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BIOSORPTION OF TRIVALENT ARSENIC AT DIFFERENT pH VALUES AND CONTACT TIMES BY UNMODIFIED AND CHEMICALLY MODIFIED FUNGAL BIOMASS

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ABSTRACT

The biosorption of As(III) from aqueous solutions by native and chemically modified biomass of *Aspergillus clavatus* and *Neosartorya fischeri* was investigated at pH 3, 4 and 5. Untreated A. clavatus exhibited substantially higher biosorption than *N. fischeri*, achieving a maximum As(III) removal of 32% compared with only 3.3%. Alkaline pretreatment with NaOH enhanced biosorption in both fungi, increasing the maximum removal efficiency to 54% and 30% for *A. clavatus* and *N. fischeri*, respectively. The highest biosorption efficiencies were obtained after $FeCl_3$ modification, reaching 81% for *A. clavatus* and 57% for *N. fischeri*. The results demonstrate that fungal species, solution pH and biomass modification significantly influence As(III) biosorption, with Fe-functionalized fungal biomass showing the greatest potential for arsenic removal.

1. INTRODUCTION

Heavy metal contamination in water is one of the most consequential environmental issues (Mishra et al., 2019). Heavy metals can enter the environment through various ways. Wastewater effluents from industrial processes, agriculture, and mining activities contain a wide range of potentially toxic metal contaminants, and anthropogenic activities are the main drivers of increased environmental toxicity (Zamora-Ledezma et al., 2021). As non-biodegradable and persistent pollutants, heavy metals bioaccumulate in living tissues and undergo biomagnification, impacting diverse organisms across the food web (Cabral Pinto et al., 2017; Kumar et al., 2020). These toxic and carcinogenic ions can induce severe multi-organ damage even at trace concentrations (Aziz et al., 2023). Arsenic is one of the most hazardous potentially toxic elements because of its high toxicity, mobility, bioavailability and persistence in the environment (Patel et al., 2023; Fattorini, 2026). It is released primarily through acid mine drainage generated during mining and ore-processing activities, as well as from legacy mining wastes and abandoned mine sites (Faria et al., 2023). Inorganic arsenic species are the predominant forms of arsenic in the environment. Arsenic occurs primarily as trivalent arsenite [As(III)] or pentavalent arsenate [As(V)] and the toxicity of arsenic is notably associated with these inorganic forms (Mandal and Suzuki, 2002; Patel et al., 2023). Arsenite predominates under reducing (anaerobic) conditions, whereas arsenate dominates in oxidizing (aerobic) environments (Barral-Fraga et al., 2020). Consequently, arsenic speciation and the stability of individual arsenic species are strongly controlled by environmental pH and redox potential (Eh) (Smedley and Kinniburgh, 2002). At moderate to high redox potentials, arsenic is mainly present as pentavalent oxyanions (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^{3-}). In contrast, under reducing conditions and lower redox potentials, particularly in acidic to mildly alkaline environments, the neutral trivalent species H_3AsO_3 predominates (Sadiq, 1997; Mandal and Suzuki, 2002; Johnston et al., 2020). Inorganic arsenic species are generally more toxic than organic forms, and trivalent arsenite exhibits substantially higher toxicity than pentavalent arsenate (Genchi et al., 2022; Visciano, 2025).

Conventional remediation of metal- and metalloid-contaminated wastewaters, including arsenic, relies primarily on physicochemical technologies such as chemical precipitation (e.g., as insoluble hydroxides), electrochemical treatment, oxidation–reduction, ion exchange, filtration, membrane separation, and evaporation (Qasem et al., 2021; Mahamallik and Swain, 2023). Despite their effectiveness, conventional physicochemical methods often involve high operational costs, incomplete contaminant removal, limited selectivity, substantial energy consumption, and the production of toxic sludge that requires further treatment and disposal (Bano et al., 2018; Daraz et al., 2023; Oladimeji et al., 2024). Alternative methods for the removal and

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recovery of metal(loid)s include biosorption, which is effective even when metal(loid)s are present at very low concentrations (Gupta et al., 2015; Priya et al., 2022). Microbial applications in arsenic remediation have gained increasing interest as a sustainable, cost-effective approach to removing arsenic from contaminated environments (Alguacil and Robla, 2023). Biosorption is defined as the passive uptake of dissolved substances from aqueous solutions by biological materials via physicochemical interactions such as absorption, adsorption, ion exchange, surface complexation and precipitation (Gadd, 2008). Microorganisms are widely recognized as effective biosorbents for removing heavy metals from wastewater in a cost-effective and environmentally friendly manner (Ramya et al., 2021). Filamentous fungi are generally more effective biosorbents than bacteria because of their higher biomass yield, larger surface area, and abundant metal-binding functional groups in their cell walls (Sar et al., 2024). Numerous fungal species are capable of adsorbing heavy metals, including Cu, Cd, Pb, Hg, and Zn, through their spores and mycelial biomass (Rajkumar et al., 2012). Because their cell walls are rich in polysaccharides, fungal species from the genera *Aspergillus, Penicillium, Rhizopus*, and *Saccharomyces* provide abundant metal-binding sites, making them highly effective biosorbents (Verma et al., 2017). The fungal cell wall carries a net negative charge over a broad pH range, providing numerous functional groups capable of binding cationic metal ions (Kleijburg et al., 2023). Because arsenic occurs predominantly as oxyanionic species in the environment, fungal biomass generally shows limited affinity for arsenic adsorption. Therefore, chemical modification of the fungal cell surface to introduce positively charged functional groups has been widely investigated as an effective strategy to enhance the biosorption capacity for arsenic species (Benis et al., 2020; Torres, 2020).

The aim of this study was to evaluate the biosorption of trivalent arsenic by unmodified and chemically modified fungal biomass under different pH conditions and contact times.

2. MATERIAL AND METHODS

2.1 Preparation of fungal biomass

Fungal strains *Aspergillus clavatus* and *Neosartorya fischeri* were isolated from mine waste collected at a site highly contaminated with arsenic and antimony.

Biomass of *A. clavatus* and *N. fischeri* was used for the biosorption experiments. Erlenmeyer flasks (250 mL) containing 50 mL of Sabouraud broth (SB; 20 g dextrose and 10 g peptone per 1000 mL of distilled water) were inoculated with 5 mL of a spore suspension of either *A. clavatus* or *N. fischeri*. The cultures were incubated on a laboratory shaker at 130 rpm for 3 days at laboratory temperature (25°C). The resulting fungal pellets were harvested by filtration through Whatman No. 1 filter paper and washed thoroughly with distilled water to remove residual culture medium. The biomass was then dried in a hot-air oven at 40°C for 24 h until a constant weight was achieved.

Both chemically unmodified and chemically modified fungal biomass were used in the biosorption experiments. The unmodified biomass consisted of the dried fungal biomass prepared as described above.

For alkali modification, the dried biomass was boiled in 50 mL of 0.5 M NaOH solution for 15 min. The biomass was then thoroughly washed with distilled water until the wash solution reached approximately neutral pH (6.8-7.2). The treated biomass was then dried overnight at 60°C.

A second chemical modification was performed by suspending the dried biomass in an $FeCl_3$ solution (15 mg L^{-1}) and shaking it for 120 min. After treatment, the biomass was thoroughly washed with distilled water to remove excess $FeCl_3$ and dried overnight at 60°C.

Biosorption experiments were performed to evaluate the effects of solution pH and contact time on the biosorption capacity of the fungal biomass.

2.2 Effect of pH and contact time

The effects of solution pH and contact time on the biosorption of As(III) by unmodified and chemically modified fungal biomass were investigated. The experiments were carried out at pH 3, 4, and 5, with the pH adjusted using 0.1 M HCl or 0.1 M NaOH.

For each experiment, 0.5 g of fungal biomass (*A. clavatus* or *N. fischeri*) was suspended in 50 mL of an As(III) solution containing 1.0 mg of arsenic, corresponding to an initial concentration of 20 mg L^{-1}. The As(III) stock solution was prepared from sodium arsenite ($NaAsO_2$). The initial As(III) concentrations used in this study were selected to assess biosorption performance under highly contaminated conditions and to allow meaningful comparisons of removal efficiency among different fungal species and biomass treatments.

The biomass was exposed to the arsenic solution for different contact times depending on the experimental design, with a maximum contact time of 240 min. Samples were collected at 30-min intervals throughout the experiment. Following each sampling, the biomass was separated from the solution by centrifugation at 3000 rpm for 10 min. The residual concentration of arsenic in the supernatant was determined by hydride generation atomic absorption spectrometry (HG-AAS).

2.3 Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical differences in As(III) removal efficiency among the tested pH conditions at the final contact time (150 or 240 min, depending on the experiment) were evaluated using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

The biosorption experiments clearly showed that As(III) removal efficiency depended not only on solution pH and contact time but also on fungal species and the type of biomass modification (Benis et al., 2020; Tripathi et al., 2022). Because this study used two isolates from mine waste, it shows that even fungi from the same contaminated environment do not necessarily have similar biosorption

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capacities. It therefore highlights that origin from a contaminated site alone does not guarantee high biosorption efficiency, as species-specific properties of the fungal cell wall and biomass surface chemistry may be the determining factors. The initial As(III) concentrations used in this study were selected to evaluate biosorption performance under highly contaminated conditions. Although these concentrations exceed those typically found in natural waters, mining-impacted environments have been documented to have comparable or substantially higher arsenic concentrations. For example, dissolved arsenic concentrations of up to 90 mg L^{-1} were reported at the Pezinok mining site in Slovakia (Flakova et al., 2012). Even higher concentrations, reaching approximately 400 mg L^{-1}, were documented in acid mine drainage from the abandoned Long Lake Gold Mine in Canada, where As(III) was identified as the predominant dissolved arsenic species (Verbuyst et al., 2024). These findings demonstrate the environmental relevance of investigating As(III) biosorption under highly contaminated conditions.

Untreated *Aspergillus clavatus* exhibited a substantially higher biosorption capacity than untreated *Neosartorya fischeri* under all experimental conditions. At pH 5, untreated *A. clavatus* removed up to 32% of the initial arsenic, whereas untreated *N. fischeri* removed only 3.3%, representing an almost ten-fold difference in biosorption capacity. These results clearly indicate that arsenic biosorption is strongly species-dependent and that fungal taxonomy alone cannot predict biosorption performance. Cárdenas-González et al. (2017) compared nine species of filamentous fungi (*Aspergillus flavus*, *A. fumigatus*, *Paecilomyces, Cladosporium, Mucor* spp.) after Fe-modification of biomass. Significant differences among species were observed, with As(V) removal ranging from approximately 60% to more than 97%, despite identical experimental conditions. The authors attributed these differences to species-specific cell wall composition and variations in the number of available binding sites. A comparative study by Ayele et al. (2021), covering a wide range of fungal species (*Aspergillus, Rhizopus, Penicillium, Trichoderma, Mucor, Saccharomyces* spp.), confirms significant species-specific differences in biosorption performance. The authors reported that metal uptake varies considerably among fungi due to differences in cell wall composition and the availability of functional groups responsible for binding, highlighting the dominant role of surface chemistry over taxonomic affiliation. Species-specific differences in fungal biosorption have been widely reported and are generally attributed to variations in cell wall composition, surface morphology and the abundance of reactive functional groups rather than phylogenetic relationships (Kapoor and Viraraghavan, 1995; Gadd, 2008; Fomina and Gadd, 2014; Lee and Sheppard, 2016). The fungal cell wall is primarily composed of chitin, β-glucans, glycoproteins and melanins containing hydroxyl, amino, carboxyl, phosphate and sulfhydryl groups that participate in metal(loid) binding (Campillo-Cora et al. 2025). However, the relative abundance, accessibility and spatial arrangement of these functional groups differ considerably among fungal species, resulting in substantial differences in sorption capacity (Wang and Chen, 2009). Moreover, cell wall composition varies with the stage of fungal growth (Lee and Sheppard, 2016). The markedly higher performance of *A. clavatus* therefore suggests a greater density of accessible binding sites and/or a cell wall architecture more favourable for As(III) complexation than that of *N. fischeri* (Figures 1-2).

The effect of solution pH on the biosorption performance of untreated biomass was relatively limited. However, As(III) uptake increased slightly with pH, reaching a maximum at pH 5. This behaviour agrees well with the aqueous chemistry of As(III). Below pH approximately 9.2, arsenic occurs predominantly as the electrically neutral species

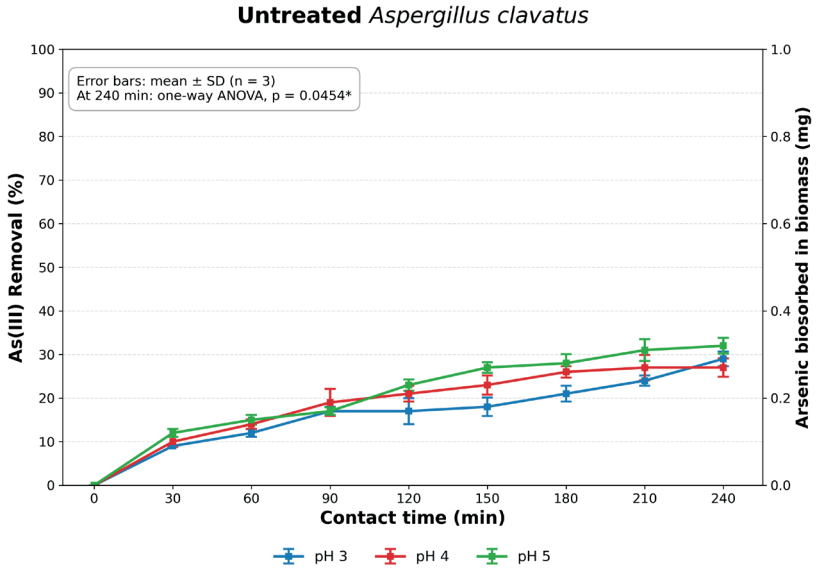


FIGURE 1: Effect of pH and contact time on the biosorption of trivalent arsenic by untreated *Aspergillus clavatus* biomass. Error bars represent the standard deviation (SD, n = 3). Differences in As(III) removal among the tested pH conditions at 240 min were evaluated using one-way ANOVA (p = 0.0454), indicating a statistically significant overall difference at the 0.05 significance level (p < 0.05).

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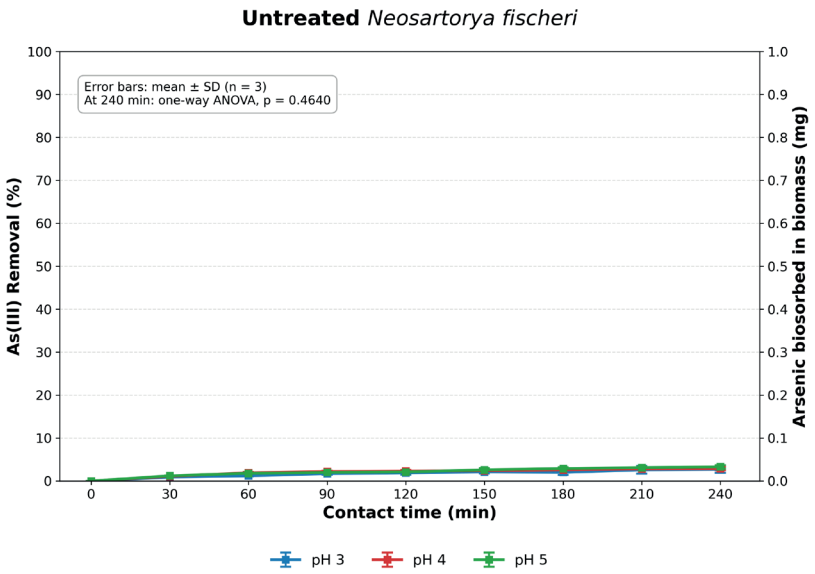


FIGURE 2: Effect of pH and contact time on the biosorption of trivalent arsenic by untreated *Neosartorya fischeri* biomass. Error bars represent the standard deviation (SD, n = 3). Differences in As(III) removal among the tested pH conditions at 240 min were evaluated using one-way ANOVA (p = 0.4640), indicating no statistically significant differences (p > 0.05).

H_3AsO_3, which minimizes electrostatic interactions with charged functional groups on the fungal surface (Mohan and Pittman, 2007; Sharma and Sohn, 2009). Consequently, biosorption is governed mainly by surface complexation, hydrogen bonding and ligand-exchange reactions rather than simple ion exchange (Caixeta et al., 2025). Nevertheless, increasing pH promotes gradual deprotonation of carboxyl and hydroxyl groups, thereby increasing the number of available coordination sites (Osman et al., 2023), which probably explains the slightly higher biosorption observed at pH 5 for untreated biomass.

Alkaline pretreatment with NaOH significantly enhanced As(III) biosorption in both fungal species. In *A. clavatus*, the maximum removal efficiency increased from 32% to 54%, whereas in *N. fischeri* it increased almost ten-fold, from only 3.3% to approximately 30%. Interestingly, NaOH treatment substantially reduced the difference in biosorption capacity between the two fungi. This finding suggests that the poor biosorption performance of untreated *N. fischeri* may be related to the lower availability of active binding sites, which became more accessible following alkaline pretreatment (Figures 3-4).

The positive effect of alkaline modification is often attributed to structural alterations in the fungal cell wall. Sodium hydroxide removes surface lipids, proteins and other extractable compounds, partially hydrolyses polysaccharide matrices, and exposes previously inaccessible hydroxyl, amino and carboxyl groups (Kapoor and Viraraghavan, 1995; Wang and Chen, 2009). In addition, partial deacetylation of chitin may generate additional amino groups with enhanced coordination ability towards metal(loid)s. Hydroxyl and amino groups are considered the principal binding sites for As(III), while carboxyl and phosphate groups may also contribute to surface complexation depending on the biomass composition and solution chemistry (Tripathi et al., 2022; Ankita and Sharma, 2026). These structural changes increase both the number and accessibility of sorption sites, thereby improving biosorption efficiency. Alkaline pretreatment modifies the surface chemistry of fungal biomass by partially hydrolyzing cell wall constituents and removing surface-bound impurities, increasing exposure of oxygen-containing functional groups. These changes improve key physicochemical properties, including hydrophilicity, ion-exchange behavior, and sorption efficiency (Fomina and Gadd, 2014). The considerably greater improvement observed for *N. fischeri* suggests that alkali treatment induced more pronounced structural changes in its cell wall than in *A. clavatus*, exposing a larger proportion of previously inaccessible binding sites. Gaur et al. (2018) reported similar findings, observing a significant increase in Pb and As uptake following NaOH pretreatment of soybean biomass. The improved adsorption capacity was attributed to the increased accessible surface area and the exposure of additional active binding sites induced by alkaline activation. Rouhollahi et al. (2014) also reported a significant improvement in the biosorption performance of fungal biomass after NaOH pretreatment. Compared with untreated biomass, which removed 64.6% of Ni(II), alkaline modification increased removal efficiency to 93.2-100%, with NaOH concentrations of 5-80 g L^{-1} achieving near-complete nickel removal.

$FeCl_3$ was selected for biomass modification because arsenic has a well-documented strong affinity for iron (hydr)oxides, which are widely recognized as effective sorbents for arsenic removal from aqueous environments (Souza and Ciminelli, 2023). In particular, ferrihydrite provides reactive surface sites that bind As(III) through the formation of inner-sphere surface complexes (Huang et al., 2024). The highest biosorption efficiencies were obtained after $FeCl_3$ modification of the biomass, indicating that iron functionalization introduced new, highly active sorption centers that dominated overall arsenic uptake.

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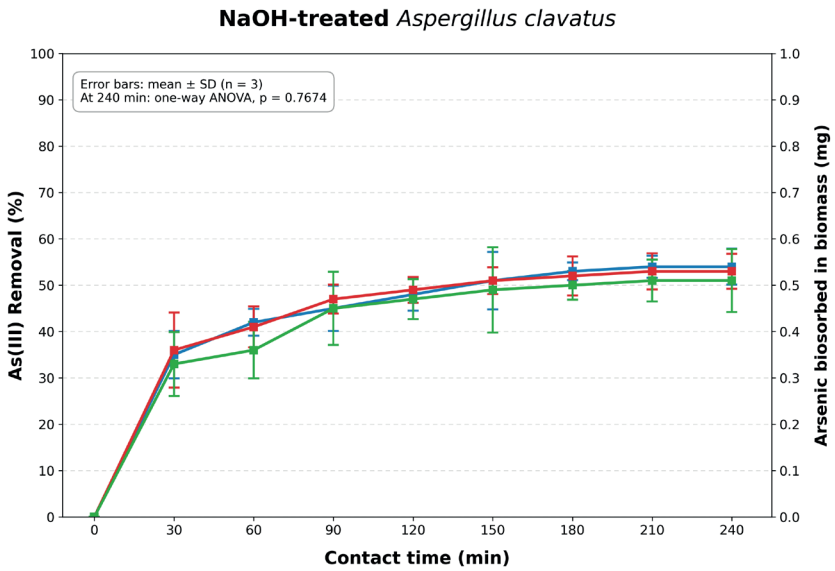


FIGURE 3: Effect of pH and contact time on the biosorption of trivalent arsenic by NaOH-treated *Aspergillus clavatus* biomass. Error bars represent the standard deviation (SD, n = 3). Differences in As(III) removal among the tested pH conditions at 240 min were evaluated using one-way ANOVA (p = 0.7674), indicating no statistically significant differences (p > 0.05).

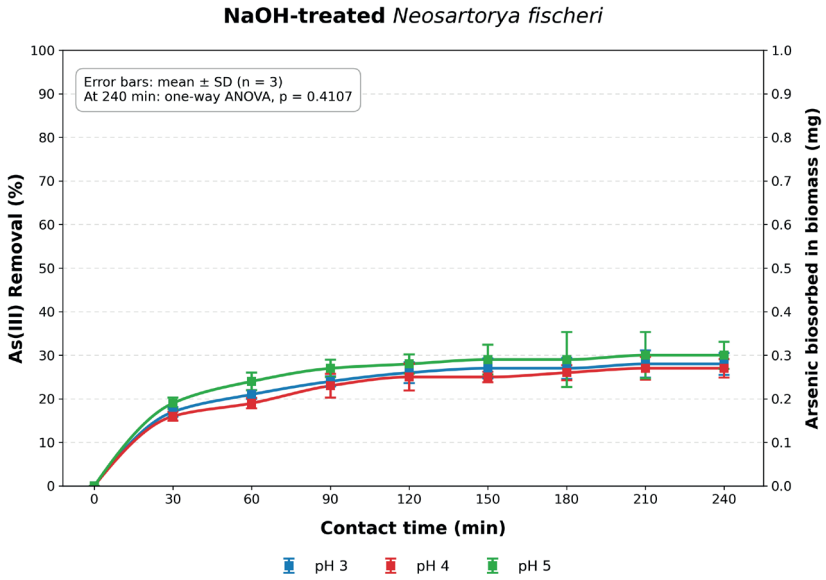


FIGURE 4: Effect of pH and contact time on the biosorption of trivalent arsenic by NaOH-treated *Neosartorya fischeri* biomass. Error bars represent the standard deviation (SD, n = 3). Differences in As(III) removal among the tested pH conditions at 240 min were evaluated using one-way ANOVA (p = 0.4107), indicating no statistically significant differences (p > 0.05).

Unlike NaOH pretreatment, which primarily enhances the availability of native functional groups, $FeCl_3$ modification introduces iron (hydr)oxide phases on the biomass surface, which act as highly reactive binding sites for arsenic through specific inner-sphere complexation mechanisms (Rahnemaie et al., 2006). Fe-treated *A. clavatus* removed up to 81% of As(III), whereas Fe-treated *N. fischeri* achieved 57% removal after only 150 min. Compared with untreated biomass, these values correspond to approximately 2.5-fold and 17-fold improvements, respectively. Moreover, both Fe-modified biomasses reached equilibrium considerably faster than untreated biomass, indicating that iron functionalization enhanced both sorption capacity and adsorption kinetics. Unlike untreated biomass, which performed slightly better at pH 5, Fe-modified biomass showed maximum biosorption at pH 4. This shift strongly suggests that iron-mediated adsorption became the dominant arsenic-removal mechanism. During $FeCl_3$ treatment, Fe(III) ions are immobilized on the fungal surface and subsequently hydrolyse to form amorphous iron oxyhydroxides possessing numerous reactive hydroxyl groups. These hydroxyl groups form stable inner-sphere surface complexes with arsenic species through ligand-exchange reactions, a mechanism fundamentally different from adsorption on untreated biomass (Dixit and Hering, 2003). Yu et al. (2014) reported a similar enhancement in sorp-

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tion capacity after $FeCl_3$ modification and successfully applied $FeCl_3$-modified granular activated carbon to remove Sb(III) from aqueous solution. Although Yu et al. (2014) investigated Sb(III) rather than As(III), their findings are relevant to the present study because arsenic and antimony exhibit similar geochemical behavior and share certain sorption mechanisms, particularly their affinity for iron (hydr)oxides. Nevertheless, differences in metalloid speciation, sorbent properties, and experimental conditions limit direct comparisons of removal efficiencies. Unlike granular activated carbon, fungal biomass represents a potentially renewable and low-cost alternative. Moreover, the present study demonstrates substantial As(III) removal at relatively high initial concentrations, highlighting the potential applicability of $FeCl_3$-modified fungal biomass to highly contaminated waters. However, further investigations are required to evaluate sorbent regeneration, long-term stability, and performance under realistic wastewater conditions. The results obtained in the present study agree well with those of Khan et al. (2018), who demonstrated that $FeCl_3$ modification was more effective than NaOH pretreatment in enhancing As(III) biosorption by fungal biomass. $FeCl_3$-treated *Aspergillus fumigatus* exhibited the highest As(III) uptake (3.2 mg g^{-1}), representing a 33.65% increase compared with untreated biomass, whereas NaOH pretreatment improved biosorption by 22.27%. These findings further support the role of iron functionalization in creating high-affinity adsorption sites for arsenic, leading to substantially higher biosorption efficiency than that achieved by alkaline pretreatment alone. Similarly, Aryal et al. (2010) demonstrated enhanced As(III) biosorption at pH 7 with an equilibrium contact time of 30 min using Fe(III)-modified *Staphylococcus xylosus* biomass (Figures 5-6).

Notably, iron modification substantially reduced the species-specific differences observed for native biomass. Whereas untreated *A. clavatus* exhibited nearly ten times higher biosorption than untreated *N. fischeri*, the difference after Fe loading decreased to approximately 1.4-fold. This indicates that, following iron functionalization, arsenic binding is governed predominantly by the immobilized iron phase rather than by the intrinsic chemistry of the fungal cell wall. Similar synergistic effects between biological substrates and iron coatings have been reported for other iron-functionalized biosorbents used for arsenic remediation (Mohan and Pittman, 2007; Fomina and Gadd, 2014).

Statistical analysis revealed differences in the influence of pH on As(III) biosorption depending on fungal species and biomass treatment (Figures 1-6). For untreated *Aspergillus clavatus*, a statistically significant overall difference in removal efficiency was observed among the tested pH conditions at 240 min (one-way ANOVA, p = 0.0454), with the highest removal efficiency at pH 5 (32%). In contrast, no statistically significant differences were observed for untreated *Neosartorya fischeri* (p = 0.4640), which exhibited consistently low biosorption efficiencies across the investigated pH range.

Following NaOH treatment, neither *A. clavatus* (p = 0.7674) nor *N. fischeri* (p = 0.4107) showed statistically significant differences in As(III) removal among the tested pH conditions at 240 min. This suggests relatively consistent biosorption performance across the investigated pH range (3-5), particularly for NaOH-modified biomass. Similarly, $FeCl_3$-treated *A. clavatus* and *N. fischeri* showed no statistically significant differences among pH conditions at 150 min (p = 0.2626 and p = 0.1698, respectively), despite differences in the observed removal efficiencies. The highest removal efficiencies were achieved at pH 4, reaching 81% for *A. clavatus* and 57% for *N. fischeri*.

Overall, these findings indicate that statistically significant differences among the tested pH conditions were detected only for untreated *A. clavatus*. In contrast, chem-

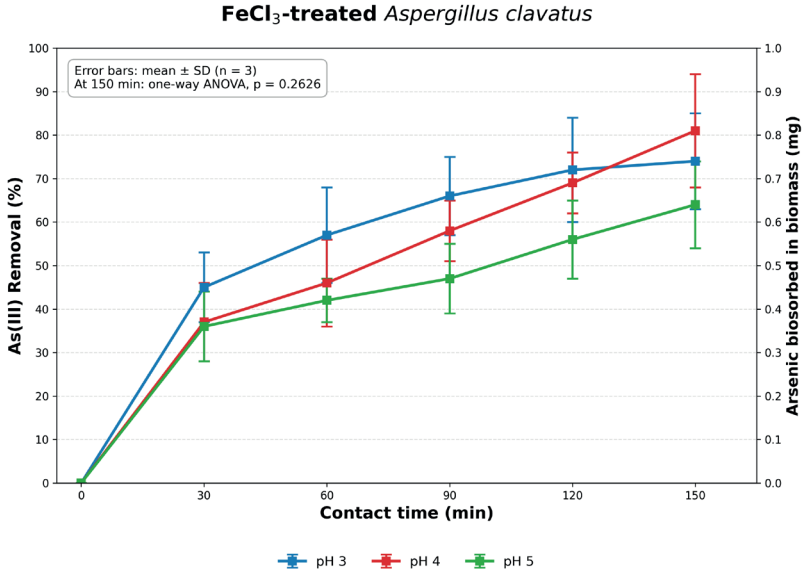


FIGURE 5: Effect of pH and contact time on the biosorption of trivalent arsenic by FeCl3-treated *Aspergillus clavatus* biomass. Error bars represent the standard deviation (SD, n = 3). Differences in As(III) removal among the tested pH conditions at 150 min were evaluated using one-way ANOVA (p = 0.2626), indicating no statistically significant differences (p > 0.05).

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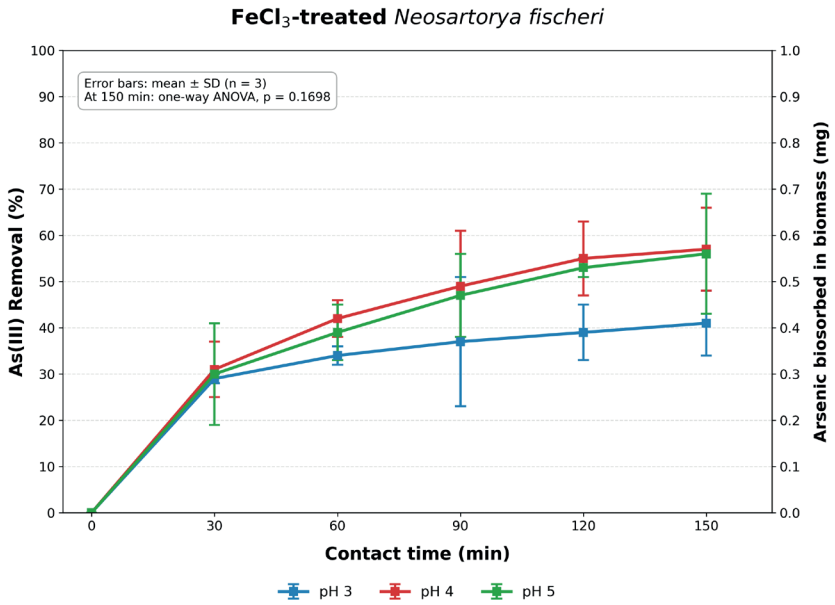


FIGURE 6: Effect of pH and contact time on the biosorption of trivalent arsenic by FeCl3-treated *Neosartorya fischeri* biomass. Error bars represent the standard deviation (SD, n = 3). Differences in As(III) removal among the tested pH conditions at 150 min were evaluated using one-way ANOVA (p = 0.1698), indicating no statistically significant differences (p > 0.05).

ically modified biomass generally exhibited no statistically significant pH-dependent differences at the final sampling times. Nevertheless, the absence of statistical significance does not necessarily imply complete independence from pH, particularly given the limited number of experimental replicates (n = 3).

4. CONCLUSIONS

The present study demonstrated that both fungal species are capable of removing As(III) from aqueous solutions; however, their biosorption performance was strongly species-dependent. Native biomass of *Aspergillus clavatus* consistently outperformed *Neosartorya fischeri*, highlighting the importance of fungal cell wall characteristics and surface chemistry in arsenic binding. Although the effect of pH was relatively limited within the investigated range, slightly higher biosorption efficiencies were generally observed at higher pH values for untreated biomass. Chemical pretreatment significantly improved the sorption performance of both fungi. NaOH modification enhanced biosorption by increasing the accessibility of active binding sites, whereas $FeCl_3$ functionalization proved to be the most effective treatment by introducing high-affinity iron-based adsorption centres for As(III). Iron-modified biomass also exhibited faster sorption kinetics and markedly reduced the differences in biosorption capacity between the two fungal species. Overall, the results confirm that fungal biomass represents an effective, environmentally friendly and low-cost biosorbent for the removal of As(III) from contaminated waters, while simple chemical modifications, particularly $FeCl_3$ treatment, substantially increase its practical application potential. However, further investigations are needed to assess the reusability, long-term stability, and performance of the modified fungal biomass under elevated temperatures, particularly in the context of climate change and its potential impact on wastewater treatment efficiency.

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