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Biosorption of Copper by *Rhizopus arrhizus* and Comparison with Other Biosorbents/Adsorbents: A Critical Review

Recep İleri

Department of Environmental Engineering, Faculty of Engineering, Sakarya University, Sakarya, Türkiye
Email: rileri@gmail.com

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Abstract

In synthetic copper solutions (10 mg/L), freely dead *Rhizopus arrhizus* achieved a removal yield of 89% within the first 10 minutes and reached 98% at equilibrium (60 minutes). Dead immobilised biomass can be used for the removal of copper from solutions of 1 - 100 mg/L initial concentration. The fungus *Rhizopus arrhizus* (immobilized dead form) 59.7 is mg Cu/g and 19.46 mg Cu/g for living form. The saturation uptake capacity was found to be 40 - 94 mg Cu/g for immobilised dead cells compared to 100 - 172 mg Cu/g for freely suspended dead cells, ranges depending on the process parameters. Use of a real wastewater effluent reduced the biomass copper uptake capacity. The saturation uptake was found to be 23.4 mg Cu (II)/g dry weight for immobilised dead cells compared to 33.8 mg Cu (II)/g dry weight for freely suspended dead cells for real wastewater effluents.

Subject Areas

Biological Engineering

Keywords

Copper, *Rhizopus arrhizus*, Biosorption, Comparison, Critical Review

1. Introduction

Copper (II) is widely used in industrial processes, and even at low concentrations, it can be toxic to aquatic organisms and humans. "To ensure safety for human consumption and the environment, copper remediation technologies aim to meet the WHO permissible limit of 2.0 mg Cu/L for drinking water and the U.S. EPA maximum goal of 1.3 mg Cu/L, while also addressing the acute toxicity to fish that can occur even at small concentrations in natural water. Exceeding this limit poses risks to human health and the environment. Therefore, effective treatment of wastewater and soil contaminated with copper is essential to reduce these risks" [1]-[5]. "Biosorption is defined as the removal of heavy metal ions from aqueous solutions using living or dead, freely or immobilised biological materials" [3] [6]. [7] "Biosorption method is particularly effective for treating wastewater with low metal concentrations (1 - 100 mg/L) where traditional methods are economically unviable" [3] [8]-[12]. Copper (II) biosorption is an eco-friendly and cost-effective separation process that utilizes biological materials-such as bacteria, fungi, algae, and agricultural wastes-to remove or recover copper ions from aqueous environments.

"Copper uptake by *Rhizopus arrhizus* biomass ranged between 50 and 150 mg/mL (de Rome and Gadd, 1987) [13] while the results by Preetha and Viruthagiri (2007) [14] recorded that under optimized process conditions (4.14 of pH, 37.75˚C of temperature, 53.84 mg/L of initial copper ion concentration, and 8.17 g/L of biomass loading) about 98.34% copper removal from aqueous solution can be achieved" [13] [14].

"Biosorption is exhibited by bacteria, algae, fungi and yeasts. Not only living organisms, but also residuals of dead bodies of microorganisms shows biosorbent properties like agricultural wastes including husk, seeds, peels and stalks of different crops. Different factors affect the rate of biosorption which includes temperature, pH, nature of biosorbents, surface area to volume ratio, concentration of biomass, initial metal ion concentration and metal affinity to biosorbent. Various models including Freundlich model and Langmuir model can be used to describe biosorption. Recovery of biosorbed metals can be done using agents like thiosulfate, mineral acids and organic acids. Choice of desorption agent should be carefully selected to prevent alteration of physical properties of a biosorbent" [15]. "This technology is particularly valuable because copper is a heavy metal that is toxic to living organisms at elevated concentrations, causing health issues like liver and kidney damage, Wilson's disease, and anemia" [16].

2. Materials and Methods

"*Rhizopus arrhizus* IMI 280098 was grown and maintained at 30˚C on GMY medium containing glucose (30 g/L), malt extract (10 g/L), yeast extract (10 g/L) and 1.2% agar for the solid medium. In order to obtain freely suspended cells, liquid medium was inoculated with *Rhizopus arrhizus* spore suspension in sterile distilled water at 0.5%(v/v) level. After cessation of growth, immobilized cultures were washed twice with double distilled water and then inactivated using 1% formaldehyde. This dead immobilized biomass was used for the removal of copper from solutions of 2 - 100 mg/L initial concentration. Copper solutions for biosorption experiments were prepared using $(NO_3)_2$. $3H_2O$ in double distilled water. Copper concentration was measured by an atomic absorption Spectrophotometer

(AA, Model IL151, Instrumentation Laboratory NC, Massachusetts, USA). A 6-litre bioreactor was employed for the copper biosorption batch kinetic studies. *Rhizopus arrhizus* cells were immobilized naturally by physical entrapment within the open pore network of reticulated polyurethane foam (Declon, Corby, Northants, UK) or stainless-steel knitted mesh material (Knit Mesh Ltd, Surrey, UK). Immobilized biomass could be used as a technical adsorbent in the same way as granular carbon or nonexchange resins in a packed bed, a batch or continuous stirred tank reactor to separate metal ions from aqueous solutions" [3] [17].

"*Rhizopus arrhizus* cell wall is approximately 90% polysaccharides, primarily composed of chitin, chitosan (polymers of N-acetyl D-glucosamine), mannans, and glucans. The wall contains various ligands that act as functional groups for metal binding, including carboxyl (-COOH), phosphate (PO_4^{3-}), amino/amine (-NH), hydroxyl (-OH), and sulfhydryl (-SH) groups" [18]. *Rhizopus arrhizus* is a zygomycete fungus recognized for its rapid growth and resilience to acidic and oxidative conditions. Biomass is typically killed and stabilized using 1% formaldehyde. While living biomass can be used, dead (non-viable) biomass is often preferred for industrial applications because it requires no nutrient supply, is resistant to metal toxicity, and can be stored for long periods (up to 10 months). To enhance structural stability and reuse in bioreactors, cells are often immobilized within reticulated polyurethane foam or stainless-steel mesh. This creates a high-porosity mycelial mat permeable to the bulk liquid. Isotherm models describe the equilibrium relationship between the concentration of the metal in the aqueous phase and the amount of metal adsorbed on the biomass at a constant temperature. "Copper solutions for the biosorption experiments were prepared using distilled water and Cu $(NO)_2 \cdot 3H_2O$ (copper nitrate) from Fisons (AR grade). The pH of the synthetic solution was adjusted to 5.5 or 7.0 using 1 M KOH and 1 M HNO_3" [3].

"The actual wastewater samples were obtained from the Kidsgrove Sewerage Treatment Works located in the town of Kidsgrove, Manchester, England. The sample I (author) collected from Kidsgrove Sewerage Treatment Works the trade effluent as discharged to sewer from ICL's factory in the town. A process which produces a copper bearing effluent. The bulk of the copper is removed by a treatment plant prior to discharge to enable ICL to meet the consent conditions laid down by North West Water Ltd. Since they had not analysed the sample supplied it could only be guessed as to what might have been presented in it. In such effluents, normally copper is in the range of 2 - 3 mg/L (measured as 3 mg/L) and there are probably lesser amounts of tin and lead, approximately 0.5 mg/l. The effluent has little organic strength, a COD of less than 100 mg/1 (measured as 90 mg/L), with only trace quantities of detergents, organic acids esters, alcohols and glycols which are used in some of their processes. There will also be inorganic salts, predominantly nitrate from the nitric acid for etching but also sulphates and chlorides (measured as 37 mg/L). pH of the effluent was 7.95. ICL's effluent is mixed with domestic sewage and they are treated together at Kidsgrove Sewage Treatment Works. The works is a conventional activated sludge plant treating 7000 m^3 per day. Sewage sludge, having been treated by an on—site digestion plant, is disposed of on farmland. The sample, taken from the influent of the advanced treatment plant, contains organic and inorganic pollutants at varying concentrations; this complex solution contains 3 mg/L of copper. Its pH is 7.95. Based on the sources, the removal efficiency for the real effluent was qualitatively described as high, even though the initial copper concentration was relatively low at 3 mg/L" [3].

The biosorption mechanism of copper by *Rhizopus arrhizus* is a complex, multi-stage process primarily involving passive physicochemical interactions with the fungal cell wall, although it can also involve active metabolic accumulation in living cells. In living systems, this stage includes active bioaccumulation, where metal ions penetrate the cell membrane and are transported into the intracellular space. The mechanism is generally categorized into the following stages and processes: Research indicates that copper uptake occurs in two distinct kinetic stages: Physical/Passive Sorption (Stage 1): This is a metabolism-independent, extremely rapid event where approximately 89% - 98% of total uptake occurs within the first 3 to 10 minutes. This phase is characterized by extracellular binding where copper ions interact with the cell wall surface through ion exchange, physical adsorption, and inorganic microprecipitation. Chemical/Biological Sorption (Stage 2): This is a slower phase (taking longer than 10 minutes) involving diffusion and chemical complexation.

2.1. Biosorption and Adsorption Isotherm Models

We may encounter different isotherm models for different biosorbents and adsorbents. Biosorption and adsorption isotherm models is given in **Table 1**.

Table 1. Biosorption and adsorption isotherm models (References: [3] [7] [10] [12] [15] [19] [42] [64] [66]; Table: Recep İleri).

Model	Characteristics and assumptions
Langmuir	Assumes a **homogeneous surface** with a finite number of identical sites, resulting in **monolayer adsorption** with no interactions between adsorbed ions.
Freundlich	An empirical model for **heterogeneous surfaces** that accounts for **multilayer adsorption** and interactions between adsorbed molecules.
Sips (Langmuir-Freundlich)	A hybrid model designed to overcome Freundlich's lack of a saturation limit; it accounts for both **surface heterogeneity** and **finite capacity**.
BET (Brunauer-Emmett-Teller)	Describes **multilayer adsorption** by applying the Langmuir equation to each successive layer of molecules.
Temkin	Assumes that the heat of adsorption decreases linearly with coverage due to **indirect adsorbate-adsorbate interactions**.
Dubinin-Radushkevich (D-R)	Used for porous materials following a **Gaussian energy distribution**; it helps distinguish between physical and chemical adsorption mechanisms.
Redlich-Peterson	A three-parameter hybrid model that approaches Freundlich at high concentrations and Henry's Law at low concentrations.

Isotherm models are used to determine the rate of metal uptake and the rate-limiting steps such as diffusion or chemical reaction) in the biosorption and adsorption process. "The Sips model (also known as the Langmuir-Freundlich hybrid) accounts for surface heterogeneity primarily through the inclusion of a specific heterogeneity parameter. While traditional models like the Langmuir isotherm assume a totally homogeneous surface with identical, finite binding sites, the Sips model is designed to describe more complex biological systems. Its approach to heterogeneity is characterized by the following mechanisms: The Heterogeneity Factor: This parameter is the defining difference between the Sips and Langmuir equations. In the Sips model, the equilibrium concentration is raised to the power (depending on the specific mathematical formulation used). The model is flexible; if the parameter power is equal to unity, the Sips model recovers the Langmuir equation, representing an ideal, homogeneous surface. The magnitude of directly reflects the system's complexity. A larger value for power indicates a higher degree of heterogeneity. This heterogeneity can stem from the physical/chemical properties of the solid biosorbent, the nature of the metal ion (adsorbate), or a combination of both" [19].

2.2. Biosorption and Adsorption Kinetic Models

We may encounter different kinetic models for different biosorbents and adsorbents. Biosorption and adsorption kinetic models is given in **Table 2**.

Table 2. Biosorption and adsorption kinetic models (References: [3] [7] [10] [12] [15] [19] [42] [64] [66]; Table: Recep İleri).

Model	Characteristics and rate-limiting step
Pseudo-first-order (Lagergren)	Assumes the rate is proportional to the number of **unoccupied sites**; typically, describes the initial rapid phase of physical adsorption.
Pseudo-second-order (Ho & McKay)	Based on the assumption that the rate-limiting step is **chemisorption**, involving electron sharing or exchange between ions and functional groups.
Intra-particle diffusion (Weber-Morris)	Analyzes the rate at which metal ions penetrate the pores of the biosorbent; multilinear plots indicate multiple diffusion stages.
Elovich	Used to describe **heterogeneous chemical adsorption** on solid surfaces.
Film diffusion	Describes the rate-limiting transport of metal ions through the liquid boundary layer surrounding the biosorbent particles.

"The pseudo-second-order (PSO) model describes copper kinetics by assuming that the rate-limiting step is chemisorption, which involves physicochemical interactions such as electron sharing or exchange between the copper (II) ions and the surface functional groups of the biosorbent. The process is typically analyzed using its linear form" [19] [20]. "In nearly all studies involving copper and other heavy metals, the PSO model consistently yields the highest correlation coefficients ($R^2 > 0.99$), providing a significantly better fit than the pseudo-first-order (Lagergren) model" [21].

3. Results and Discussions

3.1. Effect of Parameters on Biosorption of Copper by *Rhizopus arrhizus*

The biosorption of copper (II) by the filamentous fungus *Rhizopus arrhizus* is influenced by several critical physicochemical and biological factors. Optimizing these parameters is essential for achieving maximum removal efficiency and uptake capacity. “The biosorption efficiency increases with the increasing amount of biomass of test fungi and biosorption of Cu metal ions by living biomass of *Rhizopus arrhizus* is an environmentally friendly and cost-effective technology. The parameter, such as pH of the solution, temperature, and biosorbent dose significantly affects the biosorption process. The concentration of hydrogen ions affects the sorption behaviour of heavy metal ions. pH had a direct effect on the adsorption of copper metal, and maximum sorption of copper metal ions noticed at pH 7.0. The maximum biosorption of copper observed at 35˚C. The living biomass of *Rhizopus arrhizus* can be utilized as an effective component for managing heavy metal pollution” [22].

Effect of solution pH

The pH level is considered the most critical factor influencing the biosorption process. Optimal Range for *R. arrhizus*, the optimal pH for copper biosorption typically falls between 5.5 and 7.0. Some optimized processes have recorded high efficiency at a lower pH of 4.14. At highly acidic pH levels (below 4.0), the concentration of hydronium ions (H^+) is high. These ions compete with Cu^{2+} for negatively charged binding sites (like carboxyl and phosphate groups) on the fungal cell wall, significantly reducing metal uptake. As pH increases above 7.0, copper removal may appear to increase, but this is often due to the chemical precipitation of metal hydroxides ($Cu\ (OH)_2$) rather than true biosorption. “A study showed that environmental pH significantly affects the bioavailability of heavy metal ions and their affinity for cell surface ligands, making pH as a key factor in metal ion tolerance” [23].

Effect of temperature

Temperature impacts both the metabolic activity (in living cells) and the kinetic energy of the metal ions. Maximum copper uptake by *Rhizopus arrhizus* is generally observed between 30˚C and 37.75˚C. Higher temperatures can enhance biosorption by increasing the kinetic energy of the copper particles, activating more binding sites, and improving the diffusivity of the metal ions across the cell surface. Very high temperatures may physically damage the biosorbent structure or cause the desorption of previously bound ions due to increased thermal energy. “In living *Rhizopus arrhizus*, metal ions penetrate the cell membrane through active transport/absorption, a process that requires metabolic energy” [24].

Effect of contact time and kinetics

Copper biosorption by *Rhizopus arrhizus* is characterized by its remarkable speed. Approximately 89% - 90% of the total copper uptake occurs within the first

10 to 20 minutes of contact. This is attributed to the immediate availability of a large number of vacant surface binding sites. The system generally reaches a steady state or equilibrium within 60 to 120 minutes.

Effect of initial copper concentration

The concentration of copper in the initial solution acts as a powerful driving force.

Effect of biosorbent dosage

The amount of *Rhizopus arrhizus* biomass added to the solution significantly affects performance.

Effect of uptake capacity (mg/g)

As the initial concentration increases, the specific uptake (mg of metal per gram of biomass) also increases because the higher concentration gradient helps overcome mass transfer resistance. However, the amount of metal adsorbed per unit dry weight (mg/g) often decreases as the dosage increases. This is frequently due to cell aggregation, which reduces the effective surface area and causes interference between binding sites. **Equilibrium uptake:** Biosorption of copper by freely dead suspended, immobilized dead and freely living *Rhizopus arrhizus* are a rapid process, reaching 90% of the equilibrium uptake within 10 minutes and completing equilibrium in less than 60 minutes (1 hour) at 30˚C. It is uptake capacity at equilibrium time (accepted 60 minutes for *Rhizopus arrhizus* in this study). **Average uptake:** It is average of the uptake capacities obtained in repeated experiments. **First-cycle uptake:** It is initial uptake capacity in biosorption and desorption repeated experiments.

Effect of removal efficiency (%)

Conversely, the percentage of metal removed typically decreases at higher initial concentrations. This happens because the finite number of binding sites on the *Rhizopus arrhizus* surface becomes saturated, leaving more ions remaining in the solution. **Effect of total removal efficiency (%):** Increasing the biomass dosage generally leads to a higher percentage of total copper removal because more active binding sites are available. **Removal Efficiency (%):** Copper removal efficiency (%) represents the percentage of copper ions successfully sequestered by the biosorbent/adsorbent from the initial solution. It is a key indicator used to evaluate the performance of different biomass types (like algae, fungi, or agricultural waste) under various conditions. To calculate this, the following mass balance equation is used: $\{[(C_o - C_e)/C_o] \times 100\}$. C_o (Initial concentration): The concentration of copper in the solution before adding the biosorbent (mg/L) and C_e (Equilibrium/Final Concentration): The concentration of copper remaining in the liquid phase after the biosorption process is complete (mg/L).

Effect of presence of competing ions (co-ions)

"Industrial wastewaters often contain multiple metals that compete for the same non-specific functional groups on the fungal cell wall. In multi-metal systems (e.g., Cu, Ni, Pb), the presence of other cations can reduce copper uptake. *Rhizopus arrhizus* often shows a higher affinity for lead than for copper, following an order such as Pb (II) > Ni (II) > Cu (II). Interestingly, some studies show that while copper may inhibit the uptake of other metals like nickel or magnesium, the rate of copper biosorption itself can remain relatively unaffected by the presence of nickel" [25] [26].

Effect of physical state of the biomass: Living or Dead

"Non-living (dead) biomass is often preferred because it is resistant to metal toxicity, requires no nutrients, and can be stored for long periods. **Immobilization:** While immobilizing *Rhizopus arrhizus* in matrices like polyurethane foam or stainless-steel sheets improves industrial handling, it can introduce mass transfer resistances that may slightly lower the observed biosorption rate compared to freely suspended cells" [3] [6] [17] [27].

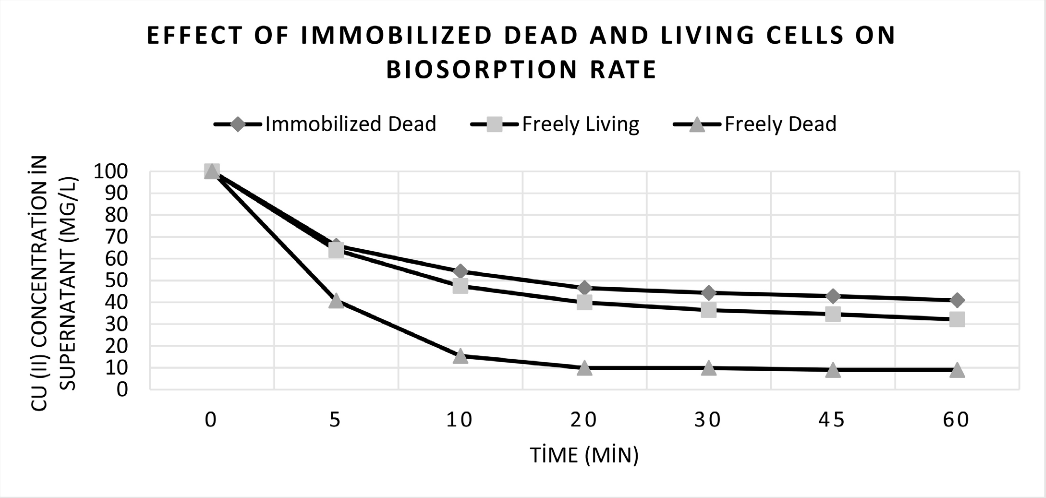
Effect of surface heterogeneity

"Pretreatment (such as with acid) can increase surface heterogeneity by creating cavities and cracks, thereby exposing more active sites and enhancing the overall capacity" [19].

Effect of immobilized and living cells on biosorption rate

Effect of immobilized dead, freely living and freely dead *Rhizopus arrhizu*s cells on biosorption rate are given in Figure 1.

Figure 1 shows the copper concentration in the supernatant as function of time for the 100 mg/L initial concentration of copper solution during biosorption by freely dead, immobilized dead and living cells. The observed copper uptake was rapid with similar kinetic behaviour has been shown in Figure 1. Biosorption of copper by freely dead suspended, immobilized dead and freely living *Rhizopus arrhizu*s are a rapid process, reaching 90% of the equilibrium uptake within 10 minutes and completing equilibrium in less than 60 minutes (1 hour) at 30˚C.

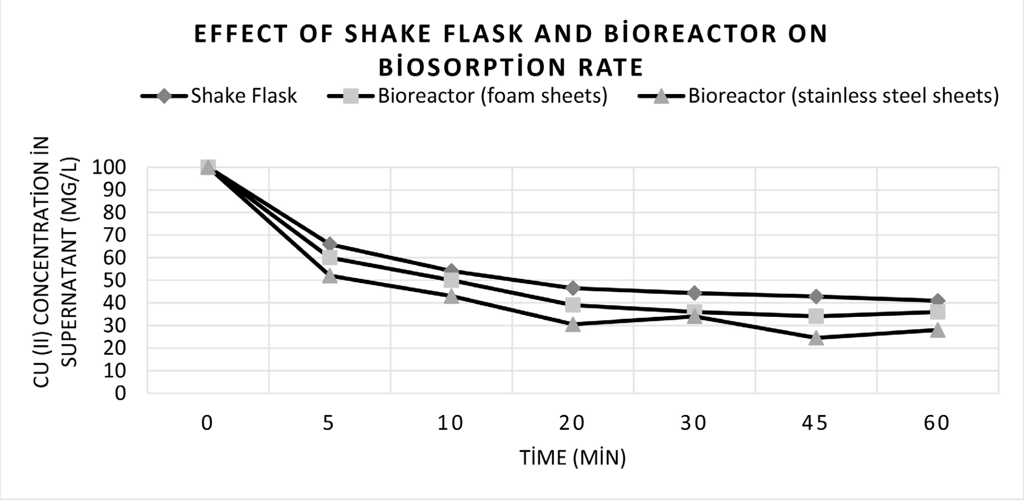


(Source: Author's own elaboration).

Figure 1. Effect of immobilized dead, freely living and freely dead *Rhizopus arrhizu*s cells on copper biosorption rate. (Shake flask experiment: pH = 5.5, T = 30˚C, C_{b0} = 100 mg Cu/L; Agitation = 200 rpm, volume = 100 mL, slab of reticulated polyurethane foam sheet = 20 pi (8 × 2.5 × 1 cm foam sheet; Immobilized dead cells biomass = 5.5 g d.w./L; Freely living cells biomass = 5.8 g d.w./L; Freely dead cells biomass = 5.7 g d.w./L).

Effect of shake flask (100 mL) and bioreactor (6 L) on biosorption rate:

Comparison of biosorption of copper by dead immobilized *Rhizopus arrhizus* in shake flask (100 mL) and bioreactor (6 liter) experiments are given in Figure 2. 60 minutes (1 hour) equilibrium time was established at 30˚C and the final solute and mycelial copper concentrations were determined. Uptake rate for all of the materials is very similar, so there were no marked differences between different support materials and therefore 20 ppi polyurethane reticulated foam and 1.6 - 2.0 stitches per cm stainless steel mesh sheet were chosen for saturation experiments.



(Source: Author's own elaboration).

Figure 2. Rate of copper biosorption by immobilized dead *Rhizopus arrhizus* in shake flask (100 mL) and Batch Sheet Bioreactor (6 L). (Shake flask experiment: pH = 5.5, T = 30˚C, C_{b0} = 100 mg Cu/L; Agitation = 200 rpm, volume = 100 mL, biosorbent: 4.90 g d.w./L, slab of reticulated polyurethane foam sheets = 20 pi (8 × 2.5 × 1 cm foam sheet). (Batch Sheet Bioreactor (BSB) experiment: pH = 5.5, T = 30˚C, C_{b0} = 100 mg Cu/L; Agitation = 400 rpm, volume = 6 L, biosorbent: 4.95 g d.w./L, reticulated polyurethane foam sheets = 20 pi (12 × 14 × 6 × 1 cm foam sheets). (Batch Sheet Bioreactor (BSB) experiment: pH = 5.5, T = 30˚C, Cb_0 = 100 mg Cu/L; Agitation = 400 rpm, Volume = 6 L, Biosorbent = 5.45 g d.w./L, Stainless steel sheets = 1.6 - 2.0 stitches per cm. (16 × 14 × 6 × 0.5 cm stainless steel sheets).

3.2. Biosorption of Copper by *Rhizopus arrhizus* and Comparison with Other Biosorbents/Adsorbents

Biosorption of copper (II) by *Rhizopus arrhizus* and comparison with other biosorbents/adsorbents are given based on pH, temperature, initial copper concentration, uptake capacity and copper removal efficiency. These chosen values by author are the most important for comparison different biosorbents and adsorbents. Biosorption (biological adsorption and biological absorption; physical, chemical/biochemical, biological; surface uptake and mass transfer and interior uptake) and adsorption (physical, chemical) phenomena are very complex to understand and comparison. But comparison is very important to choose suitable biosorbents/adsorbents removal of pollutions (including heavy metals) because application to real life and real effluents. Best or good biosorbents/adsorbents can vary depending on the conditions. One of the main aims of this study is to demonstrate that biosorption of copper (II) ions by immobilized dead *Rhizopus arrhizus* is an alternative biosorbent by making comparisons according to author's studies, author's team studies and other related literature studies. Data and information about the author's studies have been combined into figures for comparison purposes. In this critical review study; figures, tables, comparisons, comments, inferences and critical reviews are made for this review paper. Biosorption of copper (II) by different biosorbents and adsorbents are given in Table 3.

Table 3. Biosorption of copper (II) by different biosorbents and adsorbents (Data: [References] in Table; Table: Recep İleri).

Biosorbent/Adsorbent	**pH**	**Temperature (˚C)**	**Initial copper (II) concentration (mg Cu/L)**	**Uptake capacity (mg Cu/g)**	**Copper (II) removal efficiency (%)**	**References [ ]**
Rhizopus arrhizus (freely dead) (synthetic copper solutions) **FDS1990**	5.5 (5.0 - 7.0)	30	10 (2 - 100)	**104.1** (average) (100 - 172)	**80.3**	[3] [8] [9]
Rhizopus arrhizus (freely dead) (synthetic copper solutions) **FDS2008**	5.5 (5.0 - 5.5)	30	10 (2 - 100)	**100** (average)	**94** (average) 89 (in 10 minutes) 98 (at equilibrium: 60 minutes)	[19]
Rhizopus arrhizus (freely dead) (real wastewater effluents) **FDR**	5.5	30	3	**33.8**	**54**	[3] [8] [9]
Rhizopus arrhizus (immobilized dead) (synthetic copper solutions) **IDS**	5.5 (5.0 - 7.0)	30	10 (2 - 100)	**65** (average) (43 - 93.8)	**95.0**	[3] [6]
Rhizopus arrhizus (immobilized dead) (real wastewater effluents) **IDR**	5.5	30	3	**23.4**	**37**	[3] [6]
Rhizopus arrhizus (immobilized living biomass) (synthetic copper solutions) **ILS**	7.0	35	10 (2 - 100)	**19.46**	**78.6**	[3] [19]
Rhizopus arrhizus (Batch Sheet Bioreactor, immobilized dead biofilm) (synthetic copper solutions) **BSBIDBS**	5.0 - 5.5	30 - 35	10 (2 - 100)	**52.0** (average)	**88** (average) 80 - 95	[3] [28]
Rhizopus arrhizus (continuous sheet bioreactor, immobilized dead biofilm) (synthetic copper solutions) **CSBIDBS**	5.0 - 5.5	30 - 35	10 (2 - 100)	**51.0** (average) 59.7 (initially) 42.7 (after six cycles)	**83** (average) 70 - 95	[3] [28]

Continued

Mixed freely dead waste activated sludge biomass (inactivated with 1% formaldehyde) (synthetic copper solutions)	5.0 - 5.5	30	10 (2 - 100)	50	90 (average) 85 (in 10 minutes) 95 (at equilibrium: 120 minutes)	[19]
Mixed freely dead waste activated sludge biomass (inactivated with 1% sulfuric acid) (synthetic copper solutions)	5.0 - 5.5	30	10 (2 - 100) 10 (2 - 100)	50	87 (average) 82 (in 10 minutes) 92 (at equilibrium: 120 minutes)	[19]
Rhizopus arrhizus (freely living biomass) 100 mg biomass: 200 mg biomass:	7.0	35	80 80	28.725 (author) (average) 37.785 19.464	95.89 (author) (average) 94.46 97.32	[22]
Rhizopus arrhizus (dead immobilized) (synthetic copper solutions)	4 - 6	25	100	17.1	80 - 90	[13]
Rhizopus arrhizus (freely dead) (synthetic copper solutions)	4.0 - 5.0	21 - 25	10 - 100	16	80 - 90	[29]
Rhizopus arrhizus (freely dead) (synthetic copper solutions)	6.0	25	100 (50 - 250)	29.9 - 37.79	98.34	[14]
Rhizopus arrhizus (freely dead) (synthetic copper solutions)	5.0	25 - 30	100 - 200	55	90	[30]
Aspergillus terreus	5.0	30	Not in source	160 - 180	85 - 95	[31]
Rhizopus arrhizus (living freely) (synthetic copper solutions)	4.5	30	25 75 150	10.76	35 - 40 28 - 30 15 - 20	[32]
Saccharomyces cerevisiae	4.0 - 7.0	25	198	29.9 - 198	60	[15]
S. cerevisiae Perlage® BB	5.0 - 6.0	30	25 (5 - 50)	4.50 - 4.73	34 - 76	[15]
Aspergillus niger (living freely)	4.5 - 5.0	30	25 75 150	9.53	25 - 40 24 - 26 12 - 25	[32]
Parachlorella kessleri (green alga) (dead powder)	4 - 4.5	20 - 25	100 - 160	23 - 45 - 80	57.5 - 92.8	[33]
Polytrichum commune (biomass) (dead powder)	4 - 4.5	20 - 25	100 - 160	15.6	39.0 - 79.5	[33]
Shpagnum sp. (mosses) (dead powder)	-	20 - 25	100 - 160	15.1	37.7 - 85.4	[33]

Continued

Ion-exchanger (commercial cationic exchanger amberlite IR 120)	4 - 4.5	20 - 25	100 - 160	7.4 - 71	95.3 - 96.1	[33]
Lessonia berteroana (brown alga)	3.0	20	20 - 500	66.09	60 - 75	[34] [35]
Sugar beet pulp	4.0 - 5.0	25	250	28.5	90	[36]
Herbaceous peat	5.5	21	19.012 38.124	1.85 3.53	97.04 92.65	[37]
Algal-Fe_2O_3 composite	5.0 - 5.6	25 - 45	20 - 100	285.7 - 297.7	57.6 - 75.9	[38]
Sunflower hulls	5.0	25 - 30	100	57.14	75.4 (69 - 90)	[39]
Crab carapace	4.4 - 6.0	25	50 - 100 (0.5 - 100)	79.4	>95.0	[40]
Fucus vesiculosus	4.0 - 5.5	20	10 - 500	114.9 (150 - 160)	>95.0 90	[41]
Rosa damascena leaves	5.5	25 - 45	55 60 150 (30 - 150)	25.13 (17.1 - 18.6)	88.7 84 46.3 (90.1 - 90.7)	[42]
Cuttlebone powder	5.0	25	500	14.42 - 54.05	98.75 - 99.39	[43]
Magnetic thistle (EMT)	4.0 - 5.0	45	50 - 300	55.30	60.8 - 72.3	[44]
Active sludge bacteria	4.0 - 5.0	20 - 25	100	50 - 76	95	[45]
Sphagnum sp. (Moss)	5.0	22	16	71.5	85.4	[33]
Melissa officinalis L. (MO)	4.0	20	50 - 500	59.95	84	[34]
Melissa officinalis waste (M)	4.0	20	50 - 500	47.66	80	[34]
Cane papyrus	6.5	25 - 30	10 - 50	2.27	95.0	[46]
Orange peels powder	5	40	100	6.6	35.4	[47]
Lemon peels powder	5	40	100	13.2	71.3	[47]
Orange peels	5 - 6	25 - 30	5 - 100	38.2	86.6 (85 - 98)	[48]
Rosa damascena leaves powder (optimum conditions): Free biosorbent: Immobilized biosorbent:	5.5 (2 - 6)	45 45 45	55 55 55	25.13 25.13 25.13	88.7 90.1 84.0 46.3 88.7 90.7	[42]
Olive pomace	5.0	20 - 25	10	12.9	86.5	[49]
Walnut shell	5.0	20 - 25	10	8.3	88.0	[49]
Fly ash-derived zeolites	5.0	Room temperature	100 - 700	28.6 - 53.5	90	[50]
*Rossellomorea sp. ZC*255	7.0	28	1600	253.4	Not in source	[2] [51]
Paenibacillus polymyxa	4.0 - 6.0	22	2 - 100	1.602	90 - 96	[51]

Continued

Peat moss-derived biochars	4.0 - 6.0	22	50 - 250	18.2 - 60	82 - 99.3	[51]
Serratia plymuthica	4.42 - 5.03	20 - 25	200	80.5	91.5	[16]
Lavandula angustifolia	4.0	20 - 60	50 - 500	40.52 (28.08 - 59.05)	90.3	[34]
Mango peel	5.0 - 6.0	25 - 30	10 - 100	46.09	89 - 90	[5]
Banana peel	5.0 - 6.0	20 - 30	10 - 80	3.29 - 48.7	88.9 - 96.8	[5]
Alfa grass	5.5 - 6.0	25	50	25 - 35	70 - 85	[5]
Codium vermilara	5.0 - 5.28	25	48.75	54.9	85.5	[52]
Papaya seeds	3.0 - 5.0	25	5	17.24	80 - 90	[51]
Soybean hulls (modified)	4.8 (4.8 - 6.0)	25 (25 - 60)	100 - 150	20.54 - 91.5 - 154.9	80 - 99	[4]
Wood apple shell	5.5	30	50	31.15	94.2	[5]
Watermelon shell Raw (unmodified): Modified:	4.0 - 6.0 (5.0)	25 - 40 (25 - 30)	10 - 500 (50 - 100)	6.28 111.1 - 134.23 - 357.14	91.8 - 99.9	[51]
Cassava tuber bark waste Raw (unmodified): Chemical modified:	5.0 - 5.5	30	50 - 100	33.3 - 62.97 - 90.9 54.21 - 85.2	70 - 94	[4]
Neem leaves	5.0 - 6.0	25 - 30	50 - 100	62.97 (13 - 100)	74 (70 - 94)	[5]
Rice husk Raw (unmodified): Chemical modified:	4.0 - 6.0	25 - 30	10 - 100	1.62 - 7.19 17	88 - 94	[5]
Sophora alopecuroides residue	4.0 - 5.0	25 (20 - 60)	5 - 100	35.84 (1.77 - 18.98)	85 - 95	[53]
Amberlite IR 120 (rosin)	5.0	25	20 - 100	26.27 (26 - 40)	99.9	[53]
Lewatit S-100 (rosin)	5.0	25	20 - 100	46.55	99	[53]
Komagataella phaffii (Modified, X-33-Cyb5R)	4.0	30	763	14.27	Not in source	[54]
Bacillus licheniformis	4.0 - 6.0	20 - 30	10 - 200	39.37 - 61.8	70 - 99.4	[18]
Delftia tsuruhatensis (bacteria)	5.0 - 6.0	37	50 - 100 - 150	Not in source	45 - 66	[55]
Alcaligenes faecalis (bacteria)	5.0 - 6.0	37	50 - 100 - 150	Not in source	34 - 39	[55]
Klebsiella pneumoniae (bacteria)	7.0	30 (25 - 44)	5 - 10	19.26 - 28.77	93	[56]
Pantoea agglomerans (bacteria)	5.0	30	50 - 150	1.42	73.74	[56]
Coal gangue-derived zeolite	5.0 - 7.0	25 - 45	Not in source	38.46 - 41.84 - 45.05	100	[57]

Continued

Halalkalicoccus sp. Dap5	6.5 - 8.1	40	28.8 (10 - 80)	Not in source	87.3 - 90.8 - 92	[1]
Activated ganga sand	8.0 (4 - 12)	room temperature	34 (10 - 100)	48.82	97 (35 - 97)	[58]
Clinoptilolite zeolite	4.0 (2.5 - 6.0)	30 - 90	0.36 - 2.75	4.23	20 - 100	[59]
Rhizopus arrhizus	4.0 - 5.0	25 - 30	10 - 200	35 - 40	90 - 95	[60]
Rhizopus arrhizus	5.0	30	25 - 700	40 - 120	89.95	[61]
Effective with algae and modified agricultural residues, peaks	5.0 - 6.0	20 - 50	39 - 224	138	90	[62]
Lessonia berteroana (alga biomass)	4.0 - 5.0	25	20 - 500	41.1 - 66.1	95	[63]
Brown algae	4.0 - 5.5	20 - 35	10 - 1000	64 - 108	90	[64]
Agricultural wastes (rice husk, wheat straw, sugarcane), Bacteria (*Bacillus subtilis*) Activated carbon (fruit peels, nut shells) (low-cost, locally available)	5.0 - 6.0	25 - 30	50 - 150	23.3 - 42.3	90	[65]
Rhizopus arrhizus (fungus) *Bacillus subtilis* (bacteria) *Chlorella vulgaris* (green algae)	5.0 - 6.0	25 - 30	10 - 250	2.59 - 28.5	80 - –99	[66]
Pseudomonas putida (bacteria)	5.5	25 - 30	1 - 100	8.9 - 238	50 - 93	[67]
Rhizopus arrhizus (fungus)	5.5 - 7.0	25 - 35	10 - 200	7.32 - 37.79	94 - 97	[68]
Chitosan-based hydrogels Agricultural wastes	3.0 - 6.0 3.0 - 6.0	20 - 35 20 - 35	5 - 200 5 - 200	60 - 130 7 - 40	95 - 99 70 - 99	[69]
Modified lemon peel biomass	6.0	25	25 - 75	199.5	90	[70]
Agricultural residues/Plant biomass: Such as rice husk, coconut shell, orange peels, and sugarcane bagasse.	3.0 - 6.0	25 - 30	2 - 100	24.2 - 73.0	37 - 94.8	[71]
Bacteria (*Bacillus subtilis*), fungi (*Aspergillus niger*), and yeast (*Saccharomyces cerevisiae*) Agricultural/industrial wastes (rice hulls, tree bark, and chitin, alongside natural substances including peat and various algae)	4.0 - 6.0	25	2 - 50	3.92 - 18.58	20.3 - 93.5	[72]

Continued

Escherichia coli (surface-engineered microbial systems integrated with magnetic nanoparticles)	4.0 - 5.5	25 - 37	50 - 250	21	90	[73]
Sugar beet pulp	4.0	25	25 - 250	31.37	45.9	[74]
Sugar beet pulp	4.0	25 - 45	25.6 - 252.9	28.5 - 31.4	6.3 - 45.9	[75]
Chlorella vulgaris	4.0 - 4.5	25	150 - 200	43.5	80 - 95	[76]
Zoogloea ramigera	4.0 - 4.5	25	100 - 125	21.7	85 - 94	[76]
Dragon fruit peel (DFP) (chemically modified)	5.0	25	100	93	Not in source	[77]
Rambutan peel (RP) (chemically modified)	4.0	25	100	192	Not in source	[77]
Passion fruit peel (PFP) (chemically modified)	4.0	25	100	122	Not in source	[77]
Alginate-based ion Exchanger calcium alginate Hydrogen alginate	5.0	25	3 - 64	107 189	95	[78]
Waste activated sludge	4.0 - 5.5	25 - 35	10 - 100	1.5 - 4.0	90 - 99	[79]
Sphaerotilus natans	4.0 - 6.0	30	10 - 100	15 - 60	95 - 99	[80]
Olive stone	5.5 - 6.0	25	10 - 250	0.78 - 7.5 - 8.08	80 - 90	[81]
Gibberella sp. NT-1	5.0	28 - 30	10 - 500	1.6 - 7.5	20 - 85	[82]
Underia pinnatifida (treated alga)	4.0	25	5 - 50	38.82	70 - 88	[83]
Saccharomyces cerevisiae (*Perlage® BB*) (yeast)	5.5	30	5 - −100	4.73	76	[84]
Sphaerotilus natans	4.0 - 6.0	25	10 - 100	15 - 60	85 - 90	[85]
Aspergillus niger	4.0 - 6.0	25 - 30 (room temperature)	500 - 500	11.2 - 50.5	70 - 90	[86]
Fungal melanin	4.0 - 6.0	25 - 28	300 - 500	3.5 - 69.2	50 - 95	[87]
Bacterial strains *Serratia plymuthica SC5II* *Stenotrophomonas sp.* 13*a*	5.0 5.0	30 30	200 200	80.5 8.6	67 40	[88]
Azotobacter nigricans NEWG-1	8.0	20 - 30	200	Not in source	82.35 - 80.56	[89]
Bacteria (natural baseline) Bacteria (genetical modified)	1.5 - 2.5 1.5 - 2.5	25 - 35 25 - 35	2 - 50 2 - −50	0.1 - 0.5 1 - 15	80 94	[90]
Agricultural residue-derived biosorbents	4.0 - 6.0	20 - 60	10 - 500 50 - 200	91.74 - 133.33	95.5 - 99.3	[91]

Continued

Raw bagasse: Acid-modified sugarcane bagasse (ASG): Base-modified bagasse (BSG): Raw bagasse with activated carbon:	5.0 5.0 5.0 5.0	25 25 25 25	10 10 10 10	2.06 4.84 5.35 5.62	88.9 96.9 94.8 98.5	[92]
Hevea brasiliensis sawdust (unmodified) raw sawdust (RSD): Hevea brasiliensis sawdust (modified) phosphoric acid modified (PMSD4): Sodium hydroxide modified (SMSD3):	6.0 6.0 6.0	30 30 30	20 20 20	3.45 8.11 9.24	Not in source	[93]
Peanut husk (conventional): Peanut husk (ultrasound):	5.0 - 6.0 5.0 - 6.0	30 30	100 100	17.82 19.60	94.5 98	[94]
Phanerochaete chrysosporium (loofa-immobilized)	6.0	10 - 50	10 - 500	102.8	95	[95]
Natural materials like banana peel, astragalus, chestnut shell, and agricultural wastes (rice husk, sugarcane bagasse)	5.0 - 6.0	25 - 40	10 - 50	10 - 100	90	[96]
Aspergillus flavus	4.0 - 6.0	25 - 30	10 - 500	20.75 - 93.65	66.5 - 97	[97]
Aspergillus terreus	4.0 - 6.0	25 - 30	10 - 50 500	11.35	68.52 - 90 20 - 25	[98]
Aspergillus australensis	4.0 - 6.0	25 - 30	100 - 500	1.54 - 2.66	40 - 60	[99]
Aspergillus niger (dead) *Aspergillus niger* (living)	4.0 - 6.0	25 - 30	10 - 50 50 - 250 250 - 1000 50 - 100	13.4–18.05 4 - 12	95 - 99 70 - 90 20 - 50 85	[99]
Modified soybean hulls Chitosan	5.0 - 6.0 5.0 - 6.0	20 - 25 20 - 25	10 - 500 10 - 500	254.9 222	99 99	[100]
Saccharomyces cerevisiae (Baker's yeast) *Rhizopus arrhizus* (Fungus)	4.0 - 5.0 4.0 - 5.5	25 25 - 30	10 - 100 50 - 500	2.6 - 3.0 15 - 20	90 95 - 98	[10]
Thiobacillus thiooxidans (bacteria)	4.0 - 5.0	40	25 - 150	39.84	80 - 90	[101]
Banana pith (synthetic solution) (electroplating waste)	4.5	25 - 27	5 - 250 8.55	13.46 8.55	90 91.2	[102]
Rhizopus arrhizus *Aspergillus niger*	5.0 5.0	25 - 30 25 - 30	100 - 150 100 - 150	72.38 58.20	70 - 100 60 - 88	[103]

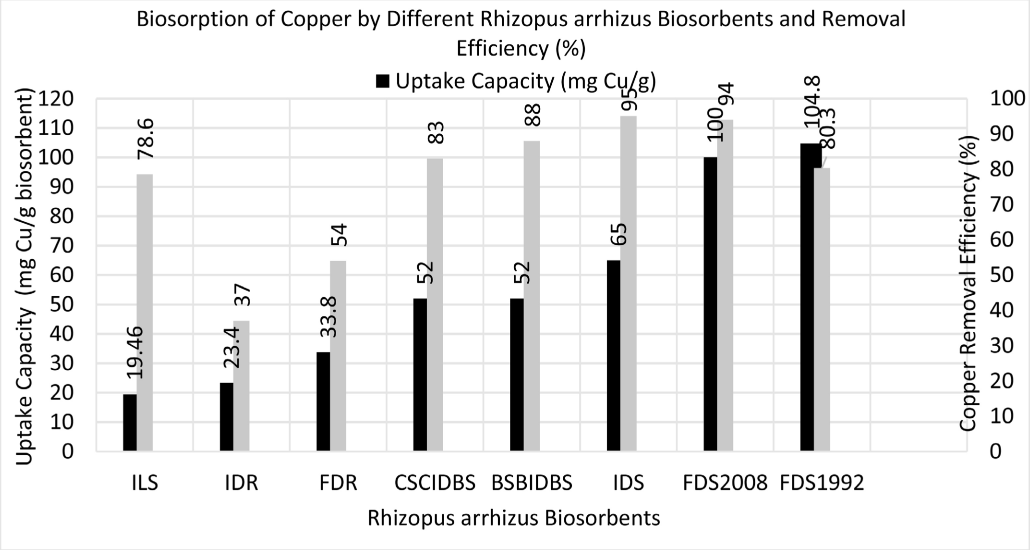
Continued

Soybean hulls (citric acid-modified)	4.0 - 5.0	25 - 30	31.77 - 2541.84	83.2	90	[104]
High-porosity activated carbon	5.0	60	98.5	195	Not in source	[105]
Kolubara lignite (adsorbent)	5.0	Room temperature	50 - 330	2.625 - 4.045	94	[106]
Activated ganga sand	8.7	25	34	80	97.7	[58]
Waste biomass	5.0	25	31.75	35	97 - 99	[107]
Modified loquat leaves	6.0	20	10 - 100	33.33	60 - 98	[108]
Carrot residues	5.0	25	10 - 100	32.74	90 - 95	[109]
Activated carbon	5.0	25 - 30	100 - 300	20 - 55	81 - 94	[110]
Silica gel-water adsorption	5.0 - 6.0	25 - 30	10 - 500	19.9 - 63.5	99	[111]
Ulothrix zonata (green algae)	4.5	20	30 - −150	176.2	90	[112]
Thuja orientalis cones	2.1 - 7.7	16 - 40 - 70	30 - 90 - 200	19.23	90 - 98	[113]
Dehydrated wheat bran	3.5 - 5.0	20 - 40 - 60	25 - 100 - 250	24.1 - 50.2	44.1 - 96.4	[114]
Sphaerotilus natans	3.0 - 6.0	25	10 - −150	2.6 - 13.98 - 27.96 - 60	15 - 95	[115]
Aspergillus hiratsukae (*LF*1) *Aspergillus terreus* (*LF*2)	4.0 - 7.0	32	100 - 500	4.63 5.95	24 - 69	[98]
Pellets made from fired coal fly ash (adsorbent)	6.0	25	50 - 300	20.92	99.6	[116]
Sugar beet pulp	4.0	25 - 45	25.6 - 258.8	11.8 - 28.5	6.3 - 45.9	[117]
Ion exchange resin amberjet 1500H Ambersep 252H	5.8 5.8	25 25	10 - 20 10 - 20	19.98 18.82	99 94 - 99	[118]
Rhizopus arrhizus	4.0 - 5.0	25 - 35	100 - 300	19.4 - 28.7	75 - 98	[119]
Rhizopus arrhizus	4.0 - 7.0	25 - 35	25 - 200	16.5 - 18.25 - 37.8	70 - 80 - 94.5	[120]
Modified jute fibres	4.0 - 5.0	35	73 - 465	7.73 - 8.40	85 - 95	[121]
Bacterial biosorbents	5.0 - 7.0	25 - 28	10 - 200	10.12–253.4	20 - 99.4	[122]
Natural zeolite (clinoptilolite) Exfoliated vermiculite	4.0	25	317.7	8.3 - 11.8 21.5	37.3 67.6	[123]
Rhizopus arrhizus (immobilized dead) (mixed metal ions)	4.0 - 5.0	25 - 35	50 - 500	16.5 - 33.4	85 - 95	[124]
Anaerobically digested biological sludge	4.0 - 5.0	25	5 - 250	13.72 - 37.68	90 - 98	[125]
Pleurotus pulmonarius *Schizophyllum commune*	4.0 4.0	50 50	5 - 200 5 - 200	13.5 10.4	80 - 90	[126]
Crab shell particles peaks	6.0	30	500 - 2000	243.9	49.4 - 75.4	[127]

Continued

Tartaric acid modified rice hulk (TARH)	5.0 - 6.0	27	100 - 800	29.0	95 - 99	[128]
Natural clay (vermiculite)	4.0 - 4.5	25 (room temperature)	8.51	1.15	70 - 99	[129]

Biosorption of copper by different *Rhizopus arrhizus* biosorbents and removal efficiency are shown in **Figure 3**.



(Source: Author's own elaboration).

Figure 3. Biosorption of copper by different *Rhizopus arrhizus* biosorbents and removal efficiency.

The removal efficiency of copper (II) from synthetic (model) solutions varies significantly depending on the type of biosorbent used, its physical form, the initial metal concentration, and optimized environmental conditions. "Copper removal efficiency is highly sensitive to pH, becoming significantly more effective as pH increases. The optimal range for biosorption using *Rhizopus arrhizus* was found to be pH 5.5 to 7.0" [3] [6] [60] [61]. "The saturation capacity depends on the state of the biomass and the type of solution. Free dead *Rhizopus arrhizus* demonstrated the highest capacity in synthetic copper solutions (2 - 100 mg Cu^{2+}/L) at 172 (average 104.1) mg Cu^{2+}/g and in real industrial wastewater effluent (copper concentration 3 mg Cu^{2+}/L), this dropped to 33.8 mg/g. Immobilized dead *Rhizopus arrhizus* are capacities in synthetic solutions ranged from 43 to 93.8 mg/g, and dropped to 23.4 mg/g in real industrial effluents" [8] [9].

"Percentage removal is generally higher at lower initial concentrations, as active sites on the biomass surface become saturated more quickly at higher concentrations" [3] [6] [62] [63]. "In synthetic solutions (10 mg/L initial concentration), freely dead *Rhizopus arrhizus* achieved a removal yield of 89% within the first 10 minutes and reached 98% at equilibrium (60 minutes)" [3]. "Based on the provided sources, the copper uptake capacity for freely living biomass of *Rhizopus arrhizus* is significantly lower than that of dead biomass, with reported maximum values ranging from 19.464 mg/g to 37.785 mg/g" [22].

"Industrial effluents typically contain a variety of metal ions and other pollutants. To implement the biosorption process in practical applications and better understand ion competition, increasing investigations are conducted on multicomponent systems. However, most studies utilize synthetic solutions, which do not fully represent real environmental conditions where effluents contain diverse pollutants, including organic compounds that interact with each other. This difference highlights the impact of other ions present in the polluted water. These ions also compete for adsorption sites on the sorbent surface, reducing the overall adsorption capacity for Cu (II) ions" [34].

"The Algal-Fe_2O_3 nanoparticle composite achieves the highest reported capacity at 297.7 mg Cu/g" [38]. "The yeast *Saccharomyces cerevisiae* [15] and the fungus *Rhizopus arrhizus* (freely dead form) [8] [9] is among the most potent biological materials, with capacities exceeding 29.9 mg Cu/gr, 170 mg Cu/g, respectively" [8] [9]. "The fungus *Rhizopus arrhizus* (immobilized dead form) 65 is mg Cu/g [3] [6] and 19.46 mg Cu/g for living form [3] [19]. "For the other study is 19.464 - 37.785 mg Cu/g for living form *Rhizopus arrhizus* biomass" [22].

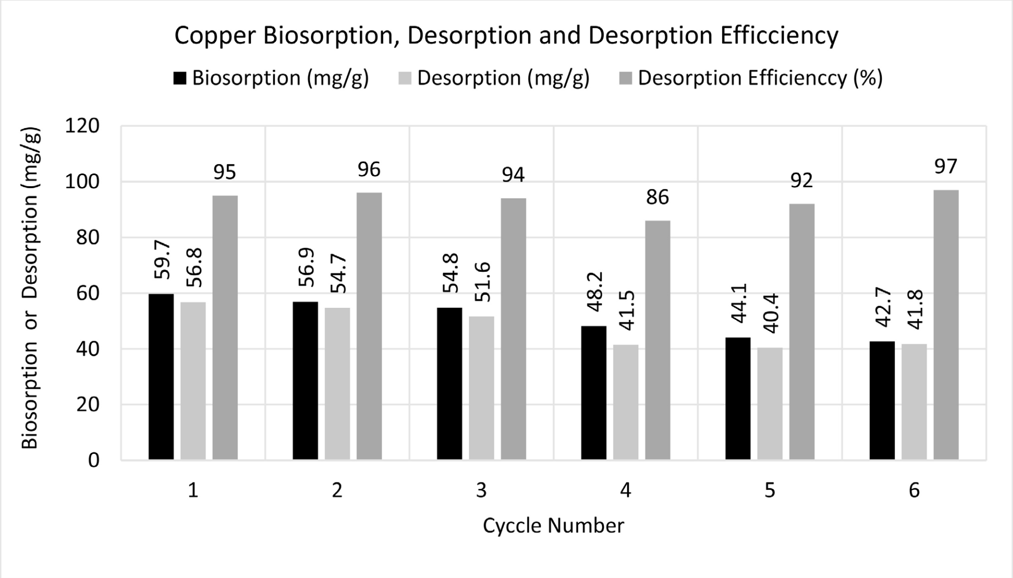
"Significant uptake for species like *Parachlorella kessleri*, *Shpagnum sp. Serratia plymuthica* and Ion-exchanger (commercial cationic exchanger Amberlite IR 120) occurs very rapidly, often within 4 to 10 minutes of initial contact. The maximum decrease of copper (II) concentration was detected in both model solutions for cationic exchanger. Copper removal efficiency of green alga *Parachlorella kessleri* was comparable to efficiency of commercial ion-exchanger under conditions of lower copper (II) concentration in solution" [33].

"The adsorption properties of four plant materials—*Melissa officinalis* L. (MO); *Lavandula angustifolia* L. (LA); and their respective waste materials (M and L)—towards Cu (II) ions were compared. Temperature and sample amount had negligible influence. The maximum adsorption capacities were found to be 59.95 mg/g (MO), 47.66 mg/g (M), 40.52 mg/g (LA), and 28.08 mg/g (L), indicating that all materials have potential as effective sorbents for copper (II) ions. Adsorption mechanisms were evaluated using Langmuir, Freundlich, and Dubinin-Radushkevich isotherm models, with non-linear chi-square (χ^2) tests confirming Langmuir's suitability for MO, M, and L, while LA aligned better with the Dubinin–Radushkevich model" [34].

"Another critical aspect concerning industrial effluents is the impact of relatively high concentrations of metal ions in real solutions, often reaching hundreds of milligrams to even several grams of heavy metals per liter. This leads to rapid saturation of the biosorbents with metals. Once saturation occurs, the sorption process must be interrupted, and a desorption operation followed by a subsequent washing step is necessary to regenerate the biosorbent for reuse while maintaining its sorption capacity. Overcoming biosorbent saturation is a challenge to ensure the continuous operation of waste treatment plants required by industries to decontaminate their effluents" [34] [62].

4. Desorption

"These factors explain why researchers observe a typical drop in uptake efficiency—approximately 25% between the first and third cycles in batch systems and 28% over six cycles in continuous bioreactors. In large-scale bioreactors, the biomass is often subjected to shear stress caused by agitation. For industrial viability, biosorbents must be regenerable. Desorbing agents such as 0.1 M HCl, 1 M HNO_3, or 0.1 M EDTA have shown up to approximately 90% - 100% recovery of bound copper, allowing materials like *Rhizopus arrhizus* to be reused for 3 (Batch Sheet Bioreactor, BSB) to 6 consecutive cycles (Continuous Sheet Bioreactor, CSB) with minimal loss (25% - 28%) in performance. The reduction in the accessibility of functional groups over several biosorption-desorption cycles is primarily caused by a combination of physical structural damage, chemical alterations from regeneration agents, and changes in the biomass's surface area" [3] [6] [28]. Continuous Sheet Bioreactor (CSB) copper biosorption and desorption results (based on the initial biomass concentration) are shown in **Figure 4**.



(Source: Author's own elaboration).

Figure 4. Continuous Sheet Bioreactor (CSB) Copper Biosorption and Desorption Results (based on the initial biomass concentration). (Continuous Sheet Bioreactor experiment: pH = 5.5, T = 30˚C, C_{b0} = 100 mg Cu/L; Agitation = 400 rpm, volume = 6 L, biosorbent = 5.45 g d.w./L, flow rate=100 mL/min, stainless steel sheets = 1.6 - 2.0 stitches per cm. (16x14x6x0.5 cm. sheets), Desorption: 0.1 M HCl.

Regeneration of exhausted biomass was possible by using 0.1 M HC1. Exhausted biomass can be regenerated using 0.1 M HCl, though uptake efficiency typically drops by approximately 25% between the first and third cycles in regeneration system in Batch Sheet Bioreactor (BSB). Research utilized Batch Sheet Bioreactors (BSB) and Continuous Sheet Bioreactors (CSB) to test *Rhizopus arrhizus* (immobilized dead biofilm, synthetic copper solutions) capacity. Saturation capacity is average 52 mg Cu (II)/g dry weight biosorbent for BSB, average 51 mg Cu (II)/g dry weight biosorbent for CSB bioreactors in optimum conditions. For industrial applications (real effluents), immobilized dead *Rhizopus arrhizus* in a BSB/CSB configuration is highly effective for removal of copper ions. The immobilized dead biomass maintains structural stability and significant functional capacity over multiple reuse cycles.

The overall uptake efficiency for the CSB system typically drops by approximately 28% between the first (59.7 mg Cu/g biomass)/and sixth cycles (42.7 mg Cu/g biomass). Despite this decline, the bioreactor maintains a high overall removal efficiency, generally ranging between 70% and 95%. The immobilised dead biomass could be used effectively in a 6-liter bioreactor continuously for at least 6 cycles of biosorption to exhaustion and then desorption with little change in the performance. The saturation uptake capacity for immobilised dead cells in the continuous system was initially 59.7 mg Cu (II)/g and after six cycles, 42.7 mg Cu (II)/g. Though uptake efficiency typically drops by approximately 28% between the first and sixth cycles in continuous sheet bioreactor (CBS) system. "Desorption studies have been performed to evaluate the regeneration potential of the selected biosorbents. Since acids are reported in the literature as the best eluents for desorption compared to other alternatives, a non-destructive method using nitric acid (0.1 M HNO_3 and 1 M HNO_3) and EDTA (0.1 M) was employed to conduct the desorption experiments. The desorption behavior confirms that the adsorption of copper ions involves reversible interactions, including ion exchange and coordination with the functional groups of the four biosorbents. This reversibility allows plant-based biosorbents to be efficiently regenerated and reused, enhancing their practicality and cost-effectiveness. From a practical and ecological perspective, EDTA is the preferred eluent. It is effective across all biosorbents while reducing the need for highly acidic conditions, making it a more environmentally friendly and less corrosive option for sorbent regeneration. The adsorption capacity of MO (*Melissa officinalis* L., plant material) decreases only slightly, from 82% in the first cycle to 77% in the fourth cycle. Meanwhile, the desorption capacity remains high, with 100% desorption efficiency for the first three cycles and 96% for the fourth cycle. These findings demonstrate that the studied plant-based materials are promising biosorbents due to their reversible adsorption mechanism, which is highly advantageous for applications in water purification and heavy metal recovery" [34].

"The capacity of dead waste sludge is highly sensitive to the pH of the solution (often optimal around pH 5.0 - 6.0 for cations) and the initial metal concentration. Additionally, chemically treated sludge (e.g., with sulphuric acid) generally exhibits higher capacities than untreated or formaldehyde-treated forms because the treatment can increase surface area and the accessibility of functional groups like carboxyl and amino groups" [3] [19].

"Other sources mention that formaldehyde is also used to inactivate fungal biomass like *Rhizopus arrhizus* to improve structural stability and resistance to chemicals, allowing the dead biomass to be used for up to 10 months without losing its activity" [17].

"The pH of biosorbents is another critical factor influencing adsorption. The studied biosorbents have pH values ranging from 5.8 to 6.3. This near-neutral pH range makes the biosorbents suitable for use in environments where extreme pH conditions might otherwise limit their effectiveness. The acidity of the environment is a critical parameter in the biosorption process. It influences the chemical state of the active centers of the biosorbent, the form and concentration of metal ions in the solution, the surface charge of the biosorbent, and the tendency to form complexes" [34].

"The removal capacity exhibited a direct proportional relationship to pH elevation, achieving optimal performance at pH 7.0. At higher pH values, the removal capacity decreased significantly. The reason for such a situation might be that cell wall ligands exhibit a strong affinity for hydronium ions at lower pH values. Temperature plays a pivotal role in heavy metal removal by live biosorbents, as it directly influences biomass growth and metabolic activity. A decline in removal effectiveness may result from departures from the optimal temperature range. the removal capacity of strain ZC255 (*Rossellomorea sp. ZC255*) for Cu (II) increased as the temperature increased from 15˚C to 28˚C but declined at higher temperatures. The maximum removal capacity was 253.4 mg/g biomass under optimum conditions (pH 7.0, 28˚C, and 2% inoculation amount). Meanwhile, kinetic analysis confirmed that adsorption follows pseudo-second-order kinetics, indicating chemisorption as the dominant mechanism. Equilibrium isotherm studies revealed that adsorption behavior aligns with both the Langmuir (monolayer adsorption) and the Freundlich (heterogeneous surface) models, suggesting complex interactions between Cu (II) and bacterial surfaces" [51].

"This scientific paper examines the process of biosorption, which utilizes nonliving biological matter to extract low-level pollutants like heavy metals from industrial wastewater. The researchers specifically investigated how dead *Rhizopus arrhizus* cells, trapped within foam or steel mesh structures, could effectively remove copper ions from a solution. Through repeated experimental cycles, the study demonstrates that these immobilized cells maintain high efficiency and stability over long periods, offering a cost-effective and renewable method for water purification. By applying mathematical models and testing various environmental conditions, the authors conclude that this biological approach provides a significant improvement for the recovery and removal of toxic contaminants in large-scale applications" [8] [9].

"This research paper details the development of a mathematical model (the orthogonal collocation method) designed to simulate how dead immobilized biomass removes copper ions from wastewater within a batch bioreactor. By utilizing the fungus *Rhizopus arrhizus* trapped in porous foam or steel mesh sheets, the authors created a system where mass transfer and biosorption kinetics work together to decontaminate aqueous solutions. The study emphasizes that internal transport, involving both diffusion and convection through the biomass, is a critical factor in determining the efficiency of metal recovery. Through sensitivity analysis, the researchers demonstrated that variables such as initial metal concentration, biomass density, and mat thickness significantly influence the speed and capacity of the biosorption process. Ultimately, the work provides a theoretical framework for designing more effective industrial systems for heavy metal removal using biological adsorbents" [3] [6] [8] [9] [29].

The removal efficiency of dead *Rhizopus arrhizus* is characterized by high performance and rapid kinetics, particularly for heavy metals like copper. In synthetic solutions (10 mg/L initial concentration), freely dead *Rhizopus arrhizus* achieved a removal yield of 89% within the first 10 minutes and reached 98% at equilibrium (60 minutes). The saturation uptake capacity was found to be 40 - 94 mg Cu (II)/g dry weight for immobilised dead cells compared to 100 - 172 mg Cu (II)/g dry weight for freely suspended dead cells, ranges depending on the process parameters. Use of a real effluent reduced the biomass copper uptake capacity. The saturation uptake was found to be 23.4 mg Cu (II)/g dry weight for immobilised dead cells compared to 33.8 mg Cu (II)/g dry weight for freely suspended dead cells.

"Researchers employ isotherm models like Langmuir, Freundlich, and Sips to describe surface saturation" [36]. "Several studies report that the Freundlich model provides the best correlation for immobilized cells, often outperforming the Langmuir and BET models. The Freundlich isotherm model prediction far the immobilised cells was close to the Langmuir and BET models; however, the former gave a better correlation" [3] [6] [8] [9].

4.1. Specific Advantages of Using Orthogonal Collocation Method

"The immobilised biomass biosorptive performance was modelled [3] [17] successfully using a mass transfer/kinetic approach using the orthogonal collocation method. Modeled copper ion biosorption by immobilized dead *Rhizopus arrhizus* in a Batch Sheet Bioreactor. A mathematical model coupling mass transfer with biosorption kinetics showed the most significant engineering parameters which affect biosorptive behaviour of the immobilised dead biomass. This sensitivity analysis indicated that the most important parameters were the maximum biosorption capacity, the initial ion concentration, the biomass concentration, the Freundlich isotherm constant, the convection velocity in the biomass and the external mass transfer coefficient. In addition, the apparent biomass density, the apparent biomass porosity and the half distance of the biomass thickness also affected metal uptake rate, but to a lesser extent. On the other hand, the effective diffusion coefficient was found to be a trivial parameter. This model can be used to help design a biosorption system with the optimum operational characteristics. The mass transfer model developed in that study was solved to simulate the concentration profiles in a batch reactor under various conditions. The values of the parameters used in this model were determined experimentally or theoretically. Comparison of experimental data with the prediction by the model is studied for a batch run (C_{bo} = 100 mg/L and C_{bo} = 2 mg/L). Comparison of experimental in Batch Sheet Bioreactor (BSB) and predicted of the model for copper solution concentration profiles (Cbo = 2 mg/L, V = 6 L, T = 30˚C, pH_i = 5.5, Agitation = 400 rpm, Biomass concentration = 4.95 g/L). The model seems to be predicting the experimental results quite well. The application of the model, as evidenced by the derivation of equations, is independent of the nature of the adsorbate. Therefore, the model can be applied to the biosorption of other adsorbates provided the equilibrium isotherm is available and the model assumptions listed in that study are valid. This model can be used to help design a biosorption system with the optimum operational characteristics and this method of biosorption offers a significant improvement in the utilization of immobilized fungal biomass for the removal of heavy metals from wastewater" [3] [17].

4.2. Specific Advantages of Using MATLAB Programme

"The use of MATLAB offers several specific advantages for biosorption modeling, primarily related to increased mathematical precision, automated statistical evaluation, and advanced predictive capabilities" [19]. "Advanced computational tools like MATLAB are utilized to solve these equations in their non-linear forms, providing high precision in predicting bioreactor performance" [19] [63].

"MATLAB improves biosorption isotherm constant calculations by allowing researchers [19] to analyze equations in their original non-linear (curvilinear) forms rather than relying on traditional linear transformations. Equilibrium data are frequently described by the Langmuir (monolayer) and Freundlich (heterogeneous) isotherm models. The Sips model (Langmuir-Freundlich hybrid) is often found to be the best statistical fit for copper systems due to its ability to describe surface saturation over wide concentration ranges using MATLAB analysis. The Sips (Langmuir-Freundlich) model has also been identified as a superior fit, yielding an R^2 of 0.9931. For copper biosorption by *Rhizopus arrhizus*, the Sips model provides the most accurate equilibrium data fit, while the pseudo-second-order model consistently shows the highest agreement with kinetic experimental results. By solving the original equations directly, MATLAB provides more sensitive and precise results for constants and correlation coefficients compared to linear approximations. The software employs the method of least squares, which ensures that the difference between experimental data points and the model-drawn curves is minimized. MATLAB automatically calculates critical accuracy parameters, such as the Sum of Square Errors (SSE) and the Correlation Coefficient (R^2). MATLAB is utilized for advanced modeling that couples biosorption kinetics with mass transfer, using methods like the orthogonal collocation method [3] [17] or Runge-Kutta method to solve the resulting differential equations. In conclusion, while the Langmuir and Freundlich models provide satisfactory results for industrial modeling, the Sips model is the most precise for describing the fundamental biosorption mechanism of copper by dead *Rhizopus arrhizus* due to its sensitivity to surface heterogeneity. For freely dead fungal cells, the Sips model was determined to be the most accurate statistical fit ($R^2 = 0.9931$) because it successfully incorporated the system's specific heterogeneity into its predictions" [19].

"In the study, biosorption kinetic and isotherm models were examined by the MATLAB for removal of copper ions from aqueous solution freely dead single *Rhizopus arrhizus* and mixed dead waste sludge biomass. Waste sludge biomass (a complex consortium of microorganisms) was obtained from the municipal wastewater treatment plant in Karaman, Sakarya, Türkiye. The copper ions removal efficiencies at equilibrium were 98% (freely dead *R. arrhizus*), 95% (inactivated with formaldehyde) and 92% (inactivated with sulfuric acid) for freely dead *R. arrhizus* and two types of dead waste sludge. The experimental data fitted to pseudo-second order kinetic model by having the best fitting degree and using the over whole range of the studies for three biosorbents. The Sips model provided a well-fitting isotherm model and described the biosorption system of the removal of Cu^{2+} ions" [19]. Summary of statistical fits (MATLAB) for copper (II) biosorption by dead *Rhizopus arrhizus* are given in **Table 4**.

Table 4. Summary of statistical fits (MATLAB) for copper (II) biosorption by dead *Rhizopus arrhizus* (Data: [19]; Table: Recep İleri).

Isotherm model	Biomass state	Correlation coefficient (R^2)
Sips	Freely suspended	0.99
Freundlich	Immobilized	0.96
Langmuir	Immobilized	0.95
BET	Immobilized	0.86

4.3. Advantages of Biosorption

"Biosorption is a process of rapid and reversible binding of ions from aqueous solutions onto functional groups that are present on the surface of biomass. This process is independent on cellular metabolism" [64].

Biosorption and the biological materials used as biosorbents offer significant technical, economic, and environmental advantages compared to traditional heavy metal remediation methods.

"Biosorption is an inexpensive alternative to conventional methods like ion exchange, membrane filtration, or chemical precipitation. The process utilizes readily available, renewable, and inexpensive biomass, such as agricultural waste (peels, husks, stalks), industrial by-products (waste sludge, spent fermentation biomass), and naturally abundant organisms like algae and fungi" [33].

"The process is generally a simple, passive physicochemical event that does not require high energy inputs or sophisticated infrastructure, making it ideal for large-scale or resource-constrained settings" [5] [65].

"Biosorption is particularly effective for treating solutions with low metal concentrations (under 100 mg/L), a range where classical methods are often technically limited or economically unviable" [2] [41].

"Because the process is often reversible, bound metals (including copper, gold, and silver) can be effectively recovered into a small, concentrated volume for potential reuse" [66]. "Metal-loaded biomass can be efficiently regenerated using inexpensive agents like dilute mineral acids or EDTA, allowing the biosorbent to be used for multiple consecutive cycles (typically 5 to 8) with minimal loss in performance" [67]. "Biosorption is recognized as an environmentally sound technology that produces minimal or no toxic sludge, significantly reducing the burden of hazardous waste disposal" [2]. "The technology facilitates the transformation of waste into valuable resources, supporting zero-waste production and sustainable industrial practices" [68]. Economic analysis and recycling strategies are given in Table 5.

Table 5. Economic analysis and recycling strategies (References: [5] [33] [38] [51] [65] [69]; Table: Recep İleri).

Method	Operational cost	Efficiency (<100 mg/L)	Secondary pollutants	Sustainability	References	*Potential environmental benefits*	*Reference*
Biosorption	Low	Very high	Minimum	Very high	[33] [69]	*High* (*waste recycling*)	[65]
Chemical precipitation	Medium	Low	High (Sludge)	Low	[5] [33]	*Low*	[65]
Ion exchange	High	Very high	Regenerative waste	Medium	[33] [51]	*Moderate*	[65]
Membrane filtration	Very high	Very high	Concentrated waste	Low	[5] [38]	*Not in source*	[65]

"The commercial success of biosorption depends on its ability to compete with traditional methods. Biosorption can offer a cost advantage of 30% to 50% compared to ion exchange resins, particularly when using inexpensive raw material sources such as agricultural waste and seaweed" [33] [38] [41], "Additionally, the recovery of the adsorbed copper through desorption using acids such as HCl or HNO_3 makes the process economically more attractive" [34] [63].

Biosorption is considered a superior 'alternative technology' to classical treatment methods primarily due to the economic and environmental limitations of traditional processes. Studies indicate that biosorption can provide a 30% to 50% cost advantage over synthetic ion-exchange resins. Biosorption minimizes or completely avoids the production of secondary hazardous waste, reducing the burden and cost of toxic sludge disposal. Biosorbents can be highly selective, targeting specific toxic metals without the need for excessive chemical additives. In many traditional methods, metal ions are trapped in sludge and cannot be easily reclaimed, whereas biosorption facilitates resource recovery. This ability to recover valuable metals like copper while extending the lifespan of the biomass aligns perfectly with circular economy principles and sustainable industrial practices.

4.4. Disadvantages of Biosorption

While biosorption is a highly efficient and cost-effective technology, it faces several technical, physical, and environmental disadvantages that hinder its widespread industrial application.

"Maintaining homogeneous mixing and adequate mass and heat transfer in large-scale bioreactors (exceeding 10,000 liters) is difficult. Excessive agitation to ensure mixing can cause shear stress that damages delicate cell structures, while insufficient agitation in large volumes can lead to oxygen transfer limitations, affecting microbial metabolism in living systems" [17]. "In continuous-flow systems, such as fixed-bed columns, biosorbents often suffer from clogging, pressure drops, and uneven flow, which increase maintenance costs and reduce the lifespan of the material" [70].

"Non-living biomass in powdered form is often difficult to separate from the reaction system after the treatment process is complete. Many biosorbents, particularly freely suspended algal or bacterial biomass, exhibit poor mechanical resistance and low chemical resistance, making them prone to disintegration under pressure" [21] [71].

"Biosorbents have a finite number of binding sites; once these sites are occupied by metal ions, the material becomes completely saturated and loses its uptake capacity" [15] [62] [72]. "Natural materials like food wastes or agricultural residues have inconsistent structures and compositions, which leads to variability and inconsistencies in adsorption efficiencies" [65]. "Biosorption systems are often highly sensitive to environmental changes, such as fluctuations in pH, temperature, and ionic strength, which can alter the sorbent structure or metal speciation" [3] [10] [12] [17] [73].

5. Conclusions and Recommendations

Different biosorbents and adsorbents uptake capacities vary depending on the conditions. Based on the provided sources (see **Table 3**), the modified watermelon shell exhibits the highest recorded copper uptake capacity, reaching up to 357.14 mg Cu/g. Among specifically designated biological composites, the Algal-Fe_2O_3 nanoparticle composite follows with a maximum capacity of 297.7 mg Cu/g. For pure bacterial strains, *Rossellomorea sp. ZC255* shows a high performance of 253.4 mg Cu/g under optimized conditions. The saturation uptake capacity was found to be 40 - 94 mg Cu (II)/g dry weight for immobilised dead cells compared to 100 - 172 mg Cu (II)/g dry weight for freely suspended dead cells ranges depending on the process parameters using shake flasks. *Rhizopus arrhizus* (immobilized living biomass) shows a lower capacity of 19.46 mg Cu/g compared to its dead forms. *Rhizopus arrhizus* (immobilized dead) in real wastewater effluents, the capacity drops significantly in complex solutions to 23.4 mg Cu/g. Dead biomass is highly resistant to metal toxicity, whereas living cells may be inhibited or killed by high concentrations of the heavy metals they are intended to remove. From a performance perspective, dead biomass typically exhibits significantly higher copper uptake capacities; for example, freely dead *Rhizopus arrhizus* can reach up to 172 mg Cu/g, while living forms of the same fungus typically range between 10.76 and 37.785 mg Cu/g. While biosorption is a highly efficient and cost-effective technology, its widespread industrial application is hindered by several significant technical, physical, and environmental challenges. Biosorption systems are highly sensitive to fluctuations in pH, temperature, and ionic strength, which can alter the structure of the sorbent or the chemical form of the metal ions. In multi-metal systems, the uptake of copper is generally reduced because various metal ions and other pollutants compete for the same non-specific functional groups (active binding sites) on the biosorbent's surface. The presence of high total concentrations of various metal ions in real solutions leads to rapid saturation of the biosorbent. Copper (II) biosorption is an eco-friendly and cost-effective separation process that utilizes biological materials—such as bacteria, fungi, algae, and agricultural wastes—to remove or recover copper ions from aqueous environments. It notes that the process is used not only to remove toxic pollutants but also to recover valuable metal resources, aligning with sustainable industrial practices discussed in this critical review.

The conclusions reached in this work can be listed as follows:

1. Copper (II) is toxic heavy metal to aquatic organisms even at low levels (1 - 100 mg/L); found in shellfish, liver, and mushrooms.
2. Biosorption is defined as the removal of heavy metal ions from aqueous solutions using living or dead, freely or immobilised biological materials. Biosorption method is particularly effective for treating wastewater with low metal concentrations (1 - 100 mg/L) where traditional methods are economically unviable.
3. Copper (II) biosorption is an eco-friendly and cost-effective separation process that utilizes biological materials-such as bacteria, fungi, algae, and agricultural wastes-to remove or recover copper ions from aqueous environments.
4. *Rhizopus arrhizus* is a zygomycete fungus recognized for its rapid growth and resilience to acidic and oxidative conditions. *Rhizopus arrhizus* (from the order Mucorales) was selected for its wide surface area, high hanging capacity, and harmlessness to humans.
5. There is very little difference between the growth patterns of immobilised and freely suspended *Rhizopus arrhizus,* showing that the method of immobilisation has no detrimental effect.
6. *Rhizopus arrhizus* immobilised in reticulated polyurethane foam and stainless-steel knitted mesh sheets can be used for the removal of copper from aqueous solutions.
7. Grown at 30˚C, inactivated (using formaldehyde or sulfuric acid), and dried

to a concentration of 4.3 g dry weight *R. arrhizus*/L for experimental use.

8. Used in its dead form to avoid the need for continuous nutrient addition, which would otherwise increase Biological Oxygen Demand (BOD) or Chemical Oxygen Demand (COD).
9. The stability of the dead immobilised *Rhizopus arrhizus* was tested by storing at room temperature in formaldehyde over a period of at least 10 months and it was found that the immobilised cells did not lose their biosorption activity.
10. Dead immobilised biomass can be used for the removal of copper from solutions of 1 - 100 mg/1 initial concentration.
11. Studies show that biosorption is a two-stage process: Physical Stage: Rapid (3 - 10 minutes), involving ion exchange and micro-precipitation at the surface. Chemical/Biological Stage: Slower (>10 minutes), involving diffusion and complexation. The Pseudo-second order rate model typically provides the highest correlation coefficients, suggesting that the rate-limiting step is chemisorption involving valency forces through electron sharing.
12. Repeated batch runs were also performed and it was found that the immobilised dead cells could be repeatedly used for 250 successive batches using shake flasks.
13. The saturation uptake capacity was found to be 40 - 94 mg Cu (II)/g dry weight for immobilised dead cells compared to 100 - 172 mg Cu (II)/g dry weight for freely suspended dead cells, ranges depending on the process parameters using shake flasks.
14. Regeneration of exhausted biomass was possible by using 0.1 M HC1. Exhausted biomass can be regenerated using 0.1 M HCl, though uptake efficiency typically drops by approximately 25% between the first and third cycles in regeneration system in Batch Sheet Bioreactor (BSB).
15. Research utilized Batch Sheet Bioreactors (BSB) and Continuous Sheet Bioreactors (CSB) to test *Rhizopus arrhizus* capacity. Saturation capacity is average 52 mg Cu (II)/g dry weight biosorbent for both bioreactors in optimum conditions. For industrial applications (real effluents), *Rhizopus arrhizus* in a BSB/CSB configuration is highly effective for copper.
16. The immobilised dead biomass could be used effectively in a 6-liter bioreactor continuously for at least 6 cycles of biosorption to exhaustion and then desorption with little change in the performance. The saturation uptake capacity for immobilised dead cells in the continuous system was initially 59.7 mg Cu (II)/g and after six cycles, 42.7 mg Cu (II)/g. Though uptake efficiency typically drops by approximately 28% between the first (59.7) and sixth cycles (42.7) in continuous sheet bioreactor (CBS) system.
17. MATLAB is essential for solving complex mathematical frameworks that couple biosorption kinetics with mass transfer. The immobilised fungal sorption isotherm is a favourable type. The Freundlich model (immobilized dead *Rhizopus arrhizus*) prediction (the orthogonal collocation method) [3] [17], was close to the Langmuir, BET and Sips (Langmuir-Freundlich hybrid) mod-

els however, the Sips gave a little better correlation by MATLAB analysis [19] for freely suspended dead *Rhizopus arrhizus*. The Sips (Langmuir-Freundlich hybrid) model has also been identified for freely suspended dead *Rhizopus arrhizus* biosorption of copper a superior fit, yielding an R^2 of 0.9931. It is used to solve the partial and first-order differential equations resulting from steady-state mass balances.

18. It employs techniques such as the orthogonal collocation method [3] [17] and the fourth-order Runge-Kutta method [19] to simulate concentration profiles over time in bioreactors. Used the orthogonal collocation method to predict copper concentration profiles in the bulk liquid, showing how the system parameters affect biosorption performance.
19. When initial copper concentration increased the removal efficiency decreased.
20. Removal of copper from the solution was more efficient with increasing pH.
21. The amount of copper adsorbed per unit weight was maximal at lower biomass concentrations and decreased with increasing concentrations of biomass.
22. The saturation capacity depends on the state of the biomass and the type of solution. Free dead *Rhizopus arrhizus* demonstrated the highest capacity in synthetic solutions at 100 - 172 mg Cu^{2+}/g and in real industrial wastewater, this dropped to 33.8 mg/g. Immobilized dead *Rhizopus arrhizus* are capacities in synthetic solutions ranged from 40 to 94 mg/g, and dropped to 23.4 mg/g in real industrial effluents. The real effluent sample, taken from the influent of the advanced treatment plant, contains organic and inorganic pollutants at varying concentrations; this complex solution contains 3 mg/L of copper. Its pH is 7.95. Real effluent also includes amounts of tin and lead, approximately 0.5 mg/L, chlorides as 37 mg/L and COD as 90 mg/L. Use of a real effluent reduced the biomass copper uptake capacity. The saturation uptake was found to be 23.4 mg Cu (II)/g dry weight for immobilised dead cells compared to 33.8 mg Cu (II)/g dry weight for freely suspended dead cells.
23. The high elution efficiency (up to 94% - 100%) using 0.1 M HCl confirms that immobilized dead *Rhizopus arrhizus* is a highly durable and regenerable biosorbent for laboratory and industrial copper removal possible applications.
24. The results have shown that immobilised dead *Rhizopus arrhizus* can successfully remove copper from aqueous solution.
25. "Biosorption systems are often highly sensitive to environmental changes, such as fluctuations in pH, temperature, and ionic strength, which can alter the sorbent structure or metal speciation" [3] [10] [12] [17] [73].

A list of suggestions for further research prompted by the work presented in this study can be listed as follows:

1. Biosorption reactor system should be applied for real effluent treatment for in larger systems in real life.
2. Immobilised or freely dead biomass should be produced from domestic, ag-

ricultural or industrial waste biomass for it to be a more applicable, reasonable, economic, cheaper, competitive, sustainable and ecofriendly biosorbent.

3. Biosorption reactor studies performed have utilized a single 6-liter bioreactor in studies. Investigations should be performed to elucidate the possible advantages that could be obtained from the use of bioreactors in series in larger systems for improved removal efficiency for conforming to standards irrigation or process water.
4. Biosorption methods should be usable as an alternative and competitive for the recovery of rare earth elements and valuable elements. In this regard, laboratory researches should not be limited; additional field studies should be increased. The transition from laboratory modeling to real-world application should be demonstrated through both industrial bioreactors and portable community filters.

Disclosure Statement

The article is single-authored. No potential conflict of interest was reported by the author(s).

Ethical Statement

The author stated that this study did not require ethical approval or informed consent because it reviewed existing literature and did not involve new data collection from human participants.

AI Statement

The author stated that control of English language has been used DeepL Translator (pro version, fee paid).

Availability of Data and Materials

All data generated from open access literature and this study and analysed during this study are available for sharing when appropriate request is directed to the author.

Conflicts of Interest

The author declares that there are no competing interests.

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