1. Introduction

Wastewaters contaminated with heavy metals have become a serious environmental problem. Biosorption of heavy metals by microbial biomass is an innovative and alternative technology of pollutant removal as opposed to conventional methods such as chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon etc. Ion exchange, membrane technologies and activated carbon adsorption process are expensive and cannot be employed in large scale. Chemical precipitation and electrochemical methods are ineffective for the treatment of wastewater containing heavy metals in low concentrations. Moreover, these methods cause the formation of large amount of sludge, the disposal of which is troublesome. Therefore, the application of microbial biomass, such as yeast seems to be more favourable for treatment of large volumes of effluents with low concentration of contaminants [1,2].

Yeast can be easily cultivated in substantial quantities using inexpensive growth media and simple fermentation techniques. Moreover, yeast is applied in a variety of industrial fermentation processes which can serve as a rich source of biomass for metal ion removal. Therefore brewing yeast can be an easily available, low-price biosorbent with a sufficiently high metal-binding capacity and selectivity for heavy metals [3]. Not only living but also dead microbial cells are able to remove heavy metals form aqueous solutions. Inactive/dead microbial biomass can passively bind metal ions via various physicochemical mechanisms. The biosorption efficiency of heavy metals by microbial biomass is mainly connected with the structure of the microorganism cell wall and consequently with cell surface properties [1-3]. The cell wall structure determines the nature of the interactions between the organisms and metals. The cell walls of different microorganisms, species or even strains vary considerably in their overall composition, which leads to varying adsorption capacity, affinity and specificity. Cell wall phosphates and carboxyl groups have been reported to be the major determinants of yeast cell surface charge [4,5]. Yeast cell walls are negatively charged and the ability of yeast cells to bind heavy metal...
cations is likely due to electrostatic interactions. The negative cell surface charge can be 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. The degree of this dye’s adsorption reflects the magnitude of the cell surface’s negative charge [6].

The cell surface hydrophobicity, which depends on the presence of various polysaccharides, proteins and lipids, may also affect biosorption capacity, facilitating hydrophobic bonds.

The aim of this work was to investigate the relationship between surface properties of yeast cells and the removal efficiency of lead, cadmium and copper by various yeast strains. Surface charge and hydrophobicity before and after biosorption were monitored. The alterations of yeast surface properties resulting from heavy metal binding by yeast were assessed.

2. Experimental Procedure

2.1. Materials

Saccharomyces pastorianus W34/78 (Hefebank Weihenstephan) and Saccharomyces cerevisiae (LOCK 0271) were cultivated from pure culture in wort broth (Merck) for 48 hours at temperature 25°C. Then biomass was harvested by centrifugation (3000×g, 5 min) and washed twice with deionised distilled water or phosphate buffer when surface charge and hydrophobicity were measured.

Waste yeast biomass was provided by a local brewery. Waste yeast was washed twice with deionised distilled water or phosphate buffer.

Metal ions solutions of cadmium, lead and copper were made by dissolving analytical grade Pb(NO₃)₂, Cd(NO₃)₂·4H₂O and CuSO₄ in deionised distilled water.

2.2. Biosorption experiments

Biosorption experiments were performed with shaking at 150 rpm at constant temperature of 10°C for 30 min using cadmium, lead and copper solutions (100 mL, 0.05 and 0.1 g L⁻¹) and 2 g of wet yeast biomass, which corresponded to 0.42 g of dry biomass. The metal concentrations in solutions were much higher than in real wastewater. However, more concentrated solutions were used in order to observe any potential changes in yeast surface properties resulting from heavy metal adsorption. pH value of the solution was adjusted to 6.0 or 5.0 with HNO₃ or NaOH (0.1 mol L⁻¹). Following cadmium, lead and copper biosorption biomaterials were separated by centrifugation (3000×g, 3 min). The concentration of residual cadmium, lead and copper ions in the supernatant was determined by atomic absorption spectrometry (AAS, GBC 932 Plus). Yeast biomass was used for measurement of cell surface charge and hydrophobicity. All experiments were conducted in triplicate and the mean values were used in data analysis.

2.3. Cell surface charge measurement

Yeast surface charge and hydrophobicity before and after heavy metal biosorption by different yeast strains were monitored. The negative cell surface charge was measured by the degree of adsorption of Alcian Blue dye [6]. Yeast cells at a concentration 5×10⁻⁷ 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 sodium acetate buffer (0.02 mol L⁻¹) and washed twice with the same buffer. Yeast was incubated with Alcian Blue tetrakis-chloride solution (1.8 mL, 50 mg 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:

\[
\text{ABR} = \left( \frac{A_{\text{solution}} - A_{\text{supernatant}}}{A_{\text{solution}}} \right) \times 100
\]

The ABR was expressed as the mean of three experiments.

2.4. Cell surface hydrophobicity measurement

The relative hydrophobicity was estimated by solvent partition assays [7]. Yeast cells were washed twice in phosphate buffer (pH = 7.0) and diluted to a concentration of 5×10⁻⁷ cells mL⁻¹. Suspension (20 mL) was mixed with xylene (5 mL), 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 (RH) was expressed as the ratio of the yeast cell number in the aqueous phase after emulsification (Nₑ) to the yeast cell number in the aqueous phase before emulsification (N).

\[
\text{RH} = \left( \frac{1 - Nₑ}{N} \right) \times 100
\]

The RH was expressed as the mean of three experiments.
3. Results and Discussion

There were differences in the surface charge and hydrophobicity among the employed yeast biomass (Tables 1-2). The negative surface charge was highest for *S. cerevisiae* and 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 [8]. A lower relative hydrophobicity and a higher negative surface charge can be related to better availability of polar/charged groups such as carboxyls, mannosylphosphates on yeast cell surface. It is likely that a higher number of negative and/or polar sites on the yeast surface results in a 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 1-3). There was a relation between the relative hydrophobicity, the surface charge of yeast biomass and the yeast 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.

By now the relation between the heavy metal sorption effectiveness and surface charge and relative hydrophobicity of biosorbent has been only shown for activated sludge. In their study, Laurent *et al.* [8] found that increase of negative surface charge and the decrease of the relative hydrophobicity of sludge due to sonication caused the increase of availability of fixation sites of metal ions on the surface of flocs and consequently an increase in cadmium and copper adsorptive capacity.

Hydrophobic properties of biosorbents have been investigated when organic compounds were bound [9]. 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 highly correlated to their hydrophobicity [9].

In this study surface properties of yeast following heavy metal binding were evaluated. Decrease in negative surface charge after biosorption of heavy metals performed at pH=5 was observed for

| Biomass  | pH  | Initial | After sorption of Cu$^{2+}$ Conc. 0.05g L$^{-1}$ | After sorption of Cu$^{2+}$ Conc. 0.1g L$^{-1}$ | After sorption of Cd$^{2+}$ Conc. 0.05g L$^{-1}$ | After sorption of Cd$^{2+}$ Conc. 0.1g L$^{-1}$ | After sorption of Pb$^{2+}$ Conc. 0.05g L$^{-1}$ | After sorption of Pb$^{2+}$ Conc. 0.1g L$^{-1}$ |
|----------|-----|---------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| *S. cerevisiae* | 5   | 85.3±1.7 | 77.1±2.4                                      | 64.7±1.4                                      | 71.4±4.7                                      | 71.3±1.3                                      | 78.7±4.8                                      | 75.2±8.3                                      |
|           | 6   |         | 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* | 5   | 66.0±5.2 | 56.9±4.1                                      | 75.0±1.6                                      | 61.9±5.9                                      | 64.0±1.3                                      | 52.6±4.8                                      | 64.4±1.8                                      |
|           | 6   |         | 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 | 5   | 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                                      |
|           | 6   |         | 69.7±2.6                                      | 72.6±1.7                                      | 59.6±0.5                                      | 60.0±3.0                                      | 71.1±2.6                                      | 63.0±2.7                                      |

| Biomass  | pH  | Initial | After sorption of Cu$^{2+}$ Conc. 0.05g L$^{-1}$ | After sorption of Cu$^{2+}$ Conc. 0.1g L$^{-1}$ | After sorption of Cd$^{2+}$ Conc. 0.05g L$^{-1}$ | After sorption of Cd$^{2+}$ Conc. 0.1g L$^{-1}$ | After sorption of Pb$^{2+}$ Conc. 0.05g L$^{-1}$ | After sorption of Pb$^{2+}$ Conc. 0.1g L$^{-1}$ |
|----------|-----|---------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| *S. cerevisiae* | 5   | 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                                      |
|           | 6   |         | 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* | 5   | 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                                      |
|           | 6   |         | 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 | 5   | 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                                      |
|           | 6   |         | 20.4±2.5                                      | 9.2±0.7                                       | 13.5±2.8                                      | 14.7±4.0                                      | 20.7±0.7                                      | 20.9±4.1                                      |
The higher metal concentration in solution the greater decrease in the negative surface charge was found. The negative charge on the 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 seen (Table 1). 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 waste yeast cells, which was the highest after sorption of copper and lead.

The relative hydrophobicity after biosorption of cadmium and lead by S. cerevisiae at pH=5 increased (Table 2). When copper was removed the increase in hydrophobicity was not statistically significant. A statistically significant increase in hydrophobicity was observed after metal uptake at a concentration of 0.05 g L^-1 by S. pastorianus. There were no statistically significant changes in hydrophobicity after biosorption by waste yeast as well as by S. cerevisiae and S. pastorianus performed at pH=6 (Table 2). But lowering of hydrophobicity of waste yeast cells was observed after metal uptake at pH=6.

### 4. Conclusions

The higher the relative hydrophobicity and lower the negative surface charge of yeast cells the effectiveness of removal of copper, lead and cadmium was found to be lower. There were not the same changes in the relative hydrophobicity and surface charge for all studied yeast biomass. The evaluation of cell surface charge and relative hydrophobicity can contribute to a better selection of heavy metal biosorbent.

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