Research article

The impact of the changes in surface properties of yeast biomass, *Saccharomyces cerevisiae*, on Pb^{2+} biosorption

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Abstract: *This study examines changes that occur in yeast cell surface properties during physical, chemical and mechanical treatment and their potential impact on the biomass uptake capacity for lead. As a result of biomass treatment, the negative surface charge decreased and relative hydrophobicity increased. A strong negative correlation was discovered between the negative surface charge of variously modified yeast cells and their relative hydrophobicity. Despite the lower negative surface charge of treated in comparison to untreated yeast cells, the treated biomass had higher lead sorption capability. No relationship was found between the effectiveness of lead sorption by treated biomass and its surface charge or relative hydrophobicity.*

Keywords: *biosorption, hydrophobicity, lead, surface charge, yeast.*

Introduction

Biosorption is a sustainable alternative to conventional technologies for the treatment of wastewater polluted with heavy metals. Biosorption is the metabolism-independent adsorption of pollutants [1-3]. Research by Parvathi et al. [4] has shown that electrostatic attraction and complexation seem to be the most important mechanisms for lead biosorption. To enhance the biosorption ability of yeast cells various processing methods have been attempted. Both physical methods and chemical treatments as well as the combination of such methods have been applied to increase the heavy metal uptake capacity of microbial biomass [3, 5-9]. In most cases, sorption by dead cells has been shown to be much more efficient than sorption by live cells, although the reason for this phenomenon is unclear [6, 7, 9, 10]. Contrary reports concerning decreases in biosorption ability in treated biomass make understanding why modification can also lead to improvements in heavy metal adsorption more difficult [5, 8].

Many types of biomaterial have the capacity to adsorb pollutants from solutions, including *S. cerevisiae* cells, which have been shown to be useful for removing heavy metal ions from aqueous solutions [3, 4, 11-13]. When

pollutants are removed using yeast biomass the cell wall is the first cellular structure that comes into contact with metal ions. The structure of the cell wall and consequently the cell surface properties thus play a key role in metal binding and have a significant influence on biosorption effectiveness [1, 11, 14-16].

Biomass treatment may bring about changes in the permeability of the cell membrane and the exposition of further metal-binding sites present inside the cells [17]. As a result, it can cause alterations in cell surface hydrophobicity and charge. The aim of our study was to assess the potential impact of these changes occurring during physical, chemical and mechanical treatment on the biomass uptake capacity for lead.

Experimental

Materials

Saccharomyces cerevisiae LOCK 0271 (Centre of Industrial Microorganisms Collection, Lodz) was cultivated from a pure culture in wort broth for 48 hours at 25°C. The biomass was then harvested by centrifugation (3000 *g*, 5 min) and washed twice with deionised water or phosphate buffer, after which the surface charge and hydrophobicity were measured. Lead ion solutions were made by dissolving analytical grade $Pb(NO_3)_2$ in deionised distilled water.

Biomass pretreatment

The native biomass was subjected to different pretreatments in order to improve its metal uptake. Physical treatment was performed by suspending 10 g of yeast slurry in 250 mL of water and heating at 120°C for 4 hours. The slurry was then centrifuged (3300 *g*, 3 min). Physicochemical modification was carried out by suspending 10 g of initial biomass in 250 mL of 4 mol/L NaOH and heating at 120°C for 4 hours. Then the slurry was centrifuged (3300 *g*, 3 min), suspended in water, neutralized with HNO_3 to reach a pH of 6 and centrifuged again. Ten grams of native biomass was also washed twice with 125 mL of deionized water, centrifuged (3300 *g*, 3 min), heated at 120°C for 4 hours and subjected to mechanical disruption by crushing with mortar and pestle (physical and mechanical treatment). In this case dry biomass was used for measurement of yeast surface charge and hydrophobicity as well as in biosorption experiments. The size of the particles was 0.5 - 1.0 mm. Untreated yeast biomass was used as the control sample. The moisture of each biomass (75.78% for physically treated yeast cells, 80.53% for physicochemically treated cells and 78.00% for untreated biomass) was measured before use by heating at 105°C for 3 hours.

Cell surface charge measurement

The yeast surface charge and hydrophobicity were recorded before and after physical, chemical and mechanical treatment of the yeast biomass. The negative cell surface charge was measured using Alcian Blue dye which is a phthalocyanine complex with four positively charged sites in the molecule. It is adsorbed by negatively charged cell surfaces, in particular the mannosylphosphate moiety.

The degree of adsorption reflects the magnitude of the negative charge [18]. The higher Alcian Blue retention rate the higher negative yeast surface charge.

Yeast cells at a concentration 5×10^7 / mL, determined using a Thoma chamber, were washed twice in phosphate buffer (pH = 7.0) and harvested by centrifugation at 1430 *g* for 5 min at 4°C. The yeast was then suspended in sodium acetate buffer (0.02 mol/L, pH = 4.0) and washed twice with the same buffer. The yeast was incubated with Alcian Blue tetrakis-chloride solution (1.8 mL, 50 mg/L) in the buffer for 30 min at 25°C. After centrifugation for 10 min at 20,000 g and 20°C the supernatant was decanted and its absorbance ($A_{supernatant}$) measured at 615 nm. The Alcian Blue retention ratio (ABR) was calculated using the following formula:

$$ABR\ (\%) = (A_{AB\ solution} - A_{supernatant}) \times 100 / A_{AB\ solution},$$

where:

$A_{AB\ solution}$ is absorbance of Alcian Blue solution (50 mg/L) at 615 nm

The ABR was calculated as the mean of three independent experiments.

Cell surface hydrophobicity measurement

Relative hydrophobicity was estimated by solvent partition assays [19]. Yeast cells were washed twice in phosphate buffer (pH = 7.0) and diluted to a concentration of 5×10^7 cells/mL. The suspension (20 mL) was mixed with xylene (5 mL), transferred to a separatory funnel, shaken for 30 seconds and allowed to stay for 30 min. When the two phases were completely separated, the number of yeast cells in the aqueous layer was determined using a Thoma chamber. The relative hydrophobicity (RH) 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).

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

The RH was expressed as the mean of three independent experiments.

Biosorption experiments

Biosorption experiments were performed while shaking at 150 rpm for 30 min using lead solutions (100 mL, 10 mg/L) and appropriate quantity of yeast biomass, which corresponded to 0.25 g of dry biomass (10°C, pH = 6.0). Biosorption was carried out for long enough to reach equilibrium in the case of each type of biomass. These experimental conditions were optimized during preliminary studies. Following lead biosorption the biomaterials were centrifuged (3300 g, 3 min). The concentration of residual lead ions in the supernatant was determined by atomic absorption spectrometry (GBC 932 Plus). All experiments were conducted in triplicate. The amount of Pb^{2+} adsorbed by the biomass at equilibrium (q) was calculated using the equation:

$$q = (C_0 - C_e)\ V / m\ (mg/g\ cell\ dry\ weight),$$

where C_0 is the initial metal concentration (mg/L), C_e is the final metal concentration (mg/L), V is the volume of metal solution (L), m is the dry weight of biosorbent (g).

Statistical analysis

A student t-test was performed to compare the various surface charges and the hydrophobicity before and after biomass treatment. A student t-test was also used to determine significant variations in the amount of Pb^{2+} adsorbed by untreated and treated biomass.

Results and Discussion

In order better to understand why modified cells showed a higher metal uptake capacity than untreated biomass, we measured the surface charge and relative hydrophobicity of yeast cells [20]. Higher yeast electronegativity can increase electrostatic attraction between biomass and metal cations. It has been shown for untreated yeasts of various species as well as for activated sludge that there is a relation between heavy metal sorption effectiveness and the surface charge and relative hydrophobicity of biosorbent [21, 22]. In a study by Laurent et al. [21] an increase in the negative surface charge and a decrease in the relative hydrophobicity of sludge due to sonication resulted in higher cadmium and copper adsorptive capacity. High negative surface charge and low relative hydrophobicity can be connected with a large number of negative and/or polar groups such as carboxyl and phosphoryl, which are active sites for binding heavy metals. Cell surface hydrophobicity may also affect metal uptake capacity by facilitating hydrophobic bonds. However, highly hydrophobic cell surface properties may limit the exposure of the cells to water-soluble compounds.

In our study, we observed differences in the surface properties of yeast cells for modified and unmodified biomass (Tables 1-2). The cell killing process was found to result in lower negative surface charge. Physico-chemical treatment caused the greatest decrease in the negative cell surface charge, and the highest alteration in hydrophobic properties. These changes can be related to the hydrolysis and dissolution of polysaccharides such as phosphomannan, which is abundant in the outermost layer of yeast cells, and which can lead to a reduction in the number of phosphoryl groups after treatment with highly-concentrated caustic soda solution. As a result of such damage to the outer layer of the cell wall, the exposition of protein in the cell wall and hence an increase in the amount of free amino and carboxyl groups has been observed [4, 23].

Table 1. The Alcian Blue retention ratio (ABR)

Biomass	ABR, %
Untreated	64.86 ± 1.56 ^{a}
Physically treated	52.06 ± 2.01 ^{b}
Physico-chemically treated	6.27 ± 2.01 ^{c}
Physically and mechanically treated	25.31 ± 1.30 ^{d}

Values expressed are the means of three replicates with standard deviations. Different letters indicate significant differences at $p < 0.001$.

A fall in the number of negatively charged phosphoryl groups on the yeast cell surface due to biomass contact with 1.0 mol/L soda solution has also been described by Zhang et al. [8]. However, this last study found a simultaneous increase in the strength of charge of caustic treated biomass.

In our research, the least significant changes in surface charge occurred after biomass heating. This could be due to the milder treatment conditions compared to the use of sodium hydroxide. A strong negative correlation was found between the negative surface charge of variously modified yeast cells and their relative hydrophobicity ($R^2 = 0.949$).

Table 2. The relative hydrophobicity of yeast biomass

Biomass	Hydrophobicity, %
Untreated	8.82 ± 2.47 ^{a}
Physically treated	36.38 ± 3.06 ^{b}
Physico-chemically treated	74.39 ± 2.54 ^{c*}
Physically and mechanically treated	62.28 ± 2.34 ^{d*}

Values expressed are the means of three replicates with standard deviations. Different letters indicate significant differences at $p < 0.001$ (*significant difference obtained at $p < 0.01$).

Modified biomass was identified as being more suitable for lead biosorption processes than untreated biomass (Table 3). However, there was no relation between lead uptake by untreated and treated biomass and its relative hydrophobicity and surface charge (Tables 1-3). Despite the lower negative surface charge of treated in comparison to untreated yeast cells, treated yeast cells had a higher capability for lead sorption. Zhang et al. [8] suggest that the hydrolysis and dissolution of polysaccharides present in the outer cell layer upon biomass treatment with sodium hydroxide could lead to better permeability of the cell wall and result in improved ion diffusion into the cells. This could explain the more effective sorption of treated biomass. Our findings are consistent with the results of Pokhrel and Viraraghavan [24] which showed no strong relationship between the surface charge of iron oxide coated *Aspergillus niger* biomass and its removal efficiency for arsenic.

Table 3. Lead uptake by yeast biomass

Biomass	Pb^{2+} uptake, mg/g
Untreated	16.26 ± 0.61 ^{a}
Physically treated	26.56 ± 0.82 ^{b}
Physico-chemically treated	24.73 ± 0.79 ^{b}
Physically and mechanically treated	24.98 ± 1.02 ^{b}

Values expressed are the means of three replicates with standard deviations. Different letters indicate significant differences at $p < 0.001$.

Based on our results, it can be supposed that the mechanism of lead biosorption varies depending not only on the microbial species or strain but also on the modifications made to the biosorbent. Despite previous reports concerning the relation between the effectiveness of heavy metal sorption and the surface charge and relative hydrophobicity of biosorbent [21, 22], such a relationship was not observed when modified biomass was applied for lead ion removal. It is likely that the mechanism of lead ion sorption by treated and untreated biomass is different due to alterations in the cell composition and properties that occur during biomass treatment. The impact of the biomass status (living or non-living) on the metal biosorption mechanism has been demonstrated previously by Wang and Chen [11]. It seems that if the surface of the adsorbent is less negatively charged the electrostatic force of attraction becomes less important in metal cation binding. This study builds on these findings, contributing to better understanding of the mechanisms behind the superior sorption levels observed following biomass treatment.

Conclusions

In this study, alterations to the cell surface characteristics of brewing yeast were observed during physical, chemical and mechanical treatment. A decrease in negative surface charge and increase in relative hydrophobicity were seen as a consequence of the cell killing process. Despite the lower negative surface charge of treated in relation to untreated yeast cells a higher capability for lead sorption of treated biomass was found.

Acknowledgments

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