A Comparative Investigation of Cu (II), Ni (II) and Molasses Sucrose Effects on the Growth of *Candida* Yeasts and Metal Bioaccumulation

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Ünsal Açikel

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Research

Keywords: *Candida lypolytica*, *Candida utilis*, *Candida tropicalis*, *Candida membranaefaciens*, Bioaccumulation, Wastewater, Cu(II), Ni(II)

Posted Date: October 9th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-87514/v1>

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Abstract

In this study, microorganism growth and bioaccumulation of Cu(II) and Ni(II) ions by *Candida* yeasts was tested as a function of sucrose, and metal ion concentration in metal-free and metal media. The growth of the yeasts and bioaccumulation of heavy metals was studied under laboratory conditions in the batch system. The optimum pH value for maximum growth and Cu(II) and Ni(II) bioaccumulation was obtained as 4.0 for the yeasts at 10 g/L constant sucrose concentration. The maximum specific growth rate and microorganism concentration increased with increasing sucrose concentration up to 20 g/L for *Candida* yeasts. It was observed that the metal uptake capacity of yeasts was increased with increasing initial metal concentrations in the range of 25-250 mg/L for Cu(II) and Ni(II) ions. Although Cu(II) and Ni(II) ions caused an inhibition effect on the growth of the yeasts, *Candida lipolytica* showed the highest specific growth rate and metal uptake capacity among the other yeasts. It was found that the inhibition effect of the Ni(II) ion was higher than Cu(II) ion on all the yeast. Monod equation was used to study the inhibition kinetics of bioaccumulation of Cu(II) and Ni(II). It was observed that saturation constant (K_s) increased with increasing the initial Cu (II) and Ni (II) ion concentrations whereas maximum specific growth rates (μ_{max}) decreased.

1. Introduction

Heavy metal pollution due to industrial activities causes major environmental problems, even if they are found in trace amounts in wastewaters (Abbas et al. 2014). Bioaccumulation which is applied to minimize the toxic effects of heavy metals on the environment is one of the low-cost, highly efficient, and eco-friendly technologies for removing heavy metals from aqueous solutions (Wang and Chen 2006; Fadel et al. 2017). Most of the studies conducted in the last few years have focused on the use of living and non-living microorganisms such as bacteria, yeast, fungi, and algae that can be used as biomass for wastewater treatment processes (Oztürk 2007; Muñoz et. al. 2012). The biosorption of metals is affected by various factors such as pH, temperature, concentration, biomass type, contact time and presence of different metal ions in solution (Sağ 2001). Bioaccumulation is a biochemical process based on the use of living cells to remove heavy metals from wastewaters (Açikel and Alp 2001). Bioaccumulation of heavy metals by microorganisms occurs in two stages. The first is the passive step in which physical adsorption and ion exchange occurs on the surface of the microorganism. This step is very fast due to surface adsorption. The second stage of metal bioaccumulation is the intracellular metal removal stage, which occurs slower due to the metabolic activity of microorganisms (Fomina and Gadd 2014). Bioaccumulation has been studied in many studies for the removal of heavy metals from wastewater due to the active growth of cells, cell regeneration, and metabolic physical adsorption of metals. Among these studies, characterization of *Saccharomyces cerevisiae* and *Candida krusei* resistant to heavy metal ions, adsorption of Cu (II), and phenol by *Candida tropicalis*, removal of Cu (II) by *Candida krusei*, isolation of Cr (VI) from tannery wastes can be given as examples (Wilde and Benemann 1993; Anh et al. 2016; Vadkertiová and Sláviková 2006; Honfi et al. 2016; Luk et al. 2017; Ramírez et al. 2004). Yeast cells are widely used as a raw biosorbent in the biotechnology and pharmaceutical industries. Compared to other microorganisms, yeasts can grow in matrices containing metal ions even if high concentrations (Banafid et al. 2017). *Candida* species, a member of the yeast family, are one of the most popular microorganisms due to satisfying biosorption performance (Muter et al 2002). It has been found that different *Candida* species such as *Candida utilis*, *Candida tropicalis*, *Candida membranaefaciens* and *Candida lipolytica* can efficiently bioaccumulate heavy metals under various experimental conditions, and the metal uptake behaviors of the yeasts are different (Aksu and Dönmez 2003; Bai et al. 2019; Yin et al. 2008). The monod equation provides a simple model for describing the growth of a cell in a given nutrient media. The equation is used to define the specific growth rate of a microbial cell as a function of a limiting substrate concentration for estimate substrate biodegradation and microbial growth (Monod 1949).

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

where μ_{max} is the maximum growth rate when there is enough substrate supplied to the cell and the value exceeds the limiting substrate concentration, $S \gg K_s$. The constant K_s is the saturation constant or half velocity constant and is equal to the concentration of the rate-limiting substrate when the specific growth rate is equal to one-half of the maximum.

If all the necessary nutrients are provided for growth media and environmental conditions are optimum, the increasing on microorganism numbers or microorganism biomass can be measured as a function of time to obtain a microbial growth curve. Different growth phases can be observed within a growth curve. These are the lag phase, exponential or lag phase, stationary phase, and death phase. Each of these phases represents a distinct growth period associated with typical physiological changes in cell culture (Ricica 1971). If Eq. 1 is rearranged:

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_s}{\mu_m} \frac{1}{S} \quad (2)$$

K_s and μ_m values can be determined from the $1 / \mu_m$ versus $1 / S$ plot. The slope of K_s / μ_m and $1 / \mu_m$ gives a line intersecting the y-axis. When molasses sucrose is used as the main substrate in growth media containing high concentrations of inhibitory (toxic) substances such as heavy metals, microbial growth is inhibited and the growth rate depends on inhibitor concentration. The steady-state specific growth rate of microbial species was assumed to fit the Monod growth kinetics described in Eq. 1. For batch cultures, growth rate:

$$\frac{dx}{dt} = \mu X \text{ OR } \ln\left(\frac{x}{x_0}\right) = \mu t \quad (3)$$

Where X_0 is the initial biomass concentration (g / L), X is the biomass concentration (g / L) at time t and μ is the specific growth rate (h^{-1}). Metal ion removal by microorganisms is calculated by the mass balance equation:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (4)$$

Where q_e is metal ion uptake per unit mass of biomass at equilibrium (mg / g biomass), C_e , metal ion concentration in solution at equilibrium (mg / L), C_0 , initial metal ion concentration in solution (mg / L), V , initial metal ion solution volume (L) of and m is the mass of biomass (g) (Horsfall and Spiess 2005).

The aim of this study is to investigate the combined effect of molasses sucrose and Cu (II) and Ni (II) ions on growth and bioaccumulation properties of *Candida* species. In present study, the inhibition effects of Cu (II) and Ni (II) ions and the specific growth rates and kinetic constants was examined. *Candida* species was chosen due to their highly resistant to Cu (II) and Ni (II) ions and can grow at acidic pH (Dönmez and Aksu 2001). Furthermore, copper and nickel are common pollutants of industrial wastewaters. Molasses sucrose contains high sucrose and other nutrients and provides low cost, easy accessibility and storage (Tchounwou et al. 2012; Joseph 2009; Gönen and Aksu 2008).

2. Material And Method

2.1 Reagents

The chemicals used in the experiments were purchased from Sigma-Aldrich. Microorganisms which was used in this study includes ATCC® 201377™ *C. membranifaciens*, ATCC® 9950™ *C. utilis*, ATCC® 13803™ *C. tropicalis*, ATCC® 9733™ *C. lipolytica*. The *Candida* type yeast strains obtained from the Biology Department of Ankara University. Molasses sucrose was supplied from the sugar factory in Ankara (Turkey).

2.2 Preparation of microorganism growth media with and without a single metal ion

Molasses sucrose, a sugar industry waste, has been used as the main carbon source for the growth of microorganism. Microorganisms showed maximum bioaccumulation at optimum pH value and bioaccumulation experiments were conducted at this pH. Yeasts were reproduced in aqueous media containing molasses sucrose (1–10 g / L) with (1 g / L) $(\text{NH}_4)_2\text{SO}_4$ and (1 g / L) K_2PO_4 at 25 °C and pH 4. The growth and cultivation media sterilized in an autoclave operating at 121 °C at 0.99 bar for 15 minutes. Subcultures were grown for 4 days at a rotating speed of 150 rpm. 1000 mg / L Cu (II) and Ni (II) stock solutions were prepared with $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ and $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ with distilled water. Working solutions (25–250 mg / L) were prepared by diluting stock solutions (1000 mg / L) to obtain different metal ion concentrations. The pH of the working solutions was adjusted using a pH meter and by adding either 0.1 N NaOH and HNO_3 as appropriate.

2.3 Metal adaptation of *Candida* yeasts

Candida lipolytica, *Candida utilis*, *Candida tropicalis*, *Candida membranifaciens* was adapted to single Cu (II) and Ni (II) ions in culture media. The adaptation was conducted exposing yeast cells to Cu (II) and Ni (II) ions during the growth stage to increase metal resistance. Subcultures was obtained through different concentrations of Cu(II) and Ni(II) ions in the range of 25–250 mg / L at changing sucrose concentrations (1–20 g/L) in growth media. Yeast cultures that resistant to 25 mg/L Cu(II) and Ni(II) ions at 10 g/L molasses sucrose was used to the first inoculation. 1 mL culture medium was used to inoculate the next culture medium containing 50 mg / L Cu (II) and Ni (II) ions at the same molasses sucrose concentration when the stressed growth culture containing *Candida* and metal reached to the exponential growth phase. In the metal adaptation process, the metal ion concentration was increased and the sucrose concentration was changed in the defined concentration range. Metal adaptation experiments were performed with 250 mL flasks containing 100 mL of media at 150 rpm and 25 °C.

2.4. Analysis of microorganism, metal and molasses sucrose concentration

5 mL samples were centrifuged at 3000 rpm for 5 min and the supernatant fluid used to determine molasses sucrose and Cu(II) and Ni(II) ion concentrations. After centrifugation, the solid residual was used to analyze yeast concentration. Molasses sucrose concentration was examined at 575 nm by Miller method. For microorganism concentration in the sample supernatant, the turbidity of the fermentation media was measured at 360 nm. Microorganism concentration was determined using a calibration curve relating the wet weight of the biomass to the dry weight of the biomass at 25 °C. Sodium diethyldithiocarbamate was used as the complexing agent for Cu(II) and Ni(II) ions. Residual metal concentrations was measured at 460 nm and 340 nm for Cu(II) and Ni(II), respectively (Snell and Snell 1959; Forouchi and Gunn 1983).

3. Results And Discussion

Bioaccumulation and growth properties of metal adapted *Candida* yeasts was investigated as a function of initial Cu(II) and Ni(II) ions and molasses sucrose concentration at pH 4 and 25 °C. The uptake yield (Uptake %) was determined as the ratio of bioaccumulated concentration of metal ion at the end of microbial growth to the initial metal ion concentration. The results are given as the units of bioaccumulated metal ion concentration ($C_{\text{acc,m}}$: mg / L) and specific metal ion uptake determined as the amount of metal ion per unit of dry weight of cells (q_m : mg / g), dried cell and accumulated metal ion concentrations in any time (X : g L; C_{acc} : mg / L), specific growth rate of yeast (μ : h^{-1}). The results showed that the metal uptake capacity of *Candida* yeasts were different from each other due to the physiological properties of the yeasts (Pabst et al 2010). Researchers reported that microorganisms demonstrate resistance to metal ions due to the environmental toxic effects of heavy metals. For efficient removal of heavy metals from wastewaters, optimum pH and temperature should be investigated. Generally, metal bioaccumulation occurs at 25–30 °C for yeasts (Abbas et al. 2014). High temperatures above 45 °C may damage cell proteins and decrease metal uptake capacity (Vadkertiová and Sláviková 2006).

3.1. The effect of initial pH on the growth of *Candida* species

The effect of pH on the specific growth rate of *Candida* yeasts and maximum dry microorganism concentrations was investigated at 25°C in aqueous medium containing molasses sucrose (1–10 g / L), (1 g / L) (NH₄)₂SO₄ and (1 g / L) K₂PO₄. The effect of pH was tested inoculating the culture medium in the pH range of 2.0–5.0. The initial pH value is a vital factor affecting the growth of *Candida* yeasts. All yeasts showed adaptation and growth in medium with pH values at 2.0–5.0. Besides some special cases, the low initial pH showed the ability to prolong the yeast lag phase, altering the consumption rate of total sugar, affecting the accumulated mass loss. Maximum specific growth rate and microorganism concentration were obtained at pH 4.0 (Fig. 1). *Candida lipolytica* showed the highest specific growth rate and microorganisms concentration (specific growth rate 0.253 h⁻¹ at pH 4.0, microorganism concentration 0.308 g / L). Optimum growth conditions for living biomass were determined as pH 4.0 and 25 °C for *Candida lipolytica*, *Candida utilis*, *Candida tropicalis*, *Candida membranifaciens* (Dönmez and Aksu 2001; Aksu and Dönmez 2000).

3.2 Effect of initial molasses sucrose concentration on growth of *Candida* species

The effect of initial molasses sucrose concentration on the growth rate of *Candida* species in metal-free media was investigated in the sucrose concentration range of 1.0–20.0 g/L, at pH 4.0 and at 25 °C. During experimental studies, molasses sucrose was used as the sole and main carbon source. It was observed that the yeast concentrations and specific growth rates increased with increasing initial sucrose concentration up to 20 g/L (Fig. 2). Substrate inhibition was not seen. As a carbon source, molasses sucrose is suitable for microbial growth (Bamforth and Cook 2019). Aksu and Dönmez (2000) used molasses sucrose as a carbon source to obtain an efficient metal removal by *Kluyveromyces marxianus* and *Candida utilis*. They observed that the specific growth rate of microorganisms and the microorganism concentration increased with increasing initial sucrose concentration (Aksu and Dönmez 2000). Kujan et al. (1995) studied the removal of Cd (II) ions by *Candida utilis* and reported that bioaccumulation depends on the organic carbon source as a substrate. When glucose was used as a carbon source, bioaccumulation capacity was ten times higher than xylose (Kujan et al. 1995).

3.3 Effect of initial Cu (II) and Ni (II) ion concentrations on growth of *Candida* species

The effect of Cu(II) and Ni(II) ion concentrations on the specific growth rate and microorganism concentration of *Candida* species were investigated at different initial Cu(II) and Ni(II) concentrations ranging from 25 to 250 mg/L. It was observed that specific growth rates and microorganism concentrations decreased with increasing Cu (II) and Ni (II) ion concentrations. Single Cu (II) and Ni (II) ions inhibited the growth of yeasts in the medium. It was found that the inhibition effect of Ni(II) ions on yeast growth was higher than Cu (II) ions for all yeasts. *Candida lipolytica* showed the highest specific growth rate and microorganism concentration while *Candida utilis* had a minimum specific growth rate and microorganism concentration among the yeasts. Growth parameters of $\mu_{\max}$ and K_s were determined using the double reciprocal plot of the Monod Eq. (1). When initial Cu(II) and Ni(II) ion concentrations were increased, the maximum specific growth rate of the yeasts decreased while saturation constants increased (Table 1). Compared to metal-free media, the decrease in the specific growth rates of the yeast was higher than yeast concentrations in medium containing 100 and 250 mg / L Cu (II) and Ni (II) at a constant 10 g / L molasses sucrose (Table 2, Table 3).

Table 1
Comparison of the maximum specific growth rates and the saturation constants in the presence of increasing concentrations of single Cu(II) and Ni(II) ions (So: 1–20 g / L; pH: 4, T: 25°C; SR: 150 rpm).

Metal Ion		<i>Candida membranefeciens</i>		<i>Candida utilis</i>		<i>Candida tropicalis</i>		<i>Candida lipolytica</i>	
C _{iCu} mg/L	C _{iNi} mg/L	$\mu_{\max}$ (h ⁻¹)	K_s (g/L)	$\mu_{\max}$ (h ⁻¹)	K_s (g/L)	$\mu_{\max}$ (h ⁻¹)	K_s (g/L)	$\mu_{\max}$ (h ⁻¹)	K_s (g/L)
25.0	0.0	0.3542	5.3990	0.3415	5.8051	0.3743	4.555	0.4223	5.0800
50.0	0.0	0.3542	5.7532	0.3314	5.7232	0.3579	4.1755	0.4254	5.3688
100.0	0.0	0.3370	5.3540	0.3414	6.4721	0.3631	4.6773	0.3885	4.5681
150.0	0.0	0.3352	6.0040	0.3148	6.1285	0.3354	4.4410	0.3907	5.2713
200.0	0.0	0.3367	6.5868	0.2995	6.0334	0.3380	4.8870	0.3781	5.3783
250.0	0.0	0.3108	6.7714	0.2851	6.5197	0.3137	4.984	0.3358	4.9207
0.0	25.0	0.3468	5.7250	0.3196	5.600	0.3525	4.2434	0.3450	4.4736
0.0	50.0	0.3314	5.4340	0.3053	5.2806	0.3651	4.7962	0.4070	5.3946
0.0	100.0	0.3337	5.7184	0.3021	5.4083	0.3566	5.0135	0.3952	5.2681
0.0	150.0	0.3177	6.6938	0.2826	6.0892	0.3048	4.2690	0.3582	5.3844
0.0	200.0	0.3097	7.0040	0.2783	6.3743	0.3099	5.1117	0.3286	4.9181
0.0	250.0	0.2671	6.2658	0.2647	7.1964	0.2840	5.2692	0.3174	5.7960

Table 2

Comparison of decrease in maximum specific growth rates obtained in the metal-free fermentation media with Cu(II) and Ni(II) ions present as the single metal at 100 and 250 mg/L (So: 10 g / L; pH: 4, T: 25 °C; SR: 150 rpm).

Metal Ion		<i>Candida membranefeciens</i>		<i>Candida utilis</i>		<i>Candida tropicalis</i>		<i>Candida lypolytica</i>	
C _{iCu} (mg/L)	C _{iNi} (mg/L)	$\mu_{\max}$ (h ⁻¹)	% Decrease	$\mu_{\max}$ (h ⁻¹)	% Decrease	$\mu_{\max}$ (h ⁻¹)	% Decrease	$\mu_{\max}$ (h ⁻¹)	% Decrease
0.0	0.0	0.245	0.0	0.238	0.0	0.289	0.0	0.308	0.0
100.0	0.0	0.223	9.0	0.207	13.0	0.258	10.7	0.278	9.7
250.0	0.0	0.189	22.9	0.175	26.5	0.218	24.6	0.236	23.4
0.0	100.0	0.215	12.2	0.199	16.4	0.249	13.8	0.268	13.0
0.0	250.0	0.170	30.6	0.157	34.0	0.197	31.8	0.215	30.2

Table 3

Comparison of decrease in maximum dried microorganism concentrations obtained in the metal-free fermentation media with Cu(II) and Ni(II) ions present as the single metal at 100 and 250 mg/L (So: 10 g / L; pH: 4, T: 25 °C; SR: 150 rpm).

Metal Ion		<i>Candida membranefeciens</i>		<i>Candida utilis</i>		<i>Candida tropicalis</i>		<i>Candida lypolytica</i>	
C _{iCu} (mg/L)	C _{iNi} (mg/L)	X _{max} (g/L)	% Decrease	X _{max} (g/L)	% Decrease	X _{max} (g/L)	% Decrease	X _{max} (g/L)	% Decrease
100.0	0.0	2.666	2.4	2.456	7.6	2.831	5.6	2.968	4.6
250.0	0.0	2.335	14.5	2.148	19.2	2.483	17.2	2.607	16.2
0.0	100.0	2.574	5.8	2.371	10.8	2.735	8.8	2.868	7.8
0.0	250.0	2.175	20.4	1.199	54.9	2.315	22.8	2.432	21.8

3.4 Bioaccumulation of Cu(II) and Ni(II) ions by *Candida* species

The effect of Cu(II) and Ni(II) ions and molasses sucrose on the bioaccumulation properties of adapted *Candida* yeasts was studied. Initial metal ion concentration in the feed medium was varied in the range 25–250 mg / L for Cu(II) and Ni(II) at 1–20 g/L molasses concentration. It was observed that bioaccumulated Cu(II) and Ni(II) ions and microorganism growth increased with increasing initial molasses sucrose concentrations for all yeasts. Results showed that the uptake efficiency of Cu (II) ions was higher than Ni (II), and *Candida lypolytica* performed the highest removal efficiency in medium containing both single Cu (II) and Ni (II) ions. It should be noted that increasing sucrose concentration significantly increased the growth and metal holding capacity of microorganisms. It was seen that the amount of Cu (II) and Ni (II) ions uptake per gram dry microorganism increased with increasing the initial metal concentrations up to 250 mg/L (Table 4 and Table 5). Dursun et al. (2003) observed the inhibition effects of metal ions on the growth of *A. niger* and obtained maximum removal capacities of 15.6 mg/g and 34.4 mg / g at initial Cu (II) and Pb (II) concentrations of 100.0 mg / L, respectively. (Dursun et al.2003b). Aksu and Dönmez (2001) investigated heavy metal bioaccumulation with *Candida sp.* They reported that specific metal uptake was 36.9 mg Cu (II)/ g at 783.6 mg / L initial Cu(II) concentration and 46.8 mg Ni(II) / g at 321.5 mg / L initial Ni (II) concentration (Dönmez and Aksu 2001). Gönen and Aksu (2008) investigated the Cu (II) removal efficiency with *Candida utilis* as a function of molasses sucrose and metal ion concentration. They found that the maximum metal removal efficiency was 34.2% in growth medium containing 15 g / L sucrose and 50 mg / L Cu (II) (Gönen and Aksu 2008). Bioaccumulated Cu (II) ion and specific Cu (II) uptake were determined as 46.80 mg/L and 17.56 mg/g, 38.4 mg/L and 15.64 mg/g, 51.48 mg/L and 18.18 mg/g, 53.60 mg/L and 18.98 mg/g respectively for *Candida membranaefaciens*, *Candida utilis*, *Candida tropicalis*, *Candida lypolytica* in fermentation media containing 10 g/L constant initial molasses sucrose and 100 mg/L Cu(II). Bioaccumulated Ni(II) ions and specific Ni(II) uptake were determined as 37.70 mg/L and 15.42 mg/g, 31.20 mg/L and 13.16 mg/g, 44.30 mg/L and 16.20 mg/g, 48.28 mg/L and 17.04 mg/g respectively for *Candida membranaefaciens*, *Candida utilis*, *Candida tropicalis*, *Candida lypolytica* in fermentation media containing 10 g/L constant initial molasses sucrose and 100 mg/L Ni(II).

Table 4

Comparison of the maximum Cu(II) biomass concentration, the bioaccumulated Cu(II), the specific Cu(II) uptake, and the Cu(II) metal ion bioaccumulation efficiency containing single Cu(II) ion (pH 4.0; T = 25 °C; SR:150 rpm)

<i>Candida membranaefaciens</i>						<i>Candida utilis</i>				<i>Candida tropicalis</i>				<i>Candida</i>
S _i (g/L)	C _i (mg/L)	X _{max} (mg/L)	C _{acc,Cu} (mg/L)	q _{Cu} (mg/g)	%uptake	X _{max} (mg/L)	C _{acc,Cu} (mg/L)	q _{Cu} (mg/g)	%uptake	X _{max} (mg/L)	C _{acc,Cu} (mg/L)	q _{Cu} (mg/g)	%uptake	X _{max} (mg/L)
1	0	1.36				1.07				1.47				1.95
5	0	2.23				1.98				2.53				2.62
10	0	2.85				2.65				2.99				3.11
20	0	3.11				3.05				3.24				3.48
1	25	1.30	2.50	1.91	10.00	1.01	2.13	2.09	8.52	1.43	2.88	2.00	11.52	1.92
5	25	2.15	8.50	3.95	34.00	1.89	7.23	3.81	28.92	2.46	9.78	3.96	39.12	2.58
10	25	2.76	14.50	5.25	58.00	2.54	12.60	4.95	50.40	2.93	16.40	5.59	65.60	3.07
20	25	3.01	17.10	5.67	68.40	2.93	15.1	5.15	60.4	3.17	19.67	6.19	78.68	3.45
1	100	1.24	6.88	5.54	6.88	0.96	5.50	5.70	5.50	1.36	7.56	5.56	7.56	1.82
5	100	2.00	29.00	14.09	29.00	1.81	23.20	12.80	23.20	2.36	35.30	14.95	35.30	2.47
10	100	2.66	46.80	17.56	46.80	2.45	38.40	15.64	38.40	2.83	51.48	18.18	51.48	2.96
20	100	2.92	58.47	19.99	58.47	2.84	46.77	16.47	46.77	3.08	64.31	20.87	64.31	3.34
1	200	1.14	14.60	12.75	7.30	0.88	8.65	9.72	23.38	1.25	16.06	12.77	8.03	1.69
5	200	1.91	50.70	26.41	25.35	1.68	41.35	24.49	4.32	2.20	58.90	26.73	29.45	2.31
10	200	2.51	79.80	31.77	39.90	2.31	68.70	29.71	20.67	2.66	88.20	33.04	44.10	2.79
20	200	2.76	95.88	34.63	47.94	2.68	83.37	31.02	34.35	2.91	105.46	36.13	52.73	3.17
1	250	1.04	11.60	11.05	4.64	0.81	8.50	10.43	3.40	1.15	15.20	13.18	6.08	1.56
5	250	1.77	57.07	32.20	22.82	1.55	47.56	30.53	19.02	2.03	66.80	32.80	26.72	2.13
10	250	2.33	94.81	40.61	37.92	2.14	79.01	36.79	31.60	2.48	104.29	42.00	41.71	2.60
20	250	2.58	115.05	44.56	46.02	2.50	95.88	38.29	38.35	2.72	126.56	46.46	50.62	2.96

Table 5
Comparison of the maximum Ni(II) biomass concentration, the bioaccumulated Ni(II), the specific Ni(II) uptake, and the Ni(II) metal ion bioaccumulation efficiency of *Candida* species in medium containing single Ni(II) ion (pH 4.0; T = 25 °C; SR: 150 rpm)

<i>Candida membranaefaciens</i>						<i>Candida utilis</i>				<i>Candida tropicalis</i>				<i>Candida lipolytica</i>
S _i (g/L)	C _i (mg/L)	X _{max} (mg/L)	C _{acc,Ni} (mg/L)	q _{Ni} (mg/g)	%uptake	X _{max} (mg/L)	C _{acc,Ni} (mg/L)	q _{Ni} (mg/g)	%uptake	X _{max} (mg/L)	C _{acc,Ni} (mg/L)	q _{Ni} (mg/g)	%uptake	X _{max} (mg/L)
1	0	1.36				1.07				1.47				1.52
5	0	2.23				1.98				2.53				2.62
10	0	2.85				2.65				2.99				3.11
20	0	3.11				3.05				3.24				3.48
1	25	1.25	2.04	3.11	8.16	0.97	1.51	2.99	6.04	1.37	2.36	3.12	9.44	1.43
5	25	2.07	7.31	6.53	29.24	1.82	5.70	6.31	22.80	2.37	8.02	6.64	32.08	2.48
10	25	2.67	12.20	8.46	48.80	2.46	10.10	8.23	40.40	2.83	13.50	8.60	54.00	2.97
20	25	2.92	14.71	9.42	58.84	2.84	12.08	8.47	48.32	3.08	16.13	10.20	64.52	3.34
1	100	1.18	5.84	6.91	5.84	0.92	4.47	5.80	4.47	1.30	6.50	7.17	6.50	1.35
5	100	1.97	24.65	16.88	24.65	1.74	18.84	14.97	18.84	2.26	30.36	17.76	30.36	2.37
10	100	2.57	39.70	20.49	39.70	2.37	31.20	18.42	31.20	2.73	44.30	22.84	44.30	2.86
20	100	2.83	49.70	22.10	49.70	2.74	38.80	19.65	38.80	2.98	55.31	24.90	55.31	3.24
1	200	1.04	10.80	10.29	5.40	0.81	6.66	8.17	3.33	1.15	12.05	10.44	6.02	1.20
5	200	1.78	37.50	21.05	18.75	1.56	31.84	20.34	15.92	2.04	44.18	21.58	22.09	2.14
10	200	2.35	61.20	25.96	30.60	2.16	53.70	24.76	26.85	2.50	65.50	26.13	32.75	2.63
20	200	2.61	72.87	27.88	36.43	2.53	65.50	25.84	32.75	2.75	79.10	28.69	39.55	2.99
1	250	0.94	8.82	9.37	3.52	0.72	6.63	9.10	2.65	1.03	10.50	10.14	4.20	1.08
5	250	1.62	43.37	26.77	17.34	1.42	37.09	26.07	14.83	1.86	50.77	27.23	20.30	1.95
10	250	2.17	72.20	33.2	28.88	1.99	62.10	31.07	24.84	2.31	79.26	34.23	31.70	2.43
20	250	2.58	115.05	44.56	46.02	2.50	95.88	38.29	38.35	2.72	126.56	46.46	50.62	2.96

4. Conclusion

The growth of *Candida* species was investigated in metal and metal-free media. Bioaccumulation experiments were carried out as a function of molasses sucrose and metal ion concentrations in a batch reactor. Initial pH and molasses sucrose directly affected the microbial growth. *Candida* yeasts showed maximum biomass concentration at pH 4.0. It was observed that the yeast concentrations and specific growth rates increased with increasing initial sucrose concentration. Cu(II) and Ni(II) ions inhibited specific growth rates and microorganism concentrations and the inhibition effect of Ni(II) ions on yeast growth was higher than Cu(II) ions for all yeasts. Maximum specific growth rate and microorganism concentration were determined as 0.253 h⁻¹ and 2.854 g / L, 0.238 h⁻¹ and 2.658 g / L, 0.289 h⁻¹ and 2.999 g / L and 0.308 h⁻¹ respectively for *Candida membranaefaciens*, *Candida utilis*, *Candida tropicalis* ve *Candida lipolytica* in metal free media at 10 g/L constant molasses sucrose concentration. Microorganism concentration was decreased from 3.111 g / L to 2.968 g / L with increasing initial Cu(II) concentration 0.0 to 100.0 mg/L for *Candida lipolytica*. *Candida utilis* showed the lowest specific growth rate and microorganism concentration among the yeasts (0.207 h⁻¹; 2.460 g / L) whereas *Candida lipolytica* showed the highest specific growth rate and microorganism concentration. The relationship between the specific growth rate, molasses sucrose, and metal concentrations was determined by inhibition kinetics. It was observed that the maximum specific growth rates of microorganisms decreased with increasing initial Cu(II) and Ni(II) ion concentrations whereas saturation constants increased. It was observed that the bioaccumulated Cu(II) and Ni(II) ions and specific Cu(II) and Ni(II) uptake was increased with increasing initial sucrose and Cu(II) and Ni(II) concentrations. The amount of Cu(II) and Ni(II) ions uptake per gram dry microorganism increased with increasing the initial metal concentration up to 250 mg/L. The microbial growth of *Candida utilis* was inhibited significantly due to increasing Cu(II) concentrations. Uptake efficiency of Cu(II) ions was higher than Ni(II), and *Candida lipolytica* performed the highest removal efficiency in medium containing both single Cu(II) and Ni(II) ions. The bioaccumulation capacity of *Candida* yeasts was increased due to the energy needs of the bioaccumulation process. The present study showed that *Candida* species appears to be useful biomass for removing heavy metal ions from wastewaters containing Cu(II) and Ni(II) ions. We hope that the study will help other researchers in choosing biomass due to metal resistance, economic, and efficient production of *Candida* yeasts for wastewater treatments.

Abbreviations

$\mu_{\max}$	: Maximum specific growth rate (h^{-1})
C_{ICu}	: Initial Cu (II) ion concentration (mg / L)
C_{INi}	: Initial Ni (II) ion concentration (mg / L)
$C_{\text{acc,Cu}}$	: Bioaccumulated copper(II) ion concentration at any time (mg / L)
$C_{\text{acc,Ni}}$	: Bioaccumulated copper(II) ion concentration at any time (mg/L)
K_s	: Saturation constant (g / L)
q_{Cu}	: Specific Cu(II) uptake defined as bioaccumulated Cu(II) ion quantity per gram of dried yeast at the end of microbial growth (mg/g)
q_{Ni}	: Specific Ni(II) uptake defined as bioaccumulated Ni(II) ion quantity per gram of dried yeast at the end of microbial growth (mg/g)
S_i	: Initial sucrose concentration (g / L)
S	: Sucrose concentration (g / L)
t	: Reaction time (h)
$X_{\max}$	: Maximum dried yeast concentration (g / L)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

Funding

The financial support provided by the Cumhuriyet University Scientific Research Projects Unit (BAP- M-354 coded project)

Authors' contributions

"ÜA analyzed the experimental data regarding the bioaccumulation and microbial growth. GM performed the experimental study, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript

Acknowledgements

The financial support provided by the Cumhuriyet University Scientific Research Projects Unit (BAP- M-354 coded project) is gratefully acknowledged.

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Figures

Figure 1

Effect of initial pH on specific growth rate and microorganism concentration for *Candida lipolytica*, *Candida utilis*, *Candida tropicalis* and *Candida membranaeficiens* yeasts (So: 10 g / L; T: 25oC; SR: 150 rpm)

Figure 2

Effect of initial sucrose concentration on specific growth rate and microorganism concentration for *Candida lypolytica*, *Candida utilis*, *Candida tropicalis* and *Candida membranaefaciens* yeasts (pH: 4, T: 25oC; SR: 150 rpm)

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