc. [g $L^{-1}$] | Metal uptake [%] | | |
|---|---|---|---|---|
| | | $Cu^{2+}$ | $Cd^{2+}$ | $Pb^{2+}$ |
| *S. cerevisiae* | 0.05 | 55±4 | 55±2 | 58±1 |
| | 0.1 | 40±2 | 39±2 | 44±1 |
| *S. pastorianus* | 0.05 | 53±2 | 51±1 | 56±1 |
| | 0.1 | 36±3 | 33±2 | 42±1 |
| Waste yeast | 0.05 | 45±4 | 45±2 | 47±2 |
| | 0.1 | 30±3 | 29±2 | 31±2 |

The decrease of the negative surface charge after biosorption of heavy metals performed at pH=5 was observed for *S. cerevisiae*. The higher metal concentration in solution the higher decrease in the negative surface charge was found. The negative charge on a surface of *S.pastorianus* cells did not have a statistically significant tendency to change due to sorption of heavy metals. In turn, there was a growth in the negative charge on the surface of waste yeast cells.

When biosorption process was conducted at pH=6 the lowering of the negative charge at the surface of *S. cerevisiae* was also seen. The magnitude of these changes was not connected with the metal concentration as it took place at pH=5. The decrease of the negative surface charge was found for *S. pastorianus* either. And again there was the increase in the negative charge at the surface of a waste yeast cells, the highest after sorption of copper and lead.

The relative hydrophobicity after biosorption of cadmium and lead by *S. cerevisiae* at pH=5 increased. When copper was removed the increase in hydrophobicity was not statistically significant. The statistically significant increase in hydrophobicity was observed after metal uptake at the concentration 0.05g $L^{-1}$ by *S. pastorianus*. There were not statistically significant changes in hydrophobicity after biosorption by waste yeast as well as by *S. cerevisiae* and *S. pastorianus* performed at pH=6. But the lowering of hydrophobicity of waste yeast cells was observed after metal uptake at pH=6.

The tendency of changes in the relative hydrophobicity and surface charge after biosorption were not the same for yeast cultivated from pure culture and waste biomass. It can result from the contamination of waste yeast.

Owing to the fact that not all the changes in the relative hydrophobicity of yeast cells and the surface charge were statistically significant it can not be concluded that after biosorption the decrease of the relative surface charge of yeast cultivated from pure yeast culture and the increase of the relative hydrophobicity was seen as well as the decrease of the negative surface charge due to biosorption of heavy metals corresponded to the increase in the relative hydrophobicity of cells.

## Conclusion

The higher relative hydrophobicity and the lower surface charge of yeast cells the lower effectiveness of removal of copper, lead and cadmium by this biomass was found. There were not the same changes in the relative hydrophobicity and the surface charge after biosorption of metals for all studied yeast biomass.

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