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Bioaccumulation of chromium(VI), copper(II) and nickel(II) ions by growing *Rhizopus arrhizus*

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Abstract

The metal resistance capacity and metal ion accumulation capacity during the growth of *Rhizopus arrhizus* and the inhibition kinetics of heavy metals, namely chromium, copper and nickel were studied in a batch reactor. A maximum percentage uptake yield of 93.84, 95.52 and 61.44% were found for 25 mg/l of initial metal ion concentrations of chromium, copper and nickel, respectively. A maximum biomass of 20.17 g/l was obtained at 20 g/l of initial dextrose concentration and in absence of heavy metals. The inhibition was found to be a competitive inhibition for the bioaccumulation of chromium. Lineweaver–Burk plot, Aiba model and Bazua and Wilke models were used to study the inhibition kinetics of bioaccumulation of heavy metals chromium, copper and nickel using *R. arrhizus* and the model parameters were evaluated using experimental data.

Keywords: Bioaccumulation; Heavy metals; *Rhizopus arrhizus*; Growth curve; Inhibition kinetics; Metal–microbe interaction

1. Introduction

The removal of heavy metal ions from industrial wastewater is a problem of increasing concern that has been mostly solved by chemical and physical methods of treatment. However, these procedures have significant disadvantages, such as incomplete metal removal, high reagent or energy requirements, or generation of toxic sludge or other waste products and are generally very expensive when the contaminant concentrations are in the range 10–100 mg/l [1]. Chromium is a toxic metal of widespread use in many industries such as metal plating facilities, mining operations and tanneries. Besides chromium, copper and nickel are also common and serious environmental pollutants. They are frequently encountered together in industrial wastewaters, e.g. electroplating, electronics wire manufacturing, oil refineries and copper sulphate manufacture [2–4]. Therefore, there is a need for the development of low cost, easily available materials, which could adsorb toxic heavy metals from wastewater. Microorganisms are potent bioremediators, removing heavy metals via active or passive uptake mechanisms. Metal accumulative bioprocesses generally fall into one of two categories, biosorptive uptake by non-living, non-growing biomass and bioaccumulation by living cells. In inactivated biomass (biosorption), the microorganisms usually sequester metal through surface bonding only; with active biomass (bioaccumulation), metals are concentrated through a combination of surface reactions, intra- and extracellular precipitation, and intra- and extracellular complexation reactions [5]. The phenomenon of metal accumulation in the microorganism will enhance the metal toxicity and in turn reduces the growth of microorganisms. Using growing cultures in bioremoval could avoid the need for a separate biomass production process such as cultivation, harvesting, drying, etc., but the requirements to maintain cell growth. The effectiveness of these processes is usually dependent on the parameters such as temperature, toxicity, oxygen level and availability of nutrients, etc. If the problem of metal toxicity to the growing cell is overcome by the use of metal-resistant organisms, the continually self-replenishing system can be left to run continuously for extended periods [6].

The objective of the present study was to investigate the effect of heavy metal ions on the growth and bioaccumulation properties of *Rhizopus arrhizus* as a function of initial metal ion concentration in batch experiments.

2. Experimental and mathematical models

R. arrhizus (MTCC 2233), a filamentous fungus was obtained from the Institute of Microbial Technology, Chandigarh, India.

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Nomenclature

K_s	saturation constant (g/l)
P	product concentration (mg/l)
P_{crit}	critical product concentration (mg/l)
S	substrate concentration (g/l)

Greek symbols

μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

The microorganism was grown aerobically in agitated enrichment media containing following composition: potato extract (200 g/l) and dextrose (20 g/l) at 25 °C. The pH of the medium was adjusted to 5.3 with dilute sulphuric acid.

Metal ion solutions of chromium(VI), nickel(II) and copper(II) were prepared by dissolving their respective salts, namely potassium dichromate ($K_2Cr_2O_7$), nickel sulphate ($NiSO_4{\cdot}6H_2O$) and copper sulphate ($CuSO_4{\cdot}5H_2O$), respectively, in double distilled water.

The factors affecting the growth and metal ion uptake rate of *R. arrhizus* were examined in 500 ml Erlenmeyer flasks with 100 ml of accumulation medium containing the respective metal ion solutions either chromium or nickel or copper ions with varying concentrations from 25 to 100 mg/l. The pH of the solution was adjusted to the desired value by using dilute sulphuric acid and autoclaved at 121 °C for 15 min. The sterilized accumulation medium was mixed with 100 ml of sterilized enrichment media containing potato extract and dextrose with desired pH. Five millilitres of inoculum was added to the above medium and the culture was grown at 25 °C and aeration was maintained by shaking at 200 rpm for 72 h. This shaking frequency supplied the culture with enough oxygen to attain logarithmic growth. The samples were drawn at pre-determined time intervals from a flask containing the same culture grown at identical conditions for measuring the residual metal ion concentrations and biomass concentrations.

Experiments were also carried out to study the growth of *R. arrhizus* in the varying dextrose concentration from 5 to 25 g/l at pH 5.6 at 25 °C in the absence of metals under identical conditions. The samples were drawn at pre-determined time intervals from a flask containing the same culture grown at identical conditions for measuring the dextrose concentration and biomass concentrations.

The residual chromium, copper and nickel ion concentration in the medium were determined spectrophotometrically at 540, 457 and 445 nm using diphenylcarbazide, neocuproine and dimethylglyoxime as the complexing agent [7]. The dextrose concentrations were also measured spectrophotometrically at 510 nm by dinitrosalicylic acid method [8]. The dry weight of the *R. arrhizus* was determined after the organism had been dried at 40 °C for 2 h.

Development of suitable mathematical models which would describe the metal inhibition and growth kinetics is very useful in designing the bioaccumulation systems using live cells. Several mathematical models have been proposed to quantify the inhibitory effects of the end product on cellular growth. Since metal ions cannot be degraded into other products, but can undergo changes in valency and/or convert into organo-metallic compounds, an attempt has been made to characterize the metal inhibition by end product inhibition.

2.1. Lineweaver–Burk plot

The well-known Monod expression for the growth kinetics does not represent the inhibitory effects of toxic end products. Lineweaver–Burk plot is the linearized form of Monod equation and is used to evaluate the kinetic parameters of bioaccumulation system accurately [9].

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}}\frac{1}{S} + \frac{1}{\mu_{max}} \quad (1)$$

where μ is the specific growth rate (h^{-1}), μ_{max} the maximum specific growth rate (h^{-1}), S the substrate concentration (g/l) and K_s is the saturation constant (g/l).

2.2. Aiba model

Though growth kinetics for most of the systems is well represented by Monod model, it fails to account for the inhibition effects. The inhibition effect mainly depends on the factors such as concentration of the metal ions, the tolerance capacity of the microorganisms and the operating conditions of the bioaccumulation process. Metal ions are considered to be the products in bioaccumulation of heavy metals by microorganism, its concentration on the microorganism increases with respect to time as bioaccumulation proceeds. The toxic effect of the metal ions ultimately affects the growth kinetics. As metal ion concentration increases specific growth rate decreases and the Monod equation must include a term for product inhibition [10].

$$\mu = f(S,\ P) \quad (2)$$

where P is the product concentration (mg/l). Aiba's exponential model has been widely used to predict the bioaccumulation kinetics in presence of inhibition due to an exponential term e^{-kp} which takes care of the product inhibition on growth kinetics of *R. arrhizus*. Aiba's exponential model, though has been widely used to analyze product inhibition, fails to give the critical value of inhibitory product concentration [9].

$$\mu = \frac{\mu_{max} S}{K_s + S} e^{-kp} \quad (3)$$

For given cell mass and substrate concentration, Eq. (3) predicts the specific growth rate more accurately for all product concentration.

2.3. Bazua and Wilke model

Bazua and Wilke's model developed for non-competitive inhibition can be used to characterize the metal inhibition kinetics of *R. arrhizus* and to find the inhibitory product concentration. The specific growth rate is affected by metal ions even