Chemical Engineering Journal 244 (2014) 561–568

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Mechanistic modelling of copper biosorption by wild type and engineered *Saccharomyces cerevisiae* biomasses

Fabrizio Di Caprio [a], Pietro Altimari [a,*], Daniela Uccelletti [b], Francesca Pagnanelli [a]

[a] Dipartimento di Chimica, Università "Sapienza" di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy
[b] Dipartimento Biologia e Biotecnologie "C. Darwin", Università "Sapienza" di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy

HIGHLIGHTS

- Copper biosorption on wild type and two engineered *S. cerevisiae* strains was studied.
- The main functional groups responsible for biosorption were identified.
- A model describing the influence of pH and metal concentration was developed.
- The influence of cell wall structure on copper biosorption was analyzed.
- Bioaccumulation could not enhance copper removal.

ARTICLE INFO

Article history:
Received 22 November 2013
Received in revised form 28 January 2014
Accepted 30 January 2014
Available online 7 February 2014

Keywords:
Biosorption
Bioaccumulation
Saccharomyces
Titration
Modelling

ABSTRACT

Copper biosorption by wild type and two engineered (Rim101Δ, Och1Δ) *Saccharomyces cerevisiae* biomasses was analyzed. Potentiometric titrations revealed that carboxylic, amino and, to less extent, phosphoric groups are responsible for the biosorption of copper. Maximum biosorption capacities of copper equal to 28.8, 8.0 and 7.5 mg/g were found at pH = 6 for wild type, Rim101Δ and Och1Δ respectively. A mechanistic equilibrium model was developed to describe the dependence of metal and proton binding on pH and metal liquid concentration. The developed model accounts for the exchange of metal ions with protons from functional groups in acidic form and for the sorption of metal ions on ionized groups. Model parameters were estimated based on potentiometric titration data and adsorption isotherms leading to satisfactory fitting for any considered strain. Metal removal by living cells was investigated to determine bioaccumulation capacity. No statistically significant difference was found among the bioaccumulation capacities of the three strains. For any strain, bioaccumulation could not enhance copper removal.

1. Introduction

Biosorption represents a competitive technological alternative for removal of heavy metals from wastewaters [1]. This technique relies on the property of certain bio-molecules (or types of biomass) to bind and concentrate selected ions or other molecules from aqueous solutions. Examples of biomasses employed in the removal of heavy metals from wastewaters include bacteria, algae, fungi and yeasts [1–4]. The main advantages of biosorption are the application of an inexpensive and biodegradable adsorbing material, and the achievement of elevated removal efficiency at low metal concentrations (0–100 ppm) [1,3].

Considerable attention has been attracted in this framework by the application of *Saccharomyces cerevisiae*. This yeast can be easily cultivated or collected as a waste from food industries and has become a benchmark in the study of biosorption. The application of *S. cerevisiae* has been proved effective in removing radionuclide, toxic and precious metals [5]. Numerous studies have investigated the influence of operating parameters (pH, temperature, presence of competing ions) on biosorption by this microorganism, and chemical pre-treatment strategies have been proposed to enhance metal removal capacity [5–8]. The application of living cells in place of dead ones has also attracted interest as it enables to remove heavy metals not only through biosorption but also ingestion by the microorganism, that is bioaccumulation [9,10].

Despite the elevated number of experimental studies focusing on the application of *S. cerevisiae* in heavy metal removal, an exhaustive mathematical model for biosorption onto this microorganism is still far from being developed. Typically, mathematical

* Corresponding author. Address: Dipartimento di Chimica, Università "Sapienza" di Roma, Piazzale Aldo Moro 5, 00185 Roma, Italy. Tel. +etf 81483368.
E-mail address: pietro.altimari@uniroma1.it (P. Altimari).

http://dx.doi.org/10.1016/j.cej.2014.01.098
1385-8947/

representations consolidated in the analysis of adsorption onto ions exchange resins or activated carbons are employed [5,11–14]. This approach cannot however account for adsorption mechanisms made possible by the complex chemistry of biomasses and that are absent in synthetic adsorbing materials. The central role played by protons in biosorption is, for example, neglected by the most popular mathematical description of the process, that is the Langmuir model [15].

A more effective mathematical representation of biosorption can be derived through analysis of the interaction between biomass functional groups and ions in solution [15,16]. This entails identifying the reactions between the main functional groups participating into biosorption and ions in solutions.

In this article, a detailed analysis of biosorption and bioaccumulation of copper onto the yeast *S. cerevisiae* is presented. The illustrated study analyzes the influence of cell wall structure on biosorption and bioaccumulation. For this purpose, two genetically modified *S. cerevisiae* strains, Rim101Δ and Och1Δ, characterized by variations in the cell wall structure were employed besides a wild type strain. Rim101Δ is a mutant defective in the transcription factor involved in the alkaline pH response [17] and is implicated in changes in membrane composition, shape and cell wall organization [18,19]. Och1Δ is a mutant with a deletion in Och1 gene. In this latter strain, the inactivation of the α-1,6-mannosyltransferase results in cell wall mannoproteins characterized by a reduced fraction of α-1,6-polymannose [20].

A mechanistic equilibrium mathematical model is derived enabling to predict the equilibrium repartition of copper between liquid and solid as the pH varies. Model parameters are separately determined for each considered strain by nonlinear regression of experimental biosorption data. In this way, valuable indications about the role played by differences in cell wall structure on copper biosorption are derived. Bioaccumulation capacities of the considered strains are determined by analysis of metal removal in cultures of living cells.

2. Material and methods

2.1. Yeast strains and growth conditions

The following *S. cerevisiae* strains were used in this study: BY4741 (*MAT*a, *met15Δ0, his3Δ0, leu2Δ0, ura3Δ0*), referred in the following to as wild type; Rim101Δ (*MAT*a, *met15Δ0, his3Δ0, leu2Δ0, ura3Δ0, rim101*::kan^R); Och1Δ (*MAT*a, *met15Δ0, his3Δ0, leu2Δ0, ura3Δ0, och1Δ*:: kan^R).

The cells were grown on YPD (1% peptone, 1% yeast extract, 2% glucose, DIFCO) at 28 °C till reaching late exponential phase. Following growth, cells were centrifuged and rinsed twice with distilled water to remove residual traces of the culture medium. The obtained pellet was incubated at 65 °C for drying.

2.2. Potentiometric titration

In potentiometric titrations, 1 g of dry biomass was suspended in 50 mL of $NaNO_3$ solution. Experiments were performed at $NaNO_3$ concentrations equal to 0.1 M and 1 M. In any performed test, the initial pH of the suspension was corrected to a value around 2–2.5 by using HCl 0.1 N. pH was measured by an Eutech pH700. NaOH 0.1 M was used as titrant solution and was added to the suspension through a burette. After the addition of any titrant solution volume, pH was measured. Titration was ended when reaching pH = 10.

Electric charges are formed at the cell wall by dissociation/protonation of weakly acid and basic groups. This makes possible to bind heavy metal ions present in solution [21,22]. The evolution of the biomass net superficial charge Q_H with pH was determined based on the results of potentiometric titration experiments. The following expression, derived by imposing electro-neutrality, was in particular employed to determine Q_H from the evolution of pH observed during titration:

$$Q_H = -\frac{([Na^+] + [H^+] - [OH^-] - [Cl^-] - [NO_3^-])V}{m} \quad (1)$$

where m is the mass of suspended biomass.

2.3. Adsorption isotherms

In order to determine the adsorption isotherms, batch experiments were performed in aqueous solution with initial copper concentration ranging between 5 and 120 mg/L. This latter solution was prepared by using a stock solution with copper concentration equal to 2 g/L realized with $CuSO_4{\cdot}7H_2O$. Experiments were conducted for each yeast strain at pH = 4 and pH = 6 under magnetic stirring. In any run, the pH was measured, controlled and corrected to 4 or 6 (±0.2) with HCl 0.1 M or NaOH 0.1 M. After 1 h, 2 mL of the suspension were taken and centrifuged at 9000 rpm. The supernatant was analyzed by atomic adsorption (analytic jena contra 300) to compute the equilibrium copper concentration. The biomass concentration employed in any performed experiment was 2 g/L (0.16 g of yeast in 80 mL of copper solution). The equilibrium adsorption capacity was determined as $Q_e = (C_0 - C_e)V/m$ where C_0 and C_e are initial and equilibrium copper liquid concentration respectively, V is solution volume and m is the weight of total suspended biomass.

It is important to note that the temporal evolution of liquid copper concentration during adsorption was monitored in preliminary experiments indicating that a time interval equal to 1 h is largely sufficient to reach equilibrium.

2.4. Extraction and digestion tests

The three different yeast strains were cultivated in 0.75 L of culture medium YPD. At the end of the exponential growth phase, an aliquot of copper stock solution (2 g/L) was added to the medium to reach a copper concentration of 500 mg/L. The pH varied during the test from 5 to 4.6. After 24 h, biomass was separated from solution through centrifugation. The supernatant was eliminated and the biomass was rinsed with distilled water to remove the residual culture medium. Then, the yeast was dried at 70 °C overnight.

In extraction tests, 0.5 g of dried biomass was suspended in 30 mL of washing solution (EDTA 1 mM, NaCl 0.1 M, pH 7) and mixed for 30 min. Biomass was then separated by centrifugation at 3000 rpm for 5 min. Metal concentration in the supernatant was determined by atomic adsorption.

In digestion tests, the pellet obtained at the end of the extraction test by separation from the washing solution was dried at 70 °C overnight, weighted and suspended in HNO_3 4 M at 90 °C for 24 h. After filtration, solution was analyzed to determinate copper concentration. As a control test, direct digestion with HNO_3 was also conducted by using 0.5 g of dried biomass not previously treated with washing solution. Two replicates were performed for each test.

3. Results and discussion

3.1. Potentiometric titration

As shown in Fig. 1, the net charge Q_H is, for each considered yeast strain, positive at low pH values and becomes negative as the pH is increased. This trend can be explained by the high