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Research Article

Comparative Biosorption of Lead Using Yeast and Lactic Acid Bacteria

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Abstract
Today, pollution caused by heavy metals is considered one of the most serious challenges to the environment and human health. Among these metals, lead (Pb) is of particular importance due to its high toxicity and ability to easily accumulate in microorganisms and the human body, especially in bones and the nervous system. Biosorption is one of the effective methods for removing Pb from the environment. Thus, this study investigated the ability of the *Saccharomyces cerevisiae* yeast and two strains of lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) to remove Pb. Factors affecting biosorption, including pH, biomass dosage, initial metal concentration, contact time, and temperature, were evaluated, and the highest Pb removal was observed by the yeast followed by the two strains of LAB under optimal conditions. The kinetic results further revealed that the adsorption process followed the pseudo-second-order model. Additionally, the analysis of the kinetic data, along with microscopic observations, indicated the significant role of the chemisorption mechanism in this process. These findings suggest that *S. cerevisiae* and LAB can serve as effective biosorbents for reducing Pb in contaminated environments and pave the way for further research at larger, real-world scales.
Keywords: Bioremediation, Lead, Kinetic Modeling, *Saccharomyces cerevisiae*, Lactic Acid Bacteria

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1. Introduction

Over the past century, population growth, economic expansion, and industrial development have posed serious risks to human health and the environment, one of which is heavy metal pollution. Heavy metals are non-biodegradable elements that exist in both organic and inorganic forms and have a density at least five times greater than that of water (i.e., > 5 g/cm^3) (1), making them environmentally persistent and allowing them to bioaccumulate through the food chain, as living organisms are unable to metabolize them effectively (2). Cadmium (Cd), chromium, lead (Pb), arsenic, manganese, copper, nickel (Ni), and mercury are among the most common heavy metals that are found in industrial wastewater and are major contributors to soil contamination (3). While some of these metals (e.g., zinc, manganese, and copper) act as essential cofactors in cellular biochemical processes, others (e.g., arsenic, Pb, and Cd) have no known biological function (4).

After being absorbed by the soil, heavy metals enter the ecological cycle, including plants, animals, and humans, and can become toxic at high concentrations (5, 6). Continuous exposure, even to low levels of these toxic elements, may lead to various health issues, such as cardiovascular disorders, neurological damage, cancer, reproductive dysfunction, and fetal developmental delays (4-7). According to reports, between 10% and 20% of Pb contamination originates from drinking water consumption (8), affecting not only the circulatory system but also kidney function in children. In addition, it has been documented that relatively high levels of metals such as Pb can cause premature births and severe mental impairments in newborns (9, 10).

Owing to increasing consumption and the persistent nature of heavy metals, heavy metal pollution has now become one of the most critical environmental challenges. Conventional methods for removing metal ions from aqueous solutions have been extensively studied, including

chemical precipitation, ion exchange, electrochemical treatment, oxidation-reduction processes, coagulation, electro-recovery, membrane technologies, and adsorption using activated carbon. However, chemical precipitation and electrochemical techniques are generally ineffective when the metal ion concentration is below 1–100 mg/L, and they often generate large volumes of sludge, leading to additional handling and disposal problems. Additionally, ion exchange, membrane filtration, and activated carbon adsorption are prohibitively expensive, making them impractical for large-scale use. As a result, it is necessary to develop cleaner, cost-effective technologies (11). One promising alternative is biosorption, which uses the natural materials of biological origin (e.g., bacteria, fungi, yeasts, and algae) as an adsorbent. These biological materials can reduce heavy metal ion concentrations in solution from parts per billion (ppb) to parts per trillion (ppt), thereby offering high efficiency and rapid removal even from complex and dilute solutions. Moreover, biosorption is less costly, simpler to operate, and more effective over a broader pH range compared to precipitation methods (12). Therefore, biosorption is considered a suitable option for the treatment of large volumes of wastewater with low metal concentrations (13).

Nonetheless, the biosorption capacity of biological materials primarily depends on the ionic properties of metals (e.g., valence, ionic radius, and atomic weight), the biological properties of biosorbents (e.g., cell age), and environmental and operational conditions (e.g., contact time, temperature, and pH) (14). The uptake of heavy metals by microbial cells occurs through biosorption and bioaccumulation. Biosorption refers to the ability of living, inactive, or dead biomass to bind heavy metals or other contaminants in dilute solutions, primarily through interactions with the cell wall. In contrast, bioaccumulation involves the metabolically active uptake of metals by living cells and typically occurs in two stages. The first stage is rapid and metabolism-independent and involves the surface binding of metal ions to the microbial cell wall. The second one is slower and metabolism-dependent, where metal cations are actively taken up into the cells, resulting in higher metal accumulation than biosorption alone (15). Among various microorganisms, yeasts are frequently preferred for heavy metal removal because of their high resistance in metal-contaminated environments, strong metal-binding capacity at the cell wall, and high intracellular uptake efficiency. The binding capacity of yeasts is largely attributed to the electrical charges generated by the partial dissociation of acidic functional groups (e.g., carboxyl, hydroxyl, and amino) in the cell wall. However, heavy metals are also toxic to microorganisms due to their high affinity for forming complexes with membrane components, which can compromise membrane integrity and impair cellular performance (16). Biological methods for heavy metal removal have several advantages, including low operational costs, food-grade safety, selective removal of specific toxic metals, minimal chemical usage (resulting in low sludge production), lack of a need for added nutrients, and high efficiency at trace concentrations (17). Furthermore, these processes are rapid, typically requiring only a few minutes under ambient pressure and temperature (18). Despite these benefits, only a limited number of wastewater treatment systems currently employ biosorption or bioaccumulation processes.

Among promising candidates for biological adsorbents are lactic acid bacteria (LAB), which are Gram-positive microorganisms classified as GRAS (generally recognized as safe) by the FDA (Food and Drug Administration). LAB have a long history of safe use as probiotic supplements and are believed to neutralize toxins due to their strong binding affinity to intestinal wall proteins (19, 20). In addition, research suggests that LAB can reduce heavy metal absorption in the body, and that the oral consumption of LAB may support in vivo detoxification (21). It should be noted that LAB strains are generally resistant to heavy metal toxicity through sequestration or aggregation (22), and their application as biological adsorbents is supported by their high metal selectivity, low cost, wide availability, and functional stability across temperature and pH ranges (23). In recent years, several LAB species have drawn significant attention for their potential use in removing heavy metals to protect human health (24, 25). In the study by Mostafidi et al, seven strains of LAB bacteria were evaluated for their ability to adsorb Cd, Pb, and Ni. The results revealed that three of the strains were capable of effectively adsorbing these metals, and their combination produced a synergistic effect, increasing the metal adsorption rate by nearly 99% (26). Similarly, in the study by Savastru et al, *Saccharomyces cerevisiae* was investigated as a low-cost biosorbent for the removal of cobalt, zinc, and copper ions from aqueous environments. Their results indicated that the maximum adsorption efficiency for all metal ions was achieved at the pH level of 5, with an adsorbent dose of 4 g/L, a room temperature of 23 $^{\circ}C$, and a contact time of 60 minutes. Moreover, the adsorption capacity and rate followed the order Co(II) > Zn(II) > Cu(II), and the adsorption mechanism was mainly attributed to the yeast cell surface (27). Likewise, Geng et al screened two LAB bacterial strains due to their high tolerance and excellent adsorption capacity for Pb, with adsorption rates of up to nearly 86% under optimal conditions. In simulated wastewater samples, Pb adsorption was 73.38% and 74.15%, respectively (28). The findings of these studies confirmed that using various biological sources (e.g., LAB and yeasts) can be an effective, eco-friendly, and low-cost approach for removing heavy metals from aquatic environments. However, a limited body of research has directly compared the heavy metal binding capacities of LAB and the *S. cerevisiae* yeast under identical experimental conditions. It is worth mentioning that these organisms were separately evaluated in most previous studies. Therefore, no comprehensive understanding of the differences in biosorption mechanisms between fungal and bacterial biomasses has been achieved so far,

and a significant scientific gap remains in this area.

Thus, this study aims to fill this knowledge gap by simultaneously comparing *S. cerevisiae* and the two selected species of LAB (*Lactobacillus plantarum* and *Lactobacillus acidophilus*) in the removal of Pb from aqueous solutions. The main innovation of this research lies in the use of identical experimental conditions for the comparative evaluation and analysis of contact time, biosorbent concentration, contaminant concentration, pH, temperature, and type of biosorbent. It is noteworthy that the obtained results can provide a scientific basis for selecting the most effective and compatible microbial biosorbents in environmental and food safety applications. Moreover, this study presents several methodological and biosorbent-selection innovations. Compared with previous studies, which mainly focused on single-species microbial evaluation, this is the first research to conduct a systematic and simultaneous comparison among three distinct microorganisms. Another notable innovation of this study is the deliberate selection and use of microorganisms classified as generally recognized as safe and already used in food and probiotic industries. This feature offers strong potential for applying these biosorbents in wastewater treatment systems related to food industries and even possible applications within the food chain, ensuring higher safety considerations.

2. Materials and Methods

This experimental laboratory investigation was performed at the Laboratory of the Faculty of Food Technology, Bahar Branch, Bu-Ali Sina University, Hamadan, over a period of three months. Experiments were conducted on synthetic Pb-containing solutions using biological adsorbents, including baker's yeast (*S. cerevisiae*; Iran Mellas Company) in the form of dried and inactivated powder and LAB strains *L. plantarum* (DSMZ 20174) and *L. acidophilus* (DSMZ 20079) purchased from DSMZ GmbH, Germany. The bacterial cell mass was preserved in 5 mL of MRS broth supplemented with 20% glycerol and stored at −80°C. For biomass preparation, the LAB strains were subcultured twice in MRS broth containing lactose and incubated under anaerobic conditions at 37°C with shaking at 150 rpm for 48 hours. After incubation, the bacterial cells were harvested by centrifugation at 10,000 rpm for 10 minutes. The biomass was then washed twice with distilled water. In addition, biomass concentrations were determined using McFarland standards and optical density measurements at 600 nm (OD_{600}). Further, the yeast powder was suspended in distilled water to remove excess soluble particles and then centrifuged. The washed yeast was subsequently dried in an oven at 60°C for 6 hours (29). To ensure the comparability of concentrations, the biomass amount for the yeast was calculated based on dry weight (g/L). Pb was introduced using Pb (II) nitrate ($Pb(NO_3)_2$), purchased from Merck Company, Germany. Other chemicals used in the experiments, including nitric acid (65%) and hydrogen peroxide (37%), were also laboratory-grade and obtained from Merck Company, Germany. All glassware and containers utilized in the study were soaked in 20% nitric acid for 48 hours prior to use to ensure decontamination.

All the experiments were conducted under aerobic conditions in a batch system using 250 mL Erlenmeyer flasks containing 100 mL of the Pb-containing solution. The biosorbent was added to each flask, and the mixtures were placed in a shaker incubator set at 150 rpm to maintain uniform agitation. Furthermore, the sample temperature was controlled by a shaker incubator. After the biosorption process, the samples were centrifuged: yeast samples at 4,000 rpm for 10 minutes and Lactobacillus samples at 10,000 rpm for 10 minutes. Then, the resulting supernatants were analyzed for residual Pb ion concentration using inductively coupled plasma optical emission spectroscopy with a Spectro Arcos model (SPECTRO, Germany).

The main experimental variables included pH (3, 4, 5, 6, 7, and 8), yeast dose (*S. cerevisiae* at 0.5 g/L, 1 g/L, 1.5 g/L, 2 g/L, and 3 g/L), initial Pb concentration (2.5 mg/L, 5 mg/L, 7.5 mg/L, 10 mg/L, and 15 mg/L), contact time (15 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes), and temperature (22°C, 28°C, 35°C, and 42°C). These parameters were individually optimized by varying one factor at a time while keeping the others constant.

In subsequent stages, the optimized Pb removal conditions were applied to biosorption experiments using *L. acidophilus* and *L. plantarum* as biosorbents. All experiments were performed in duplicate, and the average values were reported accordingly.

$$q_e = \frac{(Ci - Ct)}{m} * V \qquad \text{Eq. (1)}$$

$$\text{Removal efficiency (\%)} = \frac{(Ci - Ct)}{Ci} * 100 \qquad \text{Eq. (2)}$$

Eq. (1) was used to calculate the amount of Pb uptake by the biomass of *S. cerevisiae*, *L. plantarum*, and *L. acidophilus* in all experiments. In Eq. (1), q_e is the amount of metal uptake by the biomass (mg/g), and C_i denotes the initial concentration of metal ions in solution (mg/L). Moreover, C_f and V represent the final concentration of metal ions in solution (mg/L) and the volume of the sample that is mixed with the biomass (L), respectively, and m is the amount of biomass added based on dry weight (g). Furthermore, the removal efficiency was calculated according to Eq. (2), where R and C_i indicate the metal uptake efficiency by the biomass (%) and the initial concentration of metal ions in solution (mg/L), respectively, and C_f is the final concentration of metal ions in solution (mg/L).

To determine the adsorption kinetics of Pb by *S. cerevisiae*, experiments were performed at the pH level of 6 using an initial Pb concentration of 5 mg/L, with a biosorbent dose of 1 g/L. Additionally, contact times were set at 0 minute, 15 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes. The resulting data were used to analyze adsorption kinetics (30-32). Further, both metal-treated and untreated (control) cell pellets were examined

to investigate morphological changes in *S. cerevisiae* cells resulting from Pb adsorption. The samples were then analyzed using scanning electron microscopy (SEM) (JEOL JSM 5400 LV, Japan). Eventually, the presence of metal elements in the yeast biomass was confirmed using energy-dispersive X-ray spectroscopy (EDX; JEOL JSM 6360 LA, Japan) (33).

3. Results and Discussion

3.1. Impact of pH on Lead Removal

The effect of pH on Pb removal was studied over a 60-minute period using 1 g/L of the *S. cerevisiae* yeast powder at 28°C in 100 mL of the Pb-containing solution with an initial concentration of 5 mg/L. The experiments were conducted at pH levels of 3, 4, 5, 6, 7, and 8 (Figure 1). The highest Pb removal efficiency was observed at the pH level of 6, reaching 71%, with an adsorption capacity of 3.55 mg/g. The results indicated that Pb removal efficiency increased with rising pH from 3 to 6, while it decreased as pH was increased from 6 to 8. Thus, the pH level of 6 was identified as the optimal value. This trend is likely due to the effect of pH on the ionization of functional groups on the yeast cell surface and the chemical behavior of Pb in solution. At low pH values, a high concentration of protons leads to the protonation of the yeast's binding sites, reducing their negative charge and thus their ability to attract and bind metal ions (34). At higher (alkaline) pH levels, heavy metal ions are more prone to precipitation, which decreases their availability for biosorption. Moreover, the increased negative charge of the yeast surface at high pH levels may lead to the electrostatic repulsion of metal cations. However, this repulsion effect appears to be less substantial at higher contaminant concentrations, likely because of the greater availability of metal ions and the dominance of proton competition (35, 36). Similar findings were reported by Cui et al, who used brewer's yeast to remove Ni (II) and Cd (II) from aqueous solutions, with optimal removal also occurring at the pH level of 6. As mentioned above, the highest level of metal adsorption by yeast occurs at the pH level of 6. At lower pH values, that is, in acidic environments (e.g., pH = 1–3), the functional groups on the yeast surface (e.g., sulfonate and carboxyl groups) become protonated and gain a positive charge. This causes electrostatic repulsion with positively charged metal ions, leading to a sharp decrease in adsorption. With increasing pH and approaching neutral conditions, these groups become deprotonated and acquire a negative charge, which considerably enhances the electrostatic attraction of metal ions. At pH values higher than 6, the likelihood of metal hydroxide precipitation increases, and metal removal occurs mainly through chemical precipitation rather than surface adsorption, which was not the focus of this study. Therefore, the pH level of 6 was determined as the optimal point, as under these conditions, the maximum surface adsorption of metals occurs with minimal chemical precipitation. Similarly, Cui et al and Padmavathy et al reported a similar result regarding the effect of pH on the removal of other heavy metals using the *S. cerevisiae* yeast. This was attributed to the reduced competition between hydrogen ions and positively charged metal ions for active sites on the yeast surface and the prevention of precipitation formation (37, 38), which conforms to the findings of the present research.

3.2. Effect of Yeast Dosage

The effect of varying *S. cerevisiae* yeast doses (0.5–3 g/L) on Pb removal was evaluated at the pH level of 6, the temperature of 28°C, and an initial Pb concentration of 5 mg/L, over contact times ranging from 15 minutes to 120 minutes (Figure 2). The results demonstrated that Pb removal efficiency increased with higher yeast doses, which is attributed to the greater availability of adsorption sites. However, no remarkable improvement in removal efficiency was observed at doses above 1 g/L after 120 minutes of contact time. Consequently, 1 g/L was identified as the optimal dose. The concentration of the biosorbent is a critical factor influencing adsorption performance (39). Increasing the biosorbent dose enhances metal ion removal by providing more active binding sites for adsorption (40). Nonetheless, after reaching the optimal dosage, the adsorption capacity reaches saturation, and further increases in the amount of adsorbent have no significant effect on efficiency. This phenomenon, known as the "saturation effect", occurs due to the occupation

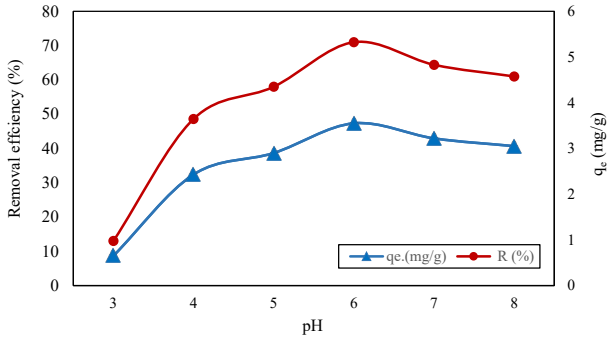

Figure 1. Effect of pH on Lead Removal in the Presence of 1 g/L of the *Saccharomyces cerevisiae* Yeast at the Temperature of 28°C and an Initial Concentration of 5 mg/L of Lead for 60 Minutes

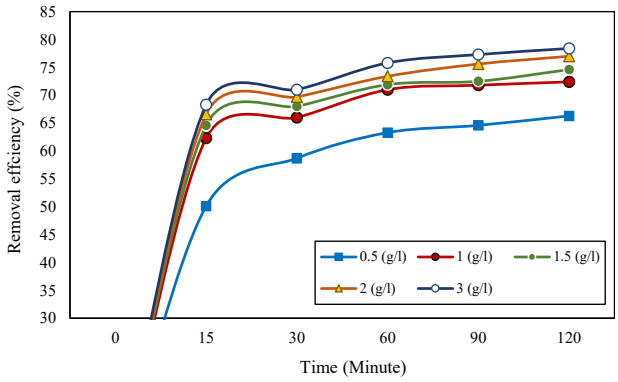

Figure 2. Effect of the *Saccharomyces cerevisiae* Yeast Dose on Lead Removal at Different Time Intervals at the pH Level of 6, Temperature of 28°C, and Initial Lead Concentration of 5 mg/L

of all available active sites and the reduction of effective contact surface resulting from particle aggregation and adhesion (agglomeration) at higher concentrations. Therefore, at the optimal point, the highest adsorption efficiency is achieved without the negative effects of crowding or reduced effective surface area. In their study aiming at modelling the biosorption and isotherms of heavy metals using reed plants, Mohammed et al reported similar findings; increasing the amount of adsorbent significantly enhanced the metal removal efficiency, but after reaching the optimal and saturation point, further increases in adsorbent dosage had no effect on metal adsorption efficiency (41). Likewise, Ren et al attempted to determine the optimization of adsorbent dosage through single-factor experiments and final optimization using the response surface method (RSM) and obtained similar results. In this study, the adsorption capacity increased with increasing adsorbent dosage, but beyond a certain point, it sharply decreased, which was attributed to cell aggregation and the saturation of all active sites at higher dosages (42).

3.3. Effect of Initial Lead Concentrations

The effect of different initial Pb concentrations (2.5–15 mg/L) on removal efficiency over various time intervals is displayed in Figure 3. The results revealed a sharp reduction in Pb concentration within the first 15 minutes for all tested concentrations. After this initial phase, no considerable further decrease was observed over the remaining contact times. After 120 minutes of treatment at concentrations of 2.5 mg/L, 5 mg/L, 7.5 mg/L, 10 mg/L, and 15 mg/L, the removal rate declined with an increase in the initial concentration. Based on these findings, 5 mg/L was identified as the optimal concentration for biosorption. Additionally, the results demonstrated that removal efficiency decreased with increasing the initial Pb concentration, likely due to the saturation of available adsorption sites. At low contaminant concentrations, metal ions bind to functional groups (e.g., carboxyl and hydroxyl) on the adsorbent surface, resulting in the removal of a significant portion of metals. As the initial concentration increases, the concentration driving force is enhanced, leading to more frequent collisions of ions with active sites, and consequently, the adsorption capacity (mg/g) increases until the optimal point is reached. However, at high concentrations, the number of metal ions exceeds the surface adsorption capacity, and the active sites become rapidly saturated; therefore, some ions remain in the solution, thereby decreasing the removal percentage. At this stage, although the percentage removal efficiency declines, the amount of metal adsorbed per unit mass of adsorbent continues to increase until complete saturation. Beyond the optimal concentration, the adsorption capacity reaches its maximum and may slightly decrease due to ion competition or excessive crowding. From a practical perspective, this behavior indicates that to achieve high removal efficiency, adsorbents should be used at low contaminant concentrations, whereas their application near saturation concentrations or with higher adsorbent doses is recommended for economic efficiency and the maximum utilization of adsorption capacity (43, 44), which is consistent with the findings of previous studies. For example, Padmavathy et al reported a decline in adsorption efficiency for divalent Ni with increasing concentration using yeast biomass (38). Likewise, Jahan et al demonstrated that increasing the initial arsenic concentration from 0.005 mg/L to 1.5 mg/L led to a decrease in the adsorption percentage from 80% to 60%, which is in line with the findings of the present study. Meanwhile, the trend of (q_e) showed that with increasing the initial metal concentration, the value of (q_e) increased in an ascending but decelerating manner until reaching saturation. Moreover, their results indicated that the highest adsorption occurred within the first hour of contact, and at longer times, the (q_e) value slightly decreased due to partial desorption (45). In contrast, in our study, the adsorption capacity (q_e) of the yeast continuously increased with an increase in the initial Pb concentration from 2.5 mg/L to 15 mg/L (Figure 4). These results corroborate the findings of Halttunen et al (45, 46). The outcomes of these studies confirm those of the present research regarding the effect of the initial metal concentration on removal efficiency, emphasizing the reliability of the obtained data.

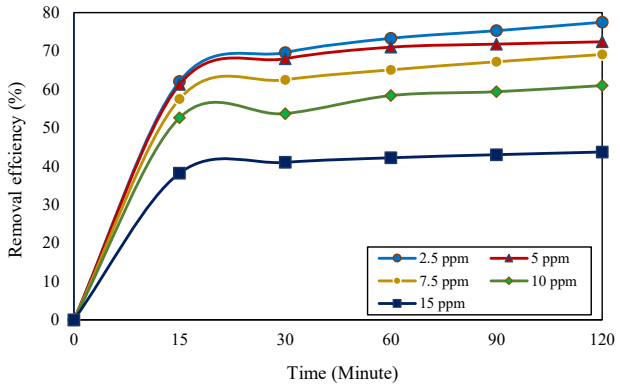

Figure 3. Effect of Changes in the Initial Lead Concentration on Removal Efficiency at Different Time Intervals at the pH Level of 6, Temperature of 28°C, and 1 g/L of the *S. cerevisiae* Yeast

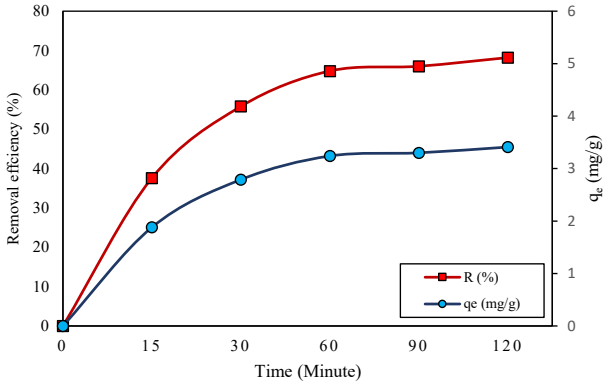

Figure 4. Lead Biosorption Capacity (q_e) of *S. cerevisiae* Biomass at Different Initial Lead Concentrations
Note. S. cerevisiae: *Saccharomyces cerevisiae*.

In this study, the performance of the biomasses used for the removal of Pb ions from aqueous solutions was investigated within a concentration range of 2.5–15 mg/L. This range was selected to simulate more realistic environmental pollution conditions with moderate intensity, with a particular focus on approximating natural and drinking water scenarios, which typically fall within this concentration range of metals. This indicates that the aim of the research was to evaluate the adsorbent's performance under conditions similar to real surface or groundwater contamination, rather than highly concentrated industrial wastewater. The results revealed that increasing the initial metal concentration up to about 5 mg/L led to a significant increase in adsorption efficiency, while at higher concentrations, the adsorption rate decreased due to the gradual saturation of active sites. Conducting experiments within the 2.5–15 mg/L range allows for assessing the sensitivity and effectiveness of the adsorbent in removing extremely low concentrations of heavy metals and demonstrates that this adsorbent can reduce contaminant concentrations below permissible limits. This trend is consistent with similar reports on heavy metal adsorption by microorganisms and inactivated biomasses. Moreover, by selecting low concentrations and controlling pH within the 3–8 range, interferences (e.g., metal hydroxide precipitation) were prevented, ensuring that the primary removal mechanism remained surface adsorption. Considering that many similar studies work within low concentration ranges, this choice aligns the study with more realistic conditions and enables more precise scientific comparisons. Overall, selecting this concentration range aimed to evaluate the performance of *S. cerevisiae* and LAB under conditions close to real water pollution and demonstrate their ability to reduce toxic metal concentrations to safe levels, indicating their practical applicability in drinking water treatment and contaminated water sources (47).

However, it should be noted that the concentration range used in this study cannot fully reveal the system's performance when exposed to highly concentrated wastewater or mineral waters (with concentrations above 100 mg/L). Thus, a decrease in efficiency due to ion competition and adsorption capacity limitations is expected under such conditions. In future studies, investigating performance at higher concentrations, along with detailed isotherm analyses and kinetic modeling, can lead to a more comprehensive understanding of the maximum adsorption capacity (q_{max}) and system stability.

3.4. Effect of Contact Time

The effect of contact time on Pb removal was studied over a period of 15–120 minutes at the optimal pH, using 100 mL of solution with an initial Pb concentration of 5 mg/L at a temperature of 28°C. The results indicated that Pb removal efficiency increases with increasing contact time. After 15 minutes, the removal efficiency was 57.3%, with an adsorption rate of 2.86 mg/g. After 120 minutes, the removal efficiency increased to 72.4%, and the adsorption rate reached 3.62 mg/g (Figure 5). In addition, the adsorption capacity increased over time. Pb adsorption onto yeast rapidly occurred within the first 15 minutes, and equilibrium was approximately reached after 60 minutes. Based on the results, the highest removal efficiency was achieved after 15 minutes, with subsequent increases in removal efficiency occurring at a slower rate. This trend highlights the significant impact of contact time on recovery efficiency, with diminishing returns as the contact time increases. A similar trend was observed in a study by Wang et al, where the time to reach equilibrium was also determined to be 1 hour. The adsorption of metal ions onto yeast follows a two-phase process; the first phase is rapid and involves passive adsorption or ion exchange on the biomass surface, which significantly contributes to the total metal adsorption. The second, slower phase involves active adsorption, contributing to a smaller portion of the overall uptake (48). The rapid phase is mainly due to the immediate occupation of available active sites on the yeast surface and ion exchange. Then, the adsorption rate decreases, and the system gradually reaches equilibrium. Once the system achieves full equilibrium, further increases in contact time are not justified scientifically or economically. The equilibrium time for metal ions is generally reported to range between 60 minutes and 180 minutes. In the slower phase, the diffusion of ions into the inner layers of the cells and less accessible sites plays a role, and increasing the contact time has a slight effect on adsorption capacity because most surface sites are already saturated. Kinetic data analysis indicates that the pseudo-second-order model provides the best description of the system's behavior, suggesting that the adsorption rate mainly depends on chemical and electrostatic interactions between metal ions and active sites (27, 29). Zinicovscaia et al evaluated the potential of the *S. cerevisiae* yeast as an efficient, low-cost, and environmentally-friendly biosorbent for the removal of heavy metals from aqueous solutions. In the initial phase of the adsorption experiments, within the first 5–45 minutes, the adsorption capacity of the biosorbent rapidly increased, with the highest increase in silver ion adsorption observed within the first 5 minutes. After this phase, the adsorption rate

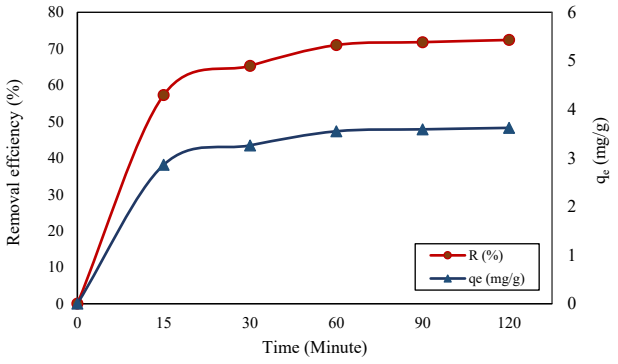

Figure 5. Effect of Contact Time Changes on Lead Removal at the pH Level of 6, Temperature of 28°C, 1 g/L of the *Saccharomyces cerevisiae* Yeast, and Initial Lead Concentration of 5 mg/L

decreased, and equilibrium was reached between 45 minutes and 60 minutes, establishing 60 minutes as the optimal contact time for maximum efficiency. The kinetic analysis of the data showed that the pseudo-second-order model best fit the data, confirming the dominant role of chemisorption and the necessity for interaction with two active centers in appropriate geometric positions. The high adsorption rate in the initial stages and the rapid attainment of equilibrium revealed that the predominant mechanism involves surface adsorption, electrostatic interactions, and ion exchange at the active sites on the cell wall; this was further supported by the release of light cations during the process (49).

3.5. Effect of Temperature

Figure 6 depicts the effect of temperature on Pb removal over a contact time of 15–120 minutes at optimal pH and an initial Pb concentration of 5 mg/L. The results indicated that increasing the temperature from 22°C to 28°C enhanced Pb removal efficiency, while a further increase from 28°C to 42°C led to a decrease in adsorption. At 28, the removal efficiency reached 64.3% and 72.4% after 15 minutes and 120 minutes, respectively. It should be noted that efficient biosorption requires maintaining the temperature within an optimal range. Elevated temperatures may inhibit microbial activity or cause cell death, while low temperatures can reduce metabolic activity and biosorption potential (50). The decrease in adsorption efficiency at temperatures between 28°C and 42°C can be attributed to physicochemical changes in the adsorbent structure and the thermodynamics of adsorption. According to findings from similar studies, in the biosorption process using dead yeast cells, increasing the temperature beyond the optimal point of 30°C leads to a significant reduction in metal adsorption capacity, which is mainly due to the physical destruction of active adsorption sites on the cell wall. In other words, high heat causes the denaturation of proteins and structural changes in the functional groups on the cell surface, which play a key role in binding metal ions. The biomass's ability to retain metals is remarkably reduced with the loss of integrity of these adsorption sites. These findings are consistent

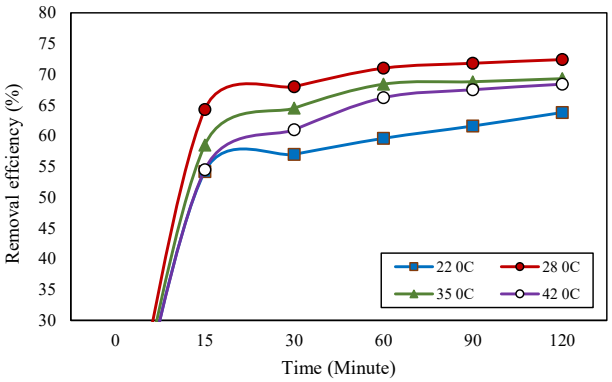

Figure 6. Effect of Temperature Changes on Lead Removal at the pH Level of 6, 1 g/L of the *Saccharomyces cerevisiae* Yeast, and Initial Lead Concentration of 5 mg/L

with the results of the present study, emphasizing that although increasing the temperature within the optimal range accelerates ion diffusion and enhances adsorption, exceeding a specific thermal threshold has detrimental effects on the structure of the biosorbent and severely reduces its efficiency (51). Previous research reported that the highest biosorption capacity for bacterial strains occurs within the temperature range of 15–40°C (52). However, the optimal temperature for biosorption varies depending on microbial species, culture conditions, and the type of heavy metal involved (53). Specifically, for *S. cerevisiae*, the highest biosorption efficiency for Pb, Ni, and chromium (Cr) was observed within the range of 15–40°C (54). Temperature plays a decisive role in the efficiency of the biosorption process, affecting both the kinetics and adsorption capacity directly and cellular metabolism indirectly. The optimal temperature for many yeast strains was reported to range between 28°C and 30°C, which usually corresponds to their natural growth temperature. One of the main mechanisms for metal removal is intracellular accumulation (bioaccumulation), which is a metabolic and energy-dependent process. Therefore, enzymatic activity and yeast metabolism decrease at temperatures below the optimum, limiting the active transport of metals into the cells. In contrast, temperatures above the optimum can lead to the denaturation of proteins and enzymes that are responsible for transport and detoxification, potentially damaging the cell or even causing cell death, thus reducing process efficiency. Additionally, temperature can influence the production of extracellular polymeric substances, which play a role in metal adsorption, affecting both the quality and quantity of produced extracellular polymeric substances. Ultimately, the study emphasized that determining the optimal temperature for each yeast strain is critical, as maintaining temperature within the appropriate range ensures both kinetic performance and cell health, enabling maximum biosorption efficiency (55). Nguyen Van et al examined the effect of temperature on two different metal removal processes by yeast: bioaccumulation by live cells and biosorption by inactivated cells and found that the optimal temperature for both processes was 30°C. At this temperature, live cells exhibited higher efficiency because they performed both surface adsorption and active ion transport, whereas dead cells solely relied on surface adsorption. Increasing the temperature beyond 30°C led to reduced efficiency and structural damage to the cells. These findings highlight the importance of determining the optimal temperature for the biosorption process (51), which is consistent with the results of the present study.

In this research, the "one factor at a time" (OFAT) method was used to evaluate the effect of variables on the biosorption process. This approach was selected due to the exploratory nature of the study and the need to gain an initial understanding of the behavior of each parameter independently. Although multivariate optimization methods (e.g., RSM) are capable of identifying interactions between factors, OFAT was

considered suitable for this stage of the research for (1) simplicity and speed for the initial screening of influential parameters, (2) lower cost and requirement for fewer samples, and (3) rapid access to the effect of each factor on adsorption efficiency. In addition, this approach serves as an initial step in process optimization and as a foundation for future studies using more advanced statistical designs (56). Furthermore, since the present study primarily aimed to compare the performance of different biomasses rather than comprehensive statistical optimization, using the OFAT method as a preliminary and efficient approach was deemed more appropriate.

3.6. Comparison of the Removal Efficiency of *Saccharomyces cerevisiae* Yeast With the Biomass of *Lactobacillus acidophilus* and *Lactobacillus plantarum*

Under the optimal conditions previously established for *S. cerevisiae* (i.e., the pH level of 6, initial Pb concentration of 5 mg/L, temperature of 28˚C, and a contact time of 60 minutes), biosorption experiments were conducted using *L. acidophilus* and *L. plantarum*. The results confirmed that the Pb removal efficiency was the highest for *S. cerevisiae*, followed by *L. acidophilus* and then *L. plantarum* (Figure 7). The high ability of *S. cerevisiae* to adsorb heavy metals arises from the unique structural and functional characteristics of this yeast, making it an efficient natural biosorbent for metal removal from aqueous solutions. The main reason for this high capacity is the cell wall structure and the presence of ligand-forming functional groups. These chemical groups on the cell surface can bind with heavy metal ions and form stable chelates, a process that constitutes the basis of passive biosorption and allows rapid and effective interaction with metal ions. Physically and structurally, the large surface area of yeast cells is another key factor that contributes to high adsorption capacity. The budding process increases the contact area of cells with metal ions, providing more opportunities for interaction. Additionally, the yeast cell wall is porous and contains significant amounts of polysaccharides, proteins, and chitin. These compounds have electron-rich functional groups, such as carboxyl ($COOH^-$), hydroxyl (OH^-), amino (NH_2), and phosphate (PO_4^{3-}), which act as active sites to capture metal ions through complexation or ion exchange. This porous structure with abundant active sites enables extensive interactions with metal ions. Functionally and biologically, the adsorption mechanisms in *S. cerevisiae* are multiple and not limited to the cell surface. They include physical surface adsorption, which is a metabolism-independent process that traps metals on the cell wall (1), chemical adsorption through complexation and ion exchange with cell wall functional groups (2), and intracellular accumulation (3). For certain metals, metabolism-dependent mechanisms are activated, transporting metals into the cytoplasm. Moreover, the genetically modified strains of *S. cerevisiae* can exhibit higher adsorption efficiency due to the increased expression of metal-binding proteins. Interestingly, inactivated or heat-killed cells occasionally adsorb metals even more effectively than live cells, as membrane permeability is enhanced and all adsorption sites on the cell wall are accessible without the impact of metal toxicity. The combination of extensive cell surface, porous wall structure, multiple functional groups, and active metabolic mechanisms ensures the high performance of *S. cerevisiae* in heavy metal adsorption. From a practical and economic perspective, this yeast is widely available as a byproduct of fermentation industries (e.g., brewing) at low cost, making it a cost-effective and suitable option for industrial-scale applications. In summary, the high performance of *S. cerevisiae* results from passive adsorption through the cell wall with multiple functional groups and active uptake mediated by intracellular metabolic processes, which trap and accumulate metals. This structural and functional combination makes *S. cerevisiae* a highly efficient natural biosorbent for removing heavy metals from aqueous solutions (57, 58).

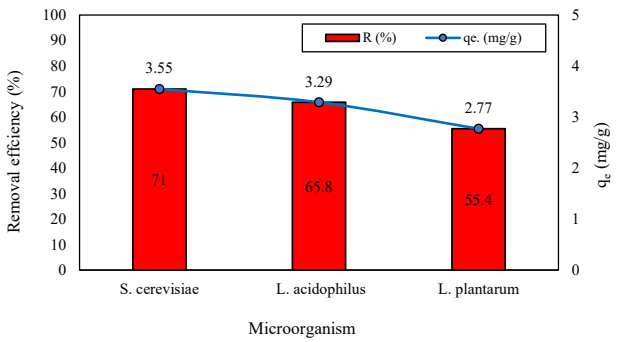

Figure 7. Comparison of the Efficiency of *Saccharomyces cerevisiae* With the Biomasses of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in Lead Removal Under Optimal Conditions*

Note. *A pH level of 6, the *Saccharomyces cerevisiae* yeast of 1 g/L, and initial lead concentration of 5 mg/L at a contact time of 60 minutes.

Conversely, LAB also have metal-binding capabilities. Their cell walls are mainly composed of peptidoglycan and lipoteichoic acid, containing negatively charged functional groups. Their adsorption mechanism is primarily non-metabolic and surface-based, with limited intracellular accumulation (26). Therefore, the higher efficiency of *S. cerevisiae* compared to LAB in heavy metal adsorption can be attributed to the unique physical and chemical structure of its cell wall and the use of active metabolic mechanisms, allowing greater metal uptake and accumulation. These features make *S. cerevisiae* a highly effective natural biosorbent for removing metals from aqueous solutions. The results of this study conform to those of Ibrahim et al; they evaluated the performance of *S. cerevisiae* in the simultaneous removal of metal ions from wastewater and reported a maximum adsorption capacity for the Pb ions of approximately 90%. The high efficiency of *S. cerevisiae* in heavy metal removal was attributed to its complex cell wall structure, which contains active functional groups (e.g., carboxyl, phosphate, amino, and hydroxyl), effectively binding metal ions through strong chemical interactions (59). Additionally, Dong et al used *S. cerevisiae* as a biosorbent for Pb and Cd removal in single-metal and binary-metal aqueous

systems and concluded that *S. cerevisiae* functioned as a rapid and effective biosorbent for both metals. The main mechanisms of heavy metal adsorption by the biomass included ion exchange, surface adsorption, complexation, chelation, and microbial precipitation, representing effective physicochemical processes for metal removal (60), which matches the findings of the present study.

Although the experimental conditions were primarily optimized for *S. cerevisiae*, the study still provides an initial and practical comparison between biomasses, allowing the identification of general trends and the relative capacity of microorganisms. As mentioned earlier, even under identical conditions, yeasts, compared to LAB, generally show higher adsorption capacity due to the unique physical and chemical structure of their cell walls, utilization of active metabolic mechanisms, and ability to uptake and accumulate heavy metals. Furthermore, the data from this study revealed that while separate optimization for each strain could improve comparison accuracy, the present research provides valuable information on relative adsorption efficiency under identical conditions. In similar studies, researchers typically adopt a screening and selection approach: first identifying the biomass with the highest metal adsorption potential and then optimizing conditions only for that sample (61, 62). This method is a common strategy in preliminary and exploratory research, where limitations in resources, materials, and cost prevent separate optimization for all samples, and is scientifically valid and reasonable. For this reason, in the present study, optimal conditions were determined only for *S. cerevisiae*, and the LAB were subsequently compared under the same conditions.

3.7. Kinetic Models

The results of Pb removal experiments conducted at various contact times (15 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes) demonstrated that the highest rate of adsorption occurred within the first 15 minutes, indicating that *S. cerevisiae* exhibits a rapid initial uptake of Pb. To better understand the adsorption behavior, the experimental data were analyzed using the pseudo-first-order and pseudo-second-order reaction models.

In this study, q_e is the Pb adsorption capacity at equilibrium (mg/g), and q_t represents the Pb adsorption capacity at time t (mg/g). Moreover, t denotes the adsorption time (minute), and parameters K_1 and K_2 are the adsorption rate constant in the first-order model (1/minute) and the adsorption rate constant in the second-order model (g/mg minute), respectively. Table 1 presents the parameters of the adsorption kinetic models as well as the correlation coefficient (R^2) for the adsorbent. The results of the experiments conducted at 28°C are summarized in Table 2, showing that the second-order kinetic model fits the Pb removal of *S. cerevisiae* better, because the R^2 value is higher than that of the first-order model. Therefore, the first-order model is unsuitable for explaining the adsorption kinetics of metal ions on the adsorbent. Similarly, the experimental data of metal ion adsorption kinetics are closely supported by the second-order model (64).

In a practical study, Parvathi et al investigated the potential of brewery waste yeast (*S. cerevisiae*) for Pb removal from the actual wastewater of a battery manufacturing unit and concluded that this biosorbent had an adsorption capacity of 2.34 mg/g under optimal conditions. In addition, equilibrium data fitted well with Freundlich and Langmuir isotherm models, representing a high theoretical capacity of the biosorbent. Mechanism analysis further highlighted the role of cell surface functional groups, revealing that carboxylic groups contributed most to Pb adsorption, with electrostatic adsorption being the predominant mechanism. This study clearly demonstrated the efficiency of an inexpensive yeast in treating a hazardous pollutant under real environmental conditions (65), which conforms to the findings of the present study. Contrarily, Teemu et al investigated the mechanisms of Cd and Pb removal from water using two ALB strains (*Lactobacillus fermentum* and *Lactobacillus longum*) and reported different results. The Pb adsorption capacities were 93 mg/g and 68 mg/g for these two strains, respectively. Comparing these results with the adsorption capacity obtained in the present study (3.67 mg/g) indicates a significant difference, primarily due to variations in the bacterial strains used and the optimized experimental conditions. In the above-mentioned study, the focus was on specific, optimized strains that, under controlled pH and metal concentration, could form metal precipitates on the cell surface, substantially enhancing adsorption capacity. In contrast, the lower capacity observed in the present work is likely due to the predominance of simple surface adsorption without precipitation, as well as the influence of less-optimized parameters, such as pH, biosorbent dose, and the biomass preparation method (66).

3.8. Effect of Lead Metal Ions on the Cell Surfaces of Saccharomyces cerevisiae Biomass

The effect of Pb metal ions on the cell surfaces of *S. cerevisiae* biomass was evaluated using SEM images. Pb metal deposits were visible on the cell surface, displaying a high Pb binding capacity by the *S. cerevisiae* cell mass (Figure 8b), while no Pb deposits were observed in the micrographs of the cells (Figure 8a). SEM images showed that yeast cells underwent significant morphological changes after absorbing the heavy metal Pb. The cell surfaces became deformed, and distinct cavities appeared on the cell walls, likely due to cytoplasmic leakage caused by contact with metal ions. Additionally, sticky particles appeared on the cell surfaces, which are presumed to be complex compounds formed between the metals and the functional groups of the biomass (67). Likewise, Kaduková et al reported structural damage on the surfaces of living cells in SEM images, observed as crater-like formations (18). Similar structural changes, described

Table 1. Characteristics of the Kinetics Investigated in This Study

Kinetic Model	Linear Form	Equation	Reference
Pseudo-first-order	$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}t$	$\frac{dq_t}{dt} = k_1(q_e - q_t)$	(63)
Pseudo-second-order	$\frac{t}{q_t} = (\frac{1}{k_2 q_e^2}) + (\frac{1}{q_e})t$	$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$	(63)

Table 2. Results of the Kinetics Study

Kinetic Model		Pseudo-First-Order	Pseudo-Second-Order
Kinetic parameters	k_1	0.0291	-
	k_2	-	0.1495
	q_e	2.38	3.67
	R^2	0.97	1/00

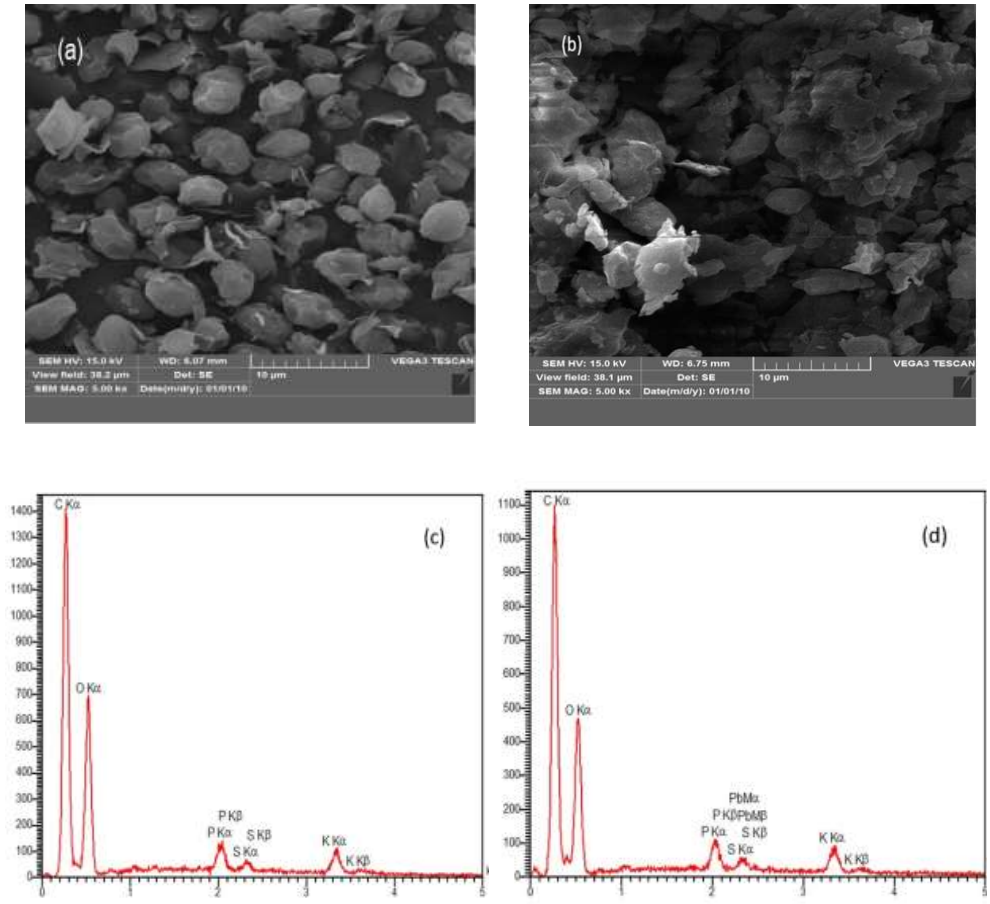

Figure 8. SEM Images of Live Yeast *Saccharomyces cerevisiae* Before and After Pb Binding: **(a)** Untreated Biomass, **(b)** Biomass After Pb binding, **(c)** EDX Spectra of Untreated Biomass, and **(d)** EDX Spectra of Biomass After Pb Binding
Note. Pb: Lead; SEM: Scanning electron microscopy: EDX: Energy dispersive X-ray.

as cave-like features, were noted in cell cross-sections obtained using transmission electron microscopy by the same researchers. Moreover, metal elements in bacterial biomass were detected using energy dispersive EDX. According to the results obtained from the analysis of the EDX micrograph, the Pb signal was found with a clear peak (Figure 8d), indicating the presence of Pb due to biological adsorption. In other words, the EDX spectrum confirmed the additional Pb peak that was not present in the control sample (Figure 8c). The morphological changes of the biomass surface of *S. cerevisiae* observed by SEM after Pb exposure are consistent with the findings of studies conducted by Teemu et al (66) and Ameen et al (33), demonstrating major Pb deposits on the surface *Lactobacillus fermentum* ME3 and *Bifidobacterium longum* 46 that were compatible after binding.

Overall, the results of this study demonstrated that *S. cerevisiae*, as an efficient biosorbent, possesses a high capacity for removing metal pollutants. Despite its promising performance at the laboratory scale, future research should focus on evaluating adsorption behavior in the presence of interfering ions, assessing performance at pilot scale, and optimizing the biosorbent regeneration process. Moreover, combining this biosorbent with

carbon-based materials or nanostructures can be a promising approach to enhance stability and adsorption capacity under real-world conditions. Previous studies have shown that the adsorption and tolerance of multiple metals by *S. cerevisiae* occur largely independently, with no direct competition for binding sites; however, the simultaneous presence of metals may increase cellular metabolic stress. Therefore, each adsorption site is specific to a particular metal, and competition occurs only at the level of cellular metabolic and energy resources (68). Furthermore, according to prior findings, *S. cerevisiae* can be regenerated and reused for up to five consecutive cycles. Although a slight reduction in adsorption capacity may be observed, overall efficiency is maintained. According to Sieber et al, the main mechanism of regeneration involves the replacement of adsorbed metal ions with protons (H^+) during washing with acids such as sulfuric acid, leading to the reactivation of the biosorbent (69). Consequently, it is recommended that future studies focus on evaluating the practical performance of *S. cerevisiae* in industrial-scale wastewater treatment systems. These investigations should include adsorption stability in the presence of interfering ions, regeneration efficiency over multiple cycles, and performance in continuous systems or bioreactors to enable the practical application of this biosorbent under real conditions.

4. Conclusion

This study systematically investigated the biosorption efficiency of Pb by three microorganisms: *S. cerevisiae*, *L. acidophilus*, and *L. plantarum*. Our findings revealed that *S. cerevisiae* exhibited the best performance with an efficiency of 72.4% under optimal conditions (pH level of 6, a temperature of 28°C, contact time of 60 minutes, an initial concentration of 5 mg/L, and an adsorbent dose of 1 g/L). In addition, the pseudo-second-order kinetic model showed a better fit with the data, and SEM/EDX observations confirmed the formation of Pb complexes on the cell surface. Moreover, kinetic data and microscopic observations indicated the significant role of the chemical adsorption mechanism in this process. Scientifically, the findings of this study demonstrated that the cell wall structure and the density of functional groups in yeasts are key factors in Pb adsorption efficiency. Practically, *S. cerevisiae*, as a low-cost, readily available, and safe biosorbent, can be used for treating Pb-contaminated wastewater at a semi-industrial scale. This approach is particularly suitable for food industries that are familiar with this yeast. Despite the favorable results, this study had limitations that should be addressed in future research. Although the OFAT method had several advantages for preliminary screening and there were logical reasons for its use in this study, it could not identify potential interactions among operational parameters affecting adsorption. Therefore, to investigate synergistic or antagonistic effects among factors, it is essential to use multivariate methods (e.g., RSM) in future studies. Additionally, resource and cost limitations prevented conducting more replicates and advanced statistical analyses (e.g., Fourier transform infrared and zeta potential). Nevertheless, the available data provide initial insights into the effects of different parameters, including temperature, reaction time, pH, biosorbent concentration, and pollutant concentration, on adsorption efficiency, and can serve as a basis for further research using multivariate statistical designs and more replicates to examine factor interactions and perform more precise statistical analyses. Finally, evaluating the recovery, regeneration, and reuse of biomass in consecutive adsorption-desorption cycles to determine the stability and cost-effectiveness of the process at a larger scale will be a priority for future studies.

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Visualization: Mojtaba Zivari Mehranfar
Supervision: Seyed Hadi Razavi

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Not applicable.

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