



Fungal Biomass for Water Softening via the Biosorption Process

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Abstract

Hardness in water, primarily caused by calcium and magnesium ions, poses significant operational, environmental, and health-related challenges. This study evaluates the potential of *Aspergillus niger* biomass (AN) and coconut-based activated carbon (AC) as low-cost biosorbents for water softening. Batch experiments were conducted to investigate the effects of contact time, shaking speed, pH, and adsorbent dosage on hardness removal from artificial hard water. The results demonstrate that both sorbents exhibit relatively rapid initial ion uptake, reaching equilibrium after 7 hours for AN and 11 hours for AC. Shaking speed showed only minor influence beyond 150 rpm, indicating that external mass transfer limitations were minimized at moderate agitation. Within the pH range of 5–9, hardness removal remained nearly constant, whereas literature data indicate a sharp increase in removal efficiency at pH values above 10 due to enhanced surface negativity. Increasing adsorbent dose improved removal efficiency up to a saturation threshold, with optimal performance observed at 15–20 g of biosorbent. Overall, *A. niger* biomass demonstrated promising softening capabilities, suggesting its potential application as an economical and sustainable alternative to conventional water softening technologies.

Keywords: water hardness, adsorption, activate carbon, biosorption, *Aspergillus niger*

1. INTRODUCTION

Water hardness, defined primarily by the presence of divalent cations such as calcium (Ca^{2+}) and magnesium (Mg^{2+}), remains one of the most widespread water quality challenges affecting domestic, industrial, and environmental systems. Hardness originates mainly from the dissolution of minerals from geological formations, particularly carbonate rocks, into groundwater and surface water sources (Helmy et al., 2017; Liu et al., 2019). Depending on the hydrogeological context, natural hardness levels may exceed recommended limits for drinking water and industrial use, leading to significant operational and health-related consequences.

From a health perspective, elevated hardness may contribute to kidney stone formation, gastrointestinal irritation, and other metabolic disorders, especially when consumed over long periods (Bindhu et al., 2021; Sengupta, 2013; Huang et al., 2019). Several studies highlight that excessive calcium and magnesium intake from untreated groundwater has been associated with increased prevalence of urolithiasis and other mineral-related disorders in affected regions (Bindhu et al., 2021). Conversely, extremely low-mineral water produced by techniques such as reverse osmosis may negatively impact electrolyte balance and drinking palatability, suggesting the need for balanced mineral content (Halpegama et al., 2021; WHO, 2011; Catling et al., 2008).

In industrial applications – particularly in boilers, cooling systems, textile production, and food processing – hard water contributes to scale formation, reduced heat transfer efficiency, pipe clogging, membrane fouling, and corrosion, ultimately increasing energy demand and maintenance costs (Kose et al., 2024; Campanile et al., 2022; Karlsson, 2021). Figures presented by Kose et al. (2024) show severe internal scale layers in biomass-fired boiler tubes, illustrating how untreated hardness leads to operational inefficiencies and production losses.

Given these risks, developing effective hardness removal technologies remains a critical research area. Traditional softening approaches include lime-soda precipitation, chemical additives, and thermal treatments; however, these methods often involve high energy input, complex operation, or sludge generation (Boonpanaid & Piyamongkala, 2023; Helmy et al., 2017). Modern water treatment has therefore shifted toward more sustainable and efficient alternatives.

Ion exchange remains one of the most widely used techniques, relying on synthetic resins (e.g., Amberlite, NuoSep) or natural zeolites capable of exchanging hardness ions for sodium or hydrogen ions (Boonpanaid & Piyamongkala, 2023; Liu et al., 2019; El-Nahas et al., 2020). Research shows that both commercial and modified resins achieve high softening efficiency, although resin regeneration generates sodium-rich brine requiring proper disposal (Boonpanaid & Piyamongkala, 2023). Zeolite-based systems have been increasingly investigated due to their mechanical stability, low cost, and reduced secondary pollution. For instance, Campanile et al. (2022) developed monolithic geopolymer-zeolite structures capable of achieving up to 90% Ca^{2+} removal through combined ion exchange and precipitation mechanisms.

Electrochemical methods, particularly electrocoagulation, have also emerged as efficient softening technologies. By generating coagulant species in situ at metal electrodes, electrocoagulation effectively removes Ca^{2+} and Mg^{2+} while minimizing chemical additives (Helmy et al., 2017). Halpegama et al. (2021) demonstrated that optimized monopolar electrocoagulation achieved up to 83% hardness removal depending on water chemistry. These systems also offer combined benefits, such as concurrent fluoride removal, sludge compaction, and reduced operational costs.

Another expanding area involves adsorption-based methods, particularly those using low-cost agricultural or biobased materials (Rolence et al., 2014; Bharadwaj et al., 2016; Gupta et

Tab. 1. Physical parameters of artificial hard water samples

Tab. 1. Parametry fizyczne próbek sztucznej twardej wody

Parametr	Artificial hard water
pH	9,23
Total hardness as mgCaCO ₃ /L	320
Calcium hardness as mgCaCO ₃ /L	180
Magnesium hardness as mgCaCO ₃ /L	140

al., 2009; Hettiarachchi et al., 2019; Kharel et al., 2016). Bindhu et al. (2021) reported that adsorbents such as elephant foot yam, rice husk ash, and coconut-shell-based activated carbon achieved hardness reductions above 80%. Such methods are particularly attractive for rural or decentralized treatment settings where cost and simplicity are essential.

Novel materials and hybrid systems – including sulfonated waste polystyrene, magnetic treatments, advanced membranes, and multi-process combinations – offer additional pathways for sustainable hardness control. Several studies demonstrate that chemically modified waste polystyrene can function as an efficient cation exchanger, enabling both plastic waste valorization and water softening (Bekri-Abbes et al., 2008; Ibrahim et al., 2016). Such circular-economy approaches reduce environmental impact while maintaining competitive performance relative to synthetic resins.

Overall, the evolution of hardness removal technologies reflects a growing emphasis on efficiency, cost reduction, environmental sustainability, and adaptability to local water quality conditions. As global water demand increases and groundwater mineralization intensifies, optimizing the selection and integration of softening methods – whether ion exchange, electrochemical treatment, adsorption, or hybrid processes – will play an essential role in protecting human health, improving industrial efficiency, and ensuring long-term water resource sustainability.

The present study describes the influence of several important operational parameters such as shaking speed, biomass/AC dosage, initial pH and contact time on the hardness removal.

2. MATERIALS AND METHODS

2.1. Preparation of biosorbent

The fungal strain *Aspergillus niger* was obtained from the Polish Collection of Industrial Microorganisms (KKP). The culture was grown in Malt Broth medium (MB; BTL P-0197) containing 17 g/L of malt extract and 3 g/L of peptone SP. The fungus was inoculated into the medium adjusted to pH 4.8 and incubated at 30°C, which were the optimal conditions for its cultivation. After ten days of incubation, the fungal biomass was harvested, washed with distilled water, and treated with a 1% formaldehyde solution. The biomass (AN) was then dried at 60°C for 24 h, pulverized, and stored in polythene bags for further experiments.

Coconut-based activated carbon (AC) was also used in the adsorption tests. The adsorbent was in the form of granular activated carbon (GAC), characterized by small and uniform pores, which makes it suitable for the removal of low-molecular-weight contaminants.

2.2. Preparation of artificial hard water

Artificial hard water was prepared by mixing 288.0 mg of CaCl₂, 220.0 mg of MgSO₄·7H₂O and 390.0 mg of NaHCO₃ in

1 dm³ of ionized water and stored in a plastic bottle. The artificial hard water parameters are shown in the Table 1.

2.3. Determination of hardness

Water hardness was determined according to PN-ISO 6059:1999, PN-ISO 6058:1999, and PN-C-04554:1999 standards. All hardness values were expressed as mg CaCO₃/L. The pH and electrical conductivity of the water were measured using a WTW inoLab® pH 7310 meter. Hardness removal efficiency was calculated using the following equation (Wang et al., 2020):

$$R = (C_i - C_f) / C_i \cdot 100\% \quad (1)$$

Where, C_i and C_f are the initial and final hardness in mg/L respectively.

2.4. Adsorption experiments

2.4.1 Effect of contact time

100 mL of artificial hard water was contacted with AN/AC in 250 mL Erlenmeyer flasks, which were shaken in an orbital shaker at a constant speed of 150 rpm, temperature 25°C and at a certain time (1, 5, 10, 20, 30, 45, 60 and 120min).

2.4.2 Effect of shaking speed

To evaluate the effect of shaking speed on adsorption process 15g of AN/AC was added to a 250 ml Erlenmeyer flask containing 100 mL of artificial hard water. The flasks were placed for 1 hour in an incubator with a shaker at different shaking speed (50, 150, 300rpm) at 25°C.

2.4.3 Effect of pH

To evaluate the effect of different pH on adsorption process 15g of AN/AC was added to a 250 ml Erlenmeyer flask containing 100 mL of artificial hard water. The assumed pH level was obtained with 0.1M/1M NaOH solution or 0.1M HCl solution. The flasks were placed in an incubator with a shaker at 25°C and 150rpm for 1 hour.

2.4.4 Effect of adsorbent dosage

To evaluate the effect of adsorbent dosage, AN/AC samples weighing 1, 5, 15, 20, and 30 g were placed in 300 mL Erlenmeyer flasks. Each sample received 100 mL of artificial hard water. The flasks were shaken for 4 hours at 150 rpm. After the contact time, suspensions were filtered, and the filtrates were analyzed using the EDTA titration method.

3. RESULTS AND DISCUSSION

3.1 Effect of contact time

Figure 1 presents the relationship between contact time and hardness removal efficiency.

Observations indicated that hardness-ion removal increased with longer contact time, reaching 75.5% and 66%

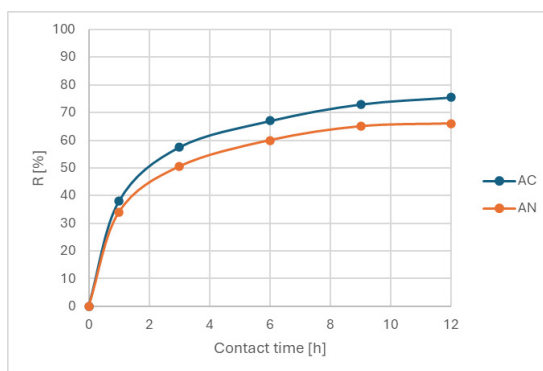


Fig. 1. Effect of contact time on hardness removal (AN/AC quantity = 15 g, T=25°C; shaking speed = 150rpm)
 Rys. 1. Wpływ czasu kontaktu na usuwanie twardości (masa AN/AC = 15 g, T=25°C; prędkość wytrząsania = 150rpm)

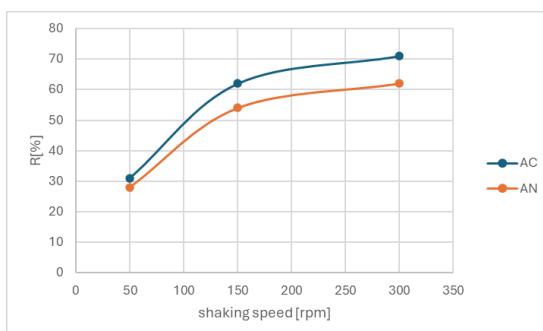


Fig. 2. Effect of shaking speed on hardness removal (AN/AC quantity = 15 g, T=25°C; t=4h).
 Rys. 2. Wpływ prędkości wytrząsania na usuwanie twardości (masa AN/AC = 15 g, T=25°C; t=4h)

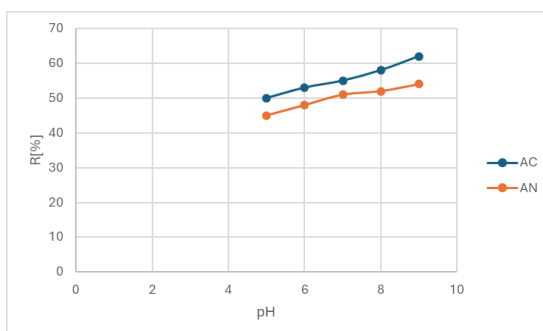


Fig. 3. Effect of pH on hardness removal (biomass/AC quantity = 15 g, T=25°C; shaking speed = 150rpm; t=4h)
 Rys. 3. Wpływ pH na usuwanie twardości (masa AN/AC = 15 g, T=25°C; prędkość wytrząsania = 150rpm; t=4h)

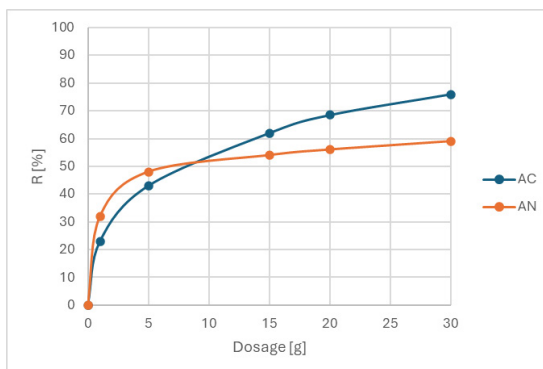


Fig. 4. Effect of adsorbent dosage on hardness removal (t=4h, T=25°C; shaking speed = 150rpm)
 Rys. 4. Wpływ dawki adsorbentu na usuwanie twardości (T=25°C; prędkość wytrząsania = 150rpm; t=4h)

for AC and AN, respectively. Equilibrium was achieved after approximately 11 hours for AC and 7 hours for AN. Analysis of the trends shows that adsorption proceeded rapidly during the initial stage and then gradually leveled off, reflecting the establishment of equilibrium. This behavior is consistent with the fact that biosorption and adsorption are surface-driven phenomena governed by mass transfer to active sites.

Previous studies (Rolence et al., 2014) suggest that the initially fast uptake results from the abundance of available surface sites. As the process continues, however, repulsive forces between solute molecules in the solid and liquid phases hinder further attachment, making it increasingly difficult for the remaining active sites to be occupied.

Biosorption is a metabolism-independent mechanism that can occur in both living cells and non-viable microbial biomass (Bilal et al., 2018; Chojnacka, 2010; Javanbakht et al., 2014). In this study, AN demonstrated effective water-softening capacity, indicating favorable surface characteristics and a high density of functional groups involved in metal binding.

As shown in Figure 1, removal efficiency improves with time; however, taking into account both the economic and operational aspects, an optimal contact time of 4 hours can be identified.

3.2 Effect of shaking speed

Figure 2 presents the variation in hardness as a function of shaking speed at 25°C.

An increased shaking speed enhances the diffusion of ions toward the active adsorption sites, thereby improving the overall efficiency of the adsorption process. A slight improvement in adsorption was observed at 300 rpm; however, it was not significant enough to justify the higher agitation rate. This suggests that external mass transfer resistance becomes negligible at elevated shaking speeds. Therefore, a shaking speed of 150 rpm was selected for subsequent experiments.

3.3 Effect of pH

Figure 3 presents the influence of pH on the efficiency of the softening process.

The pH selected for the batch studies were from 5 to 9 that is near to the standard pH of the drinking water. The results of the study showed that, within the tested range, the removal of hardness-causing ions was only slightly influenced by the pH of the solution. Previous studies (Jimoh et al., 2012) have demonstrated that changes in hydrogen-ion concentration affect the number of available metal-binding sites on the adsorbent surface. At lower pH values, the surface of the adsorbent becomes surrounded by hydrogen ions, increasing competition between hydronium ions (H_3O^+) and the positively charged Ca^{2+} and Mg^{2+} ions for active sites. As pH increases, the concentration of hydronium ions decreases, allowing hardness ions (Ca^{2+} and Mg^{2+}) to be adsorbed in greater amounts.

In addition, an exponential increase in removal efficiency was observed in the pH range of 10–12 (Rolence et al., 2014). This may be attributed to the higher concentration of hydroxide ions (OH^-), which enhances the negative charge of the adsorbent surface and thus promotes the attraction of positively charged ions.

Within the tested pH range of 5–9, the hardness-removal efficiency remained nearly constant. This trend is likely relat-

ed to the presence of relatively balanced concentrations of H^+ and OH^- ions, which moderate the surface polarity of the adsorbent, resulting in a surface that is insufficiently charged to enhance further ion adsorption.

3.4 Effect of adsorbent dosage

Figure 4 presents the relationship between adsorbent dose and hardness removal efficiency at 25°C.

The results show that hardness removal increases with higher adsorbent doses. A dose of 20 g achieved removal efficiencies of 69% for AC and 56% for AN. Notably, for AN, a 15 g dose already produced a high softening efficiency of 54%. Further increases in adsorbent mass led to only marginal improvements. This plateau effect can be attributed to the greater availability of exchange and adsorption sites at higher adsorbent concentrations. Once a critical adsorbent dose is reached, adsorption approaches its maximum capacity, and additional AC/AN no longer contributes significantly to ion removal (Rolence et al., 2014).

4. CONCLUSIONS

The experimental findings not only confirm the effectiveness of both *A. niger* biomass and activated carbon in hardness removal but also clearly illustrate the fundamental principles governing biosorption processes. The observed trends – rapid initial uptake, subsequent equilibrium, limited influence of agitation, and dosage-dependent saturation – are fully consistent with the physicochemical nature of biosorption, in which functional groups on the sorbent surface interact with dissolved metal ions through ion exchange, complexation, and electrostatic attraction. These results therefore align with the broader theoretical framework of metabolism-independent biosorption and reinforce the role of fungal biomass as a versatile, surface-active material suitable for water treatment applications.

Because of their unique characteristics – including ease of cultivation, high biomass yield, and considerable chemical and genetic flexibility – fungi are regarded as highly promising biosorbents for applications that are both economically and environmentally advantageous. The fungal cell wall exhibits remarkable binding properties due to structural components commonly found in various fungal species, such as chitin, lipids, polyphosphates, and proteins. Their cell walls also contain abundant polysaccharides and glycoproteins, which provide diverse metal-binding functional groups, including amine, phosphate, carboxyl, and hydroxyl groups. These features make fungi versatile tools in numerous fermentation processes and allow their biomass to be produced readily on an industrial scale for biosorption purposes. Moreover, fungal biomass can be obtained inexpensively from low-cost growth media or recovered as a by-product from fermentation industries. Fungi are also less sensitive than many other microorganisms to fluctuations in nutrients or process parameters such as pH, temperature, and aeration. Their filamentous morphology further facilitates separation by filtration (Patil and Paknikar, 1999; Dinçer et al., 2007; Dey et al., 2024).

Biosorption itself is a metabolically passive process and therefore does not require energy input. The amount of pollutant that a biosorbent can remove depends on kinetic equilibrium and the composition of its cell surface. Because the

process is governed by equilibrium, biosorption is strongly influenced by environmental pH, biomass concentration, and interactions among metal ions. Metal accumulation inside living cells involves transport across the cell membrane and depends on metabolic activity; thus, only viable cells can participate in metabolism-dependent uptake. In contrast, metabolism-independent biosorption – typical for dead or inactivated biomass – results from physicochemical interactions between metal ions and the functional groups present on the biosorbent surface. This uptake mechanism is consistent with ion exchange, physical adsorption, and chemical sorption theories (Dey et al., 2024).

Summarizing the results of the study, it can be concluded that:

1. Both AC and AN are effective biosorbents for water hardness removal, with removal efficiencies reaching 75.5% (AC) and 66% (AN) at extended contact times. The rapid initial uptake observed for both materials confirms that ion exchange and surface adsorption are the dominant mechanisms driving hardness reduction.
2. Contact time plays a key role in biosorption performance. The adsorption curves indicate a fast initial phase followed by a gradual plateau, consistent with active-site saturation. Although full equilibrium required 7–11 hours depending on the adsorbent, a practical operating time of 4 hours emerged as a reasonable compromise between performance and process efficiency.
3. The effect of shaking speed was minimal beyond 150 rpm, suggesting that boundary-layer resistance becomes negligible at moderate mixing intensity. Therefore, increasing agitation above this threshold does not significantly enhance mass transfer and is not economically justified.
4. Hardness removal was largely insensitive to pH within the drinking-water range of 5–9. This behavior is likely due to competing concentrations of H^+ and OH^- , which neutralize surface charge effects

and maintain a near-constant biosorption potential. The literature, however, indicates strongly improved performance under alkaline conditions (pH 10–12), where negatively charged sites on the adsorbent surface become more abundant.

5. Adsorbent dosage exhibited a clear saturation effect. Increasing AN or AC mass enhanced hardness removal up to approximately 15–20 g, beyond which only marginal improvements were observed. This plateau indicates that the number of available ions in solution becomes the limiting factor once a critical adsorbent concentration is reached.
6. AN biomass displayed competitive performance relative to AC, despite its simpler preparation and lower cost. The functional groups present in fungal cell walls – such as carboxyl, phosphate, amine, and hydroxyl groups – likely contributed to its ability to bind Ca^{2+} and Mg^{2+} ions effectively. These findings support the feasibility of repurposing fungal biomass, including waste biomass from industrial fermentations, for sustainable water treatment.
7. The study highlights the practical potential of biosorption for decentralized or low-resource settings, where conventional softening technologies (e.g., ion exchange resins or electrochemical systems) may be cost-prohibitive. AN biomass, as a renewable and low-maintenance sorbent, represents a promising alternative for environmentally friendly hardness control.
8. Further research should explore sorbent regeneration, adsorption isotherms, and kinetic modeling, which would provide deeper insights into the interaction mechanisms and support the design of scalable biosorption systems. Evaluating performance using natural hard water and continuous-flow systems would also strengthen the applicability of the method.

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Wykorzystanie biomasy grzybowej do zmiękczenia wody metodą biosorpcji

*Twardość wody, wynikająca głównie z obecności jonów wapnia i magnezu, stanowi istotne wyzwanie eksploatacyjne, środowiskowe oraz zdrowotne. W niniejszym badaniu oceniono potencjał biomasy *Aspergillus niger* (AN) oraz węgla aktywnego (AC) jako niskokosztowych biosorbentów do zmiękczenia wody. Przeprowadzono eksperymenty okresowe mające na celu określenie wpływu czasu kontaktu, prędkości wytrząsania, pH oraz dawki sorbentu na usuwanie twardości ze sztucznie przygotowanej twardej wody. Wyniki wykazały, że oba sorbenty charakteryzują się stosunkowo szybkim początkowym wychwytem jonów, osiągając stan równowagi po 7 godzinach dla AN i po 11 godzinach dla AC. Prędkość wytrząsania miała jedynie niewielki wpływ powyżej 150 rpm, co wskazuje, że ograniczenia transportu masy w warstwie zewnętrznej były zminimalizowane przy umiarkowanej intensywności mieszania. W zakresie pH 5–9 usuwanie twardości pozostawało praktycznie niezmiennie, podczas gdy dane literaturowe wskazują na gwałtowny wzrost efektywności przy pH powyżej 10 ze względu na zwiększoną ujemną polaryzację powierzchni. Zwiększenie dawki sorbentu poprawiało efektywność usuwania do momentu osiągnięcia progu nasycenia, przy czym optymalną wydajność obserwowano przy 15–20 g biosorbentu. Ogólnie rzecz biorąc, biomasa *A. niger* wykazała obiecujące właściwości zmiękczające, wskazując na jej potencjalne zastosowanie jako ekonomicznej i zrównoważonej alternatywy dla konwencjonalnych technologii zmiękczenia wody.*

Słowa kluczowe: *twardość wody, adsorpcja, węgiel aktywny, biosorpcja, *Aspergillus niger**