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Research Article

Bioaccumulation of Nickel Ions by *Rhizopus delemar*

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ABSTRACT

The current research investigated the bioaccumulation of Ni(II) ions by *Rhizopus delemar* in molasses-containing fluids in a batch reactor. Due to the low pH requirements that *R. delemar* requires, it may grow in wastewater, which has an acidic pH. In the absence of Ni(II) ions, the influence of pH and molasses concentration on the growth rate and concentration of *R. delemar* were examined. The highest level of microbial growth occurred at a pH of 4.0. Up to 20 g/L of sucrose content increased the maximum *R. delemar* concentration and the specific growth rate. While the substrate content in each growing medium including molasses was kept constant at 10 g/L, initial concentrations of metal ions were changed between 50 and 250 mg/L to evaluate the bioaccumulation of Ni(II) ions. It was discovered that when metal ions existed, the rate of microorganism growth slowed down as the metal ion concentration increased. The maximum growth rates were discovered to be 0.257 h^{-1} in the presence of 50 mg/L Ni(II). When media containing 50 mg/L Ni(II) ions, the efficiency of Ni(II) bioaccumulation was found to be 51.8%.

1. Introduction

Along with the growth of industry and human activities, the concentration of heavy metals in wastewater has been growing. Heavy metal-containing wastewater that enters the environment endangers both the ecology and the health of people. Heavy metals are dangerous because they cannot biodegrade and may result in cancer [1]. The heavy metals from different industries that are of the most concern include lead, zinc, copper, cadmium, chromium, and nickel. These substances come from a variety of sources, including metal complex dyes, insecticides, fertilizers, textile fixing agents, and bleaching agents [2]. Tolerable limits for various heavy metals in drinking water have been established by the WHO [3]. Heavy metal removal from industrial effluents is accomplished using conventional treatment methods such as chemical precipitation, ion exchange, coagulation/flocculation, adsorption, and electrochemical removal.

The ineffective removal of heavy metals and the production of toxic sludge are just two of the significant drawbacks of these low-cost systems. Reverse osmosis, nanofiltration, and ultrafiltration, among other membrane separation techniques, have all been applied to the treatment of water in recent years [4]. Because the production of activated carbon and ion exchange resins is dependent on fossil fuels such as coal and oil, they are not sustainable. However, the bioaccumulation technique is commonly favoured for its affordability, environmental friendliness, and ease of usage [5].

Heavy metals enter microorganisms' interior spaces via bioaccumulation, a metabolically active process involving the importer compounds that form a translocation channel across the

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bilayer of lipids. The heavy metals can be trapped inside the intracellular space by proteins and peptide ligands [6]. Metal is actively bioaccumulated by microorganisms. Numerous microorganisms, such as bacteria, algae, and fungi, have bioaccumulated metals from polluted habitats. The microorganisms that are utilized for bioaccumulation should be capable of withstanding one or more contaminants at increasing concentrations.

Additionally, they might be able to biotransform hazardous chemicals into less toxic or benign forms, reducing the toxicity of the pollutant and keeping it confined [7]. The bioaccumulation process involves two steps. In the first stage, metal ions cover the cell's surface, and metabolism is passive. The cell is then entered by metal ions. Only metabolically active cells can complete the second stage. If the second stage's ideal circumstances for organism growth are maintained, biomass production rises. This makes it possible for larger concentrations of metal ions to bind [8].

Bioremoval cultivating cultures may eliminate the requirement for other sources of biomass technologies such as cultivation, harvesting, drying, etc., but the uses of these methods are limited by the requirements of sustaining cell growth [9]. Additionally, environmental factors like temperature, pH, and biomass content always have a substantial influence on how well living cells can take in metal ions [10].

Fungi's cell walls are largely made up of polysaccharides which account for around 80% of their dry weight and may make up an additional 30% of their dry weight. According to Mir-Tutusaus et al. [11], fungi contain a large number of cell wall components with a high capacity to bind metals, making them efficient bio-sorbents. Fungi's cell walls include significant amounts of chitin, chitosan, glucan, and mannan in addition to a small quantity of glycoprotein. These polymers commonly contain hydroxyl (OH), carboxyl (R-COOH or R-CO_2H), amine (NH_2), and phosphate (PO_4^-, PO_3^-) functional groups as metal-binding ligands [12]. Fungi are inexpensive, kind to the environment, and abundant in nature [13]. It is known that the fungus uses both metabolism-dependent and independent mechanisms to survive in environments with high metal stress.

R. delemar is an opportunistic pathogen that lives in the soil on decaying plants [14] and is known as a superb metal accumulator. The maximum Cu(II) bioaccumulation was measured by *R. delemar* at pH 6.0, 32.6°C, with an initial concentration of 50 mg/L of Cu(II) [15]. Similar to this, *R. delamar* determined the maximal Zn(II) removal to be 26.31 mg/L in the bioaccumulation medium containing 30 mg/L Zn(II) initially at pH 5.0 and 35°C [16]. *R. arrhizus* was able to remove the most Cu(II) at an initial concentration of 75 mg/L, with a maximum specific intake of 10.76 mg/g [9].

Nickel is a common industrial chemical that is also regarded as a prominent pollutant of aquatic ecosystems. Basically, Ni forms soluble salts with other chemicals in aquatic ecosystems, which can adsorb onto other substances and have a variety of synergistic and antagonistic effects. Numerous factors, such as Ni concentration, water quality, and an organism's physiological state, affect the degree of Ni toxicity. In aquatic systems, nickel is a major contaminant. It has been stated that fish exposed to nickel collect nickel in their gills. Fish have suffered from compromised digestive and respiratory systems as a result. Ion regulation in fish is inhibited by Ni poisoning, which results in oxidative stress [17]. The World Health Organization (WHO) has established a Ni limit of 0.07 mg/L in drinking water [18].

The uptake of Ni(II) ions by *R. delemar* in a molasses medium was studied in this work both with and without Ni(II) ions. It was found that *R. delemar* may be used as an efficient biomass for metal ion bioremoval, such as Ni(II).

2. General Methods

2.1. *R. delemar* growth and preparation for bioaccumulation

The Fungi, *R. delemar,* was provided for the work by the NRRL of the US Department of Agriculture. The growing medium for *R. delemar* contains 2.0 g/L yeast extract, 0.5 g/L K_2HPO_4, 0.2 g/L $MgSO_4.7H_2O$, and 10 g/L molasses

sucrose. To accomplish the sterilization procedure, the growth medium was autoclaved (t: 15 min T: 121°C P: 1.2 atm). We bought KH_2PO_4 and $MgSO_4.7H_2O$ from Sigma Aldrich Company. A sugar plant in Ankara (Türkiye) offered the molasses sucrose. For the *R. delemar* development, molasses served as the only carbon source.

2.2. Bioaccumulation media preparation

$Ni(NO_3)_2$ was added to in distilled water at an average concentration of 1.0 g/L to make Ni(II) solution from stock. The prepared fermentation media had a range of 50 to 500 mg/L for metal concentrations. By adding HNO_3 (0.1 N) and NaOH (0.1 N) the fermentation media's pH was set to 4.0.

2.3. Bioaccumulation studies and measurements of the concentrations of *R. delemar*, molasses sucrose, and Ni(II)

In a batch system with a working capacity of 100 mL, bioaccumulation tests were conducted at a temperature of 25°C. The experiments employed a shaker running at 150 rpm. Once the cells had entered the exponential growth phase, sterilization was done to keep additional microorganisms out of the growth medium. The cells were modified with metal ion-containing media before inoculum. A 1 mL solution of microorganisms was added to begin the bioaccumulation tests. At time periods, samples from growing media were removed and centrifuged. The quantity of sucrose in the collected liquid was determined using a UV-Vis spectrophotometer (CHEBIOUS UV-spectrophotometer) set at 575 nm. Dinitrosalicylicacid was employed to color sucrose complexes. The quantity of unbioaccumulated Ni(II) ions in the effluent was measured at 232 nm using an AAS (GBC Avanta) with an air-acetylene flame. At 360 nm, the culture medium was measured spectrophotometrically [19].

2.4. Modelling growth and bioaccumulation of *R. delemar*

Models of microbial growth often depict variation in the maximum specific growth rate (μm), which is a reflection of metabolic activity [20]. Using microorganism cultures, microbial growth can be examined and modeled. The growth of a microorganism isolate is depicted by a sigmoidal curve made up of distinct and distinguishable growth phases including lag, exponential (log), stationary, and death (Fig. 1) [21].

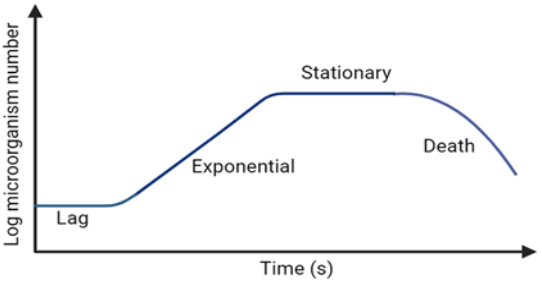

Figure 1. The microorganism batch-culture growth curve

In the batch system, the specific growth rate is used to indicate how the concentration of microorganisms changes over time during the exponential growth phase.

$$\frac{dX}{dt} = \mu X \quad (1)$$

Equation 2 is obtained by integrating Equation 1 at the boundary conditions of $X=X_o$ at time t=0 and X=X at time t=t.

$$In\frac{X}{X_0} = \mu t \quad (2)$$

where μ is specific growth rate (h^{-1}), X is dry microorganism concentration (g /L), and t is time (h).
The connection between the quantity of the rate-limiting nutrient (sucrose) and the specific growth rate may be determined using the Monod growth kinetics expression in the absence of inhibition. The formula is shown as follows:

$$\mu = \frac{\mu_m S}{K_s + S} \quad (3)$$

where Ks is the saturation constant (g/L), and μ_m is the maximum specific growth rate when S>>Ks [22].

The uptake of Ni(II) by *R. delemar* was determined using the following equation 4:

$$q = \frac{C_i - C_f}{X} = \frac{C_{acc}}{X} \quad (4)$$

where q is bioaccumulated Ni(II) ions per unit weight of dried biomass (mg/g mo), C_i is the initial Ni(II) ion concentration (mg/L), C_f is the residual Ni(II) concentration in solution (mg/L), X represents the amount of microorganisms per liter (g/L), and C_{acc} is the amount of bioaccumulated Ni(II) ions per liter (mg/L) [23]. The removal percentages of Ni(II) were determined with an equation as below:

$$\%removal = \frac{C_i - C_{eq}}{C_i} x100 \quad (5)$$

where C_o is the initial Ni(II) concentration (mg/L) and C_{eq} is the residual metal concentration at equilibrium (mg/L).

3. Results and Discussion

3.1. Influence of pH on microbial growth of *R. delemar*

Environmental elements necessary for microbial survival and development are influenced by pH. By altering the salinity and composition of aqueous solutions, it controls the bioavailability of nutrients and trace elements. Additionally, pH affects the reactivity of naturally occurring organic matter as well as the activities of extracellular enzymes [24]. pH could inhibit microbial metabolism. The majority of laboratory cultures are found in a pH range of 3–4 units, which corresponds to a 3–4 order of magnitude difference in proton chemical activity [20]. Based on their optimal growth pH, microorganisms may be divided into three groups: alkaliphiles, which grow quickly above pH 9, neutrophiles, which grow best between pH 5 and 9, and acidophiles, which grow best at pH 5 [25]. When the ambient pH deviates from the optimal pH ranges, microbial growth rates decrease. The topologies of microbial populations can be affected by pH through changing the thermodynamics and kinetics of redox reactions. Numerous redox activities produce or consume protons, hence pH affects the free energy outputs of these processes [26].

The pH of wastewater is significantly impacted by the presence of heavy metals in natural circumstances [27]. To study the impact of pH on microorganisms concentration and specific growth rate, the growth media's pH was changed between 2.0 and 5.0. At pH 4.0, the highest concentration and growth rate of *R. delemar* were found to be 3.76 g/L and 0.297 h^{-1} respectively as shown in Fig. 2. According to Evirgen and Sağ Acikel [16], the greatest biomass concentrations of *R. delemar* were achieved on days 4 and 5 of development, at pH 5.0 and temperatures of 25 and 35 °C, respectively, in the metal-free medium. These concentrations were 1.09 and 1.16 g/L, respectively [16].

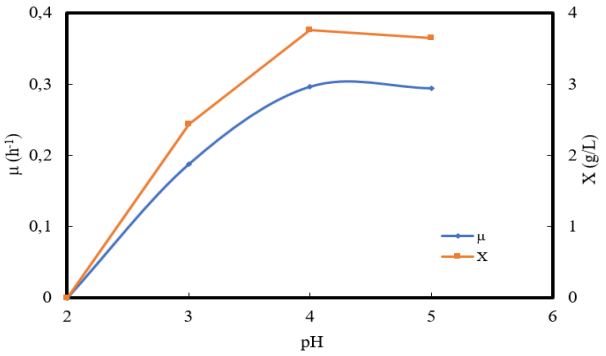

Figure 2. Effect of the initial pH value on the maximum concentration of microorganisms and the specific growth rate (S_o: 10 g/L)

Functional groups on the fungal cell surface, such as COOH, OH, NH_2, and PO_4^{3-}, are important for the sorption of heavy metal ions and display different behaviors depending on the medium's pH. The chemical properties of the biomaterial and their surface functional groups (including COOH, PO_4^{2-}, and NH_2) were significantly influenced by the pH of the medium [28].

3.2. The effect of initial sucrose content on *R. delemar* microbiological growth

Molasses, which contain a high concentration of sugars, is a low-cost feedstock for the generation of value-added bioproducts via bioconversion [29]. It can be used as a resource for ethanol production since it contains carbon sources required for yeast strain growth and metabolism [30]. Research shows that molasses is a suitable medium for the cultivation of microorganisms such as algae [31], yeast [32] and bacteria [33].

Table 1 shows the results of testing the effects of molasses sucrose concentration on specific growth rates and maximum microorganism concentrations at concentrations ranging from 1 to 20 g/L. The specific growth rate and maximum

microorganisms concentration were found a 0.365 h^{-1} and 4.55 g/L, respectively at 20 g/L sucrose concentration. Using the Monod equation, the maximum specific growth rate and saturation constant were estimated to be 0.406 h^{-1} and 24.182 g/L, respectively. According to Aksu and Dönmez, raising the sucrose content from 5 to 20 g/L enhanced the specific growth rate of *Kluyveromyces* cells from 0.090 to 0.222 h^{-1} [34]. Authors in another research achieved similar results. *Candida* cell specific growth rate increased from 2.28 to 4.80 day^{-1} when sucrose concentration was raised from 5 to 15 g/L in the absence of dye anions [35].

Table 1. Specific growth rates and maximum concentrations of *R. delemar* at different molasses concentration

S_o (g/L)	μ (h^{-1})	X (g/L)
1	0.085	0.98
2	0.145	1.67
5	0.212	2.55
10	0.297	3.76
15	0.350	4.24
20	0.365	4.55

3.3. The influence of initial nickel concentration on *R. delemar* growth

The majority of microorganisms have a biphasic reaction to a variety of heavy metals. Growth is promoted at low metal concentrations, but as the metal concentration grows, growth is hindered and finally ceases. The "transition" area between metal excitation and inhibition is often in a relatively small concentration range [36].

The bioaccumulation of Ni(II) ions in *R. delemar* was studied at initial molasses sucrose concentrations of 10 g/L, pH 4.0, and initial metal ion concentrations ranging from 50 to 500 mg/L. Fig. 3 depicts the relationship between the specific growth rate, the maximum microorganism concentration, and initial metal ion concentrations. As the initial Ni(II) ion concentration grew up to 500 mg/L, the specific growth rate and maximum microorganism concentration dropped. Metal ion concentrations rose in the growth medium, impeding microorganism development. At 100 mg/L initial Ni(II) ion concentration, the specific growth rate and maximum microorganisms concentration were 0.222 h^{-1} and 2.36 g/L, respectively. Açıkel and Alp [37] stated that as the initial metal ion concentration increased, *R. delemar* growth was inhibited. *Aspergillus niger* growth was detected in the presence of Cu(II), Pb(II), and Cr(VI) ions by Dursun et al. [38].

Maximum biomass production by *A. niger* has been documented in the absence of metal ions, but all tested amounts of Cr(VI) hindered growth. Additionally, according to Acikel and Ersan [39], the specific growth rates of *R. delemar* dramatically decreased when Ni(II) ion concentrations increased in the region of 0–50 mg/L. Naskar et al. [40] studied the bioaccumulation of Ni(II) in developing *Bacillus sp.* cells. They reported that no increase in microbial growth at metal ion concentration over 50 mg/L.

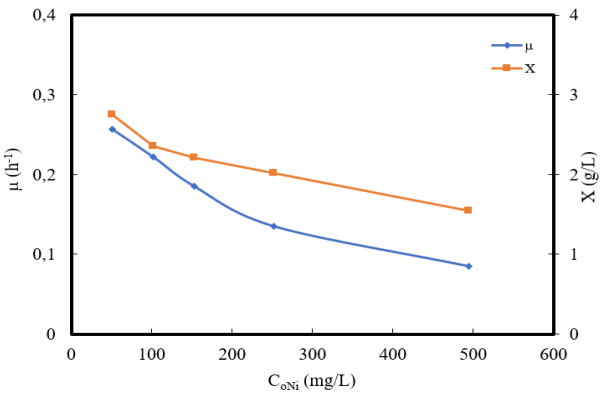

Figure 3. The effect of initial nickel content on the specific growth rate and maximum microorganism concentration of *R. delemar* (S_o: 10 g/L)

Through various interactions with enzymes involved in biodegradation or general metabolism, metal ions have been demonstrated to inhibit microbial processes [41]. Metal ions (e.g., Cd^{2+}) are capable of binding to DNA bases to break single-stranded DNA or binding to sulfhydryl (-SH) groups of enzymes [42]. Metal ions may substitute for physiologically essential cations within an enzyme, for example, Cd^{2+} substitutes for Zn^{2+} [43]. Three patterns of the detrimental effects of metal ions on cytoplasmic biodegradation have been observed at the level of microbial communities. (1) As metal ion concentration rises, inhibition rises gradually. (2) High metal ion concentrations impede biodegradation, while low metal ion concentrations promote it. (3) Biodegradation is less inhibited by high metal concentrations than

by low amounts of metal. So, in order to resist hazardous metals, microbes have evolved to use a variety of techniques, such as metal reduction, metal efflux pump, and metal chelate synthesis. As a result, various biological systems may be more or less tolerant of certain metal ions depending on their toxicity and effect [44].

3.4. The influence of the initial concentration of nickel on bioaccumulation

The correlation between bioaccumulated Ni(II) ion concentrations and the quantity of Ni(II) bioaccumulated per unit dry weight of *R. delemar* versus initial Ni(II) ion concentration is depicted in Fig. 4. The bioaccumulated Ni(II) ion concentrations and quantities of Ni(II) bioaccumulated per unit dry weight of fungal cell at 100 mg/L initial Ni(II) ion concentration were 48.8 mg/L and 20.67 g Ni(II)/g dry weight of microorganism, respectively. Li et al. [45] investigated cadmium bioaccumulation in *Zygosaccharomyces rouxii* and *Saccharomyces cerevisiae* cultures. They found that both yeasts had a high cadmium removal rate at low cadmium concentrations. At the same initial cadmium concentration, *Z. rouxii* had a greater removal rate than *S. cerevisiae*. Total, intracellular and cell-surface cadmium bioaccumulation of both yeasts increased when cadmium concentrations rose in the medium.

The bioaccumulation efficiency of *R. delemar* decreased as the initial metal ion concentration increased (Table 2). The removal efficiency decreased from 51.75% to 20.44% when initial metal ion concentration increased from 50 mg/L to 500 mg/L. Heat-treated *Saccharomyces cerevisiae* were tested for their ability to remove Cu(II) ions from aqueous solutions by Stanescu et al. [46].

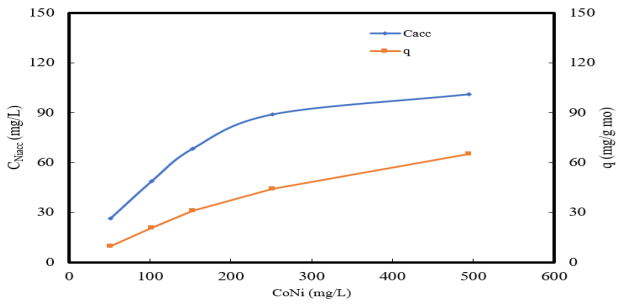

Figure 4. The effect of initial nickel concentration on bioaccumulated nickel concentration and bioaccumulated nickel quantity per unit dry weight of microorganism (S_o: 10 g/L)

They discovered that as the initial metal concentration increased, the removal efficiency declined (55.89-39.04%) for the most concentrated samples (100-250 mg/L). This is due to Ni(II) ions in solution not being connected to the biomass at greater concentrations due to biomass saturation induced by an increase in the number of ions competing for available binding sites.

Table 2. Bioaccumulation efficiency of nickel in the growth medium (S_o=10 g/L)

C_{oNi} (mg/L)	S_o (g/L)	Bioaccumulation efficiency %
50	10.12	51.75
100	9.95	47.70
150	10.15	44.91
250	9.98	35.30
500	9.95	20.44

Toxic contaminants in soil or water can be reduced or removed using fungus. According to the species, type, and concentration of the heavy metal, the threshold for the fungus's tolerance to it varies, ensuring that the inhibition of growth by a low concentration of one metal does not pose a barrier to its tolerance of another metal with a high concentration. Most likely, inherent physiological mechanisms are to blame for the variance in how different fungi react to trace metals. Most fungi species are not universally sensitive to all metals, even if they are sensitive to one or more types of metals [47].

4. Conclusion

The bioaccumulation of Ni(II) ions by *R. delemar* in molasses media in the presence and absence of Ni(II) ions was investigated in this work. The removal of comparatively non-toxic metals through bioaccumulation is very promising, but the detrimental effects of toxic metals make this process complex. *R. delemar* was able to remove Ni(II) at low concentrations, which was mostly due to intracellular Ni(II) bioaccumulation. Microbial growth was greatest at pH: 4 and 25°C. Increases in molasses sucrose content up to 20 g/L resulted in increases in growth rate and microorganism concentration. The rate of microorganism growth slowed when metal ion concentration increased. The removal % of Ni(II) bioaccumulation by *R. delemar* reduced as initial Ni(II) concentrations increased.

We think that *R. delemar* might be used as a biomass for Ni(II) removal in wastewater. A more complete research of operational parameters such as temperature, biomass content, agitation, and so on should be carried out to better understand *R. delemar*'s Ni(II) bioaccumulation.

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