Comparative Analysis of Hexavalent Chromium Biosorption Efficiency Using Dead and Live *Aspergillus nomius* Biomass

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Abstract

Daily industrial activities especially in developing countries produce and discharge wastes containing heavy metals into the water resources making them polluted, threatening human health and the ecosystem. One such heavy metal is Chromium, the hexavalent form of which is extremely toxic and carcinogenic. Biosorption, the process of passive cation binding by dead or living biomass, represents a potentially cost-effective way of eliminating toxic heavy metals from industrial wastewater. The potential of microorganisms to remove metal ions in solution has been extensively studied; in particular, live and dead fungi have been recognized as a promising class of low-cost adsorbents for the removal of heavy metal ions. Fungal biomass has various advantages; hence, it needs to be explored further to take its maximum advantage in wastewater treatment. In this study, we discuss the live and dead fungi characteristics of sorption, factors influencing heavy metal removal. Biosorption studies were performed with both dead and live biomass and the effectiveness of Cr (VI) biosorption was compared for each parameter. It was observed that biosorption was maximum (approximately): 82% while using sulfuric acid as the pre-treatment agent (hence only dead biomass) and also maximum of 96.5% at 1 N. The optimum pH for maximum biosorption was 6 when dead biomass was used, while it was 2 when live biomass was used. Maximum Chromium removal of 86% was obtained using 2 g live biomass whereas 0.5 g of dead biomass was enough to obtain the maximum efficiency.96% chromium was removed at 25° C using dead biomass, whereas, maximum removal of about 84% was obtained when live biomass was used for biosorption and it took place at 35° C. Maximum Cr (VI) removal of about 95% was obtained when dead biomass was used and 69% when live biomass was used, both at 1mg/L metal concentration. 0.5 g of dead biomass in 100 ml, 1 mg/L solution, was optimum for Cr (VI) removal, while for live biomass, maximum Cr (VI) biosorption of 63% was obtained when 1.5 g of it was used in 300 ml solution. It was finally concluded that dead fungal biomass has better biosorption potentials and also some other inherent advantages over live biomass.

Keywords: Biomass, biosorption, fungi, heavy metal, hexavalent chromium

1. Introduction

Water bodies getting contaminated by heavy metals is becoming a great global concern. Heavy metals reach the environment through two main sources, natural (volcanic emissions, forest fires, deep-sea vents, and geysers) and anthropogenic (mining and smelting sites, tanneries, metal-manufacturing plants, and painting and coating industries). Industrial expansions lead to an uncontrollable release of heavy metals in the environment. The problem is primarily observed for developed countries that produce huge quantities of waste-waters,that may contain a high concentration of heavy metals. Due to the lack of technologies, advanced manpower, and low policy enforcement, the challenge is stronger in developing countries. (Gadd, 2009; Singh and Gauba, 2014; Irawati et al., 2016; Lata et al., 2019).

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Chromium, the heavy metal can exist in hexavalent as well as in trivalent forms. The hexavalent form is very toxic, carcinogenic, and mutagenic in humans and animals but the trivalent form is comparatively less toxic, less soluble and thus a lesser hazard (Philip et al., 1998). Various adverse health effects can result from hexavalent Chromium exposure, including growth inhibition, cancer, nervous system damage, organ damage, and in extreme cases, death (Akpor and Muchiem, 2010).

Various conventional methods have been used to remove these contaminants from water bodies including filtration, chemical precipitation, ion exchange, reverse osmosis, evaporation, membrane technology, carbon adsorption, electrowinning, preconcentration, coagulation of wastewater, chelation, redox, and electrochemical treatment (Yahaya and Don, 2014; Cai et al., 2016; Jin et al., 2018; Joshi, 2018; Ferreira et al., 2019; Khan et al., 2019). It has been concluded by many researchers, that these technologies have certain limitations on cost-effectiveness, cause of secondary pollution, complexity, and alteration of the physical and chemical nature of the environment. These techniques are usually very expensive for usage at a large scale and also tedious for constant monitoring and controlling, due to their incomplete and unpredictable metal removal efficiencies (Ferreira et al., 2019; Ayete et al., 2021). The above processes may also remove non-target useful microbial biota such as nitrogen-fixing bacteria as well as other fauna species which is undesirable (Siddiquee et al., 2015). For overcoming those limitations, biological treatment (bioremediation) techniques are highly recommended because they are environmentally friendly, fast, and cost-effective (Javanbakht et al., 2014; Joshi, 2018; Ferreira et al., 2019; Ayete et al., 2021). These biological methods can also be implemented for treating a large volume of effluent with low biomass concentration within a short operation time (Sharma et al., 2016). Most common bioremediation techniques include biofilters, biosorption, bioaugmentation, bioventing, biotransformation, composting, land farming, bioreactor, and biostimulation.

Biosorption is one of the biological techniques that utilize microbes as a biosorbing agent to detoxify and remove environmental pollutants such as heavy metals (Siddiquee et al., 2015). Microbes uptake heavy metals through two processes, intracellular accumulation through their living biomass, and extracellular binding, through both living and dead biomass (Kapoor, 1998; Gadd, 2009; Javanbakht et al., 2014). The filamentous fungal strains are of great interest for biotechnological applications such as wastewater treatment because they have some polymers in the structure of their cell wall which are responsible for the adsorption of pollutants (Silah and Gul, 2014).

This study aims to investigate the biosorption potential of living and dead biomass of *Aspergillus nomius* isolated from tannery effluent for Chromium (VI) at a batch-scale level to assess its application as a low-cost biosorbent.

2. Materials and Methods

2.1 Microorganism

Sixteen fungal strains (S1-S16) were isolated from tannery effluent of Bantala Leather Complex area (Kolkata, West Bengal, India) and their Cr (VI) tolerance capacity was tested at concentrations of 1,000-2,000 mg/L. Among the 16 strains, S12 showed the maximum Cr (VI) tolerance capacity of 2,000 mg/L and so this strain was subjected to fungal ITS sequencing analysis and phylogeny which revealed that the S12 strain has 99.82% similarity with *A. nomius*. (Guha et al., 2020). So this S12 strain is being selected here for the biosorption study and it will be referred to as *A. nomius*. The strain is maintained on a Czapekdox agar medium with regular subculturing after every 15 days and preserved at 4° C.

2.2 Biomass production

The fungus was cultured in a liquid medium (Czapekdox broth) in 100 mm glass Petri-plates. The growth medium consisted of (g/l of distilled water): Sucrose 30.000, Sodium nitrate 2.000, Di-potassium phosphate 1.000, Magnesium sulfate 0.500, Potassium chloride 0.500, Ferrous sulfate 0.010.

The pH of the medium was adjusted to 5, before autoclaving by using 0.5 M HCl. Once inoculated, the plates were incubated in an incubator for 2 weeks at 30° C. for the production of sufficient fungal mat.

2.3 Bio-sorbent preparation

The dead and live biomass of *A. nomius* was used as a biosorbent for the sorption of Cr (VI) from an aqueous solution. After incubation, the biomass was collected from the medium and washed with warm distilled water. To make it dead the biomass was then pre-treated by immersing it in 500 ml 0.5 (N) solutions of various acids and alkali (Sulfuric acid, Hydrochloric acid, Nitric acid, Acetic acid, and Sodium hydroxide) and then kept in a water bath at 100° C for 15 minutes. For experiments with live biomass this pretreatment step was skipped (the biomass was only washed with cold water). The biomass was then washed with deionized water until the pH of the wash solution was in the near-neutral range (pH 6.8-7.2). It was then dried at 60° C in a hot-air oven for 12 hours, grounded using mortar and pestle, and stored in an air-tight container (Santhi and Guru, 2014).

2.4 Chromium (VI) solution preparation

Aqueous (stock) solution of Chromium (VI) concentration 1,000 mg/L was prepared by dissolving 2.83 g of potassium dichromate salt in 1,000 ml distilled water (Reya et al., 2012). The concentration of Chromium was varied from 1 mg/L to 5 mg/L.

3. Optimization of Various Biosorption Parameters

3.1 Effect of pre-treatment (for dead biomass)

Fungal biomass was modified by pre-treatments with acids and alkali. 0.5 N, 500 ml solutions of Sulfuric acid, Hydrochloric acid, Nitric acid, Acetic acid, and Sodium hydroxide were used for this purpose. The percentage removal of the metal ion with this pre-treated fungal biomass was studied spectrometrically at 540 nm using Diphenylcarbazide (DPC) assay method (USEPA, 1992).

3.2 Effect of pre-treatment agent concentration (for dead biomass)

The effect of pre-treatment agent concentrations was observed for biosorption of 1 mg/L of Cr (VI) using different concentrations of Sulfuric acid as pre-treatment agent (0.3, 0.5, 0.8, and 1 N). The adsorbent dosage was 0.5 g in 100 ml solution in 250 ml conical flask and pH 6.0. The flasks were then incubated for 24 hours at 37° C and 150 rpm. After 24 hours, the samples were taken out, filtered, and analyzed using DPC assay at 540 nm (Reya et al., 2012).

3.3 Effect of pH (determined with both dead and live biomass)

The effect of pH on biosorption efficiency was determined using both live and dead *A. nomius* biomass. 100 ml of 1 mg/L concentration of Chromium solution was adjusted to various pH (2, 4, 6, 8, 10, and 12), and to it, 0.5 g of biomass was added and incubated at 37° C, at 150 rpm for 24 hours. After 24 hours, the samples were taken out, filtered, and analyzed using DPC assay at 540 nm (Goyal et al., 2003)

3.4 Effect of contact time (determined with both dead and live biomass)

To determine the effect of contact time 0.5 g of biomass (live and dead biomass were used separately) was added in a 100 ml solution of 1mg/L of Cr (VI) in a 250 ml conical flask and incubated for 12, 24, and 48 hours respectively in a shaker incubator at 37° C and 150 rpm. After the incubation period samples were taken out, filtered, and analyzed using DPC assay at 540 nm (Sethuraman and Balasubramanian, 2010). The pH of the Cr (VI) solution was adjusted to 6 when dead biomass was being used and pH was kept at 2 when optimization experiments were done with live biomass (as such was the optimum values, shown in the result section). This was followed for the rest of the experiments as well.

3.5 Effect of biomass loading (determined with both dead and live biomass)

0.5g, 1 g, and 2 g fungal biomass were added separately in 100 ml of 1 mg/L concentration of Chromium solution and then incubated at 37° C for 24 hours at 150 rpm. After 24 hours, the samples were taken out, filtered, and analyzed using DPC assay at 540 nm (Reya et al., 2012).

3.6 Effect of temperature (determined with both dead and live biomass)

The effect of temperature on biosorption efficiency was observed with a Chromium (VI) concentration of 1 mg/L. 0.5g of biomass was added to 100 ml solution of 1 mg/L concentration of Cr (VI) and incubated at various temperatures (20° C, 25° C, 30° C, 35° C, 40° C, and 45° C) for 24 hours at 150 rpm. After 24 hours samples were taken out, filtered, and analyzed using DPC assay at 540 nm (Reya et al., 2012).

3.7 Effect of initial metal ion concentration (determined with both dead and live biomass)

0.5 g of fungal biomass was added in 100 ml solution of different concentrations (1, 2, 3, 4, and 5 mg/L) of Chromium (VI) in 250 ml conical flasks The flasks were then incubated in a shaker incubator at 37° C at 150 rpm for 24 hours. After 24 hours, the samples were taken out, filtered, and analyzed using DPC assay at 540 nm (Reya et al., 2012).

3.8 Effect of volume differences (keeping metal concentration constant) (determined with both dead and live biomass)

50, 100, 150, 200, and 250 ml solution of Chromium (VI) of 1 mg/L concentration were prepared. To it, 0.5 g of biomass was added and incubated at 37° C with an agitation rate of 150 rpm for 24 hours. After 24 hours, the samples were taken out, filtered, and analyzed using DPC assay at 540 nm.

3.9 Effect of varying combinations of biomass load and volume (for dead and live biomass)

1 mg/L concentration of Chromium solutions was exposed to various combinations of biomass load and volume (0.5 g in 100 ml, 1 g in 200 ml, and 1.5 g in 300 ml). Then the flasks were agitated in a shaker at 150 rpm and incubated at 37° C for 24 hours. After 24 hours, the samples were taken out, filtered, and analyzed using DPC assay at 540 nm.

4. Biosorption Efficiency Calculation

Biosorption efficiency (%) was calculated using the following equation (Bajpai and Rai, 2010).

$$E = \frac{(C_i - C_f) \times 100}{C_i} \quad (1)$$

where:
E=Percentage removal of hexavalent Chromium
C_i=Initial metal ion concentration (mg/L)
C_f=Final metal ion concentration (mg/L)

Each experiment was repeated three times to get accurate results and the average was taken for the calculation of biosorption efficiency.

5. Results

5.1 Effect of pre-treatment (determined for dead biomass)

Metal adsorption to biomass can be manipulated by pre-treating the biomass with alkali, acid, and heat which may increase the amount of metal sorbed. The comparison for Chromium adsorption capacities by *A. nomius* biomass when pre-treated with acids and alkalis shown in Figure 1. It is seen that when the biomass was pre-treated with sulfuric acid, the adsorption efficiency was maximum (82%) whereas when pretreated with alkali (NaOH) the adsorption efficiency was minimum (1%).

Adsorption of Chromium may vary because of the action of chemical agents contributing to modifications in the cell wall structure. A Similar observation was reported by Reya et al., (2012) where they used *A. oryzae* and *A. sojae* biomass and the acid treatment yielded better results than alkali treatment.

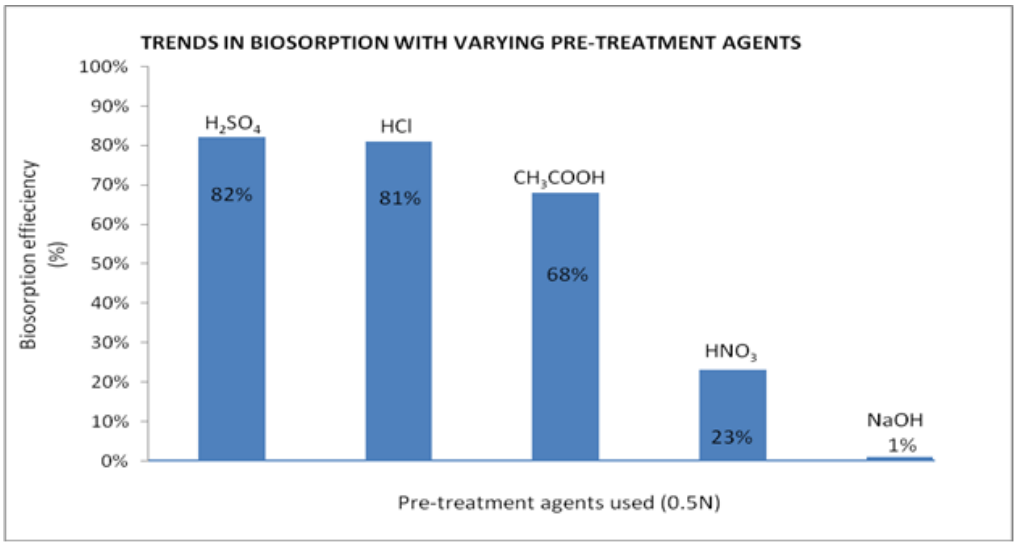

Figure 01. Trends in biosorption with varying pre-treatment agents

5.2 Effect of pre-treatment agent (Sulfuric acid) concentration (determined for dead biomass)

The effect of various concentrations of sulphuric acid on biosorption by dead *A. nomius* biomass was studied and results are shown in Figure 2. It was observed that the percentage removal of Chromium increased with increase in the concentration of the pre-treatment agent and was maximum at 96.5% when 1N solution was used.

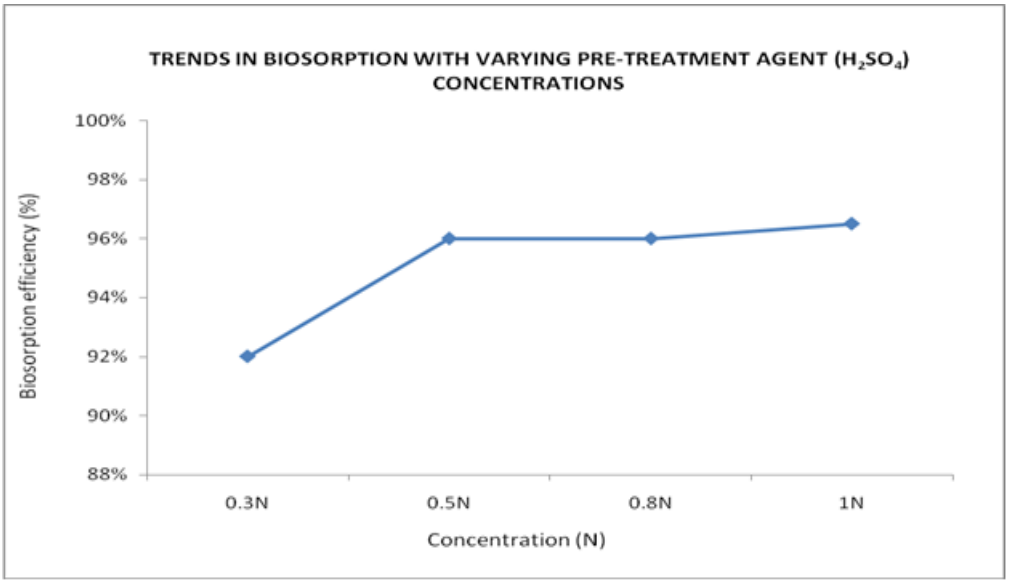

Figure 02. Trends in biosorption with varying pre-treatment agent (H_2SO_4) concentrations

5.3 Effect of pH (determined with both dead and live biomass)

The effect of pH on biosorption was studied and results are shown in Figure 3. It was observed that the percentage removal of Chromium was maximum at pH 6.0 and was found to be 98% (for dead biomass). As the pH increases from pH 2.0 to pH 6.0, there was an increase in the removal efficiency and after reaching pH 6.0 (optimum) it started to decline. (Visoottiviseth and Panviroj, 2001; Jaglan et

al., 2020). The cell wall of *A.* species contains a large number of surface functional groups. Biomass has live sites capable of binding metal ions and such bond formation could be done by displacement of protons which can be determined by pH (Marandi, 2011). Positively charged metal ion uptake was increased because the charge of the fungal surface becomes negative under high pH values such as 6 (Allaboun and Abu Al-Rub, 2008). For live biomass, maximum biosorption efficiency was observed at pH 2 (93%) and then gradually decreased with increase in pH level. The percentage removal of Chromium was maximum at pH 2.0 and was found to be 89% and 85% for *A. oryzae* and *A. sojae* respectively (Reya et al., 2012).

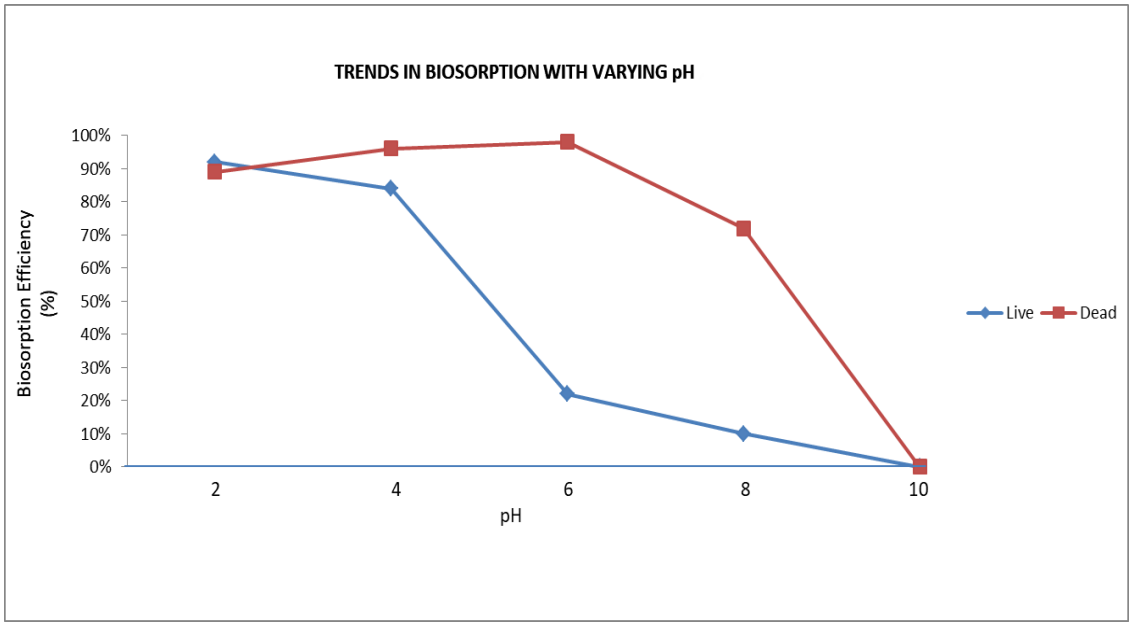

Figure 03. Trends in biosorption with varying pH

5.4 Effect of contact time (determined with both dead and live biomass)

The effect of contact time using dead *A. nomius* biomass was studied and results are shown in Figure 4. For dead biomass, initially there was a gradual increase in the adsorption efficiency as contact time increased followed by a decrease in efficiency after 24 hours. The percentage removal was around 98% and 90% respectively for durations of 24 hours and 48 hours. As time passed, the metal uptake by the adsorbent surface slowed down, due to the competition for decreasing availability of live sites occupied by the metal ions remaining in the solution.

Santhi and Guru reported synonymous results where they recorded maximum biosorption at 24 hours, using *A. niger* biomass as the biosorbent (Santhi and Guru, 2014).For live biomass, initially there was a gradual increase in the adsorption efficiency as contact time increased with the highest biosorption efficiency of 84% observed after 48 hours and further change was not observed, similarly to what has been reported by Silah and Gul (2017).

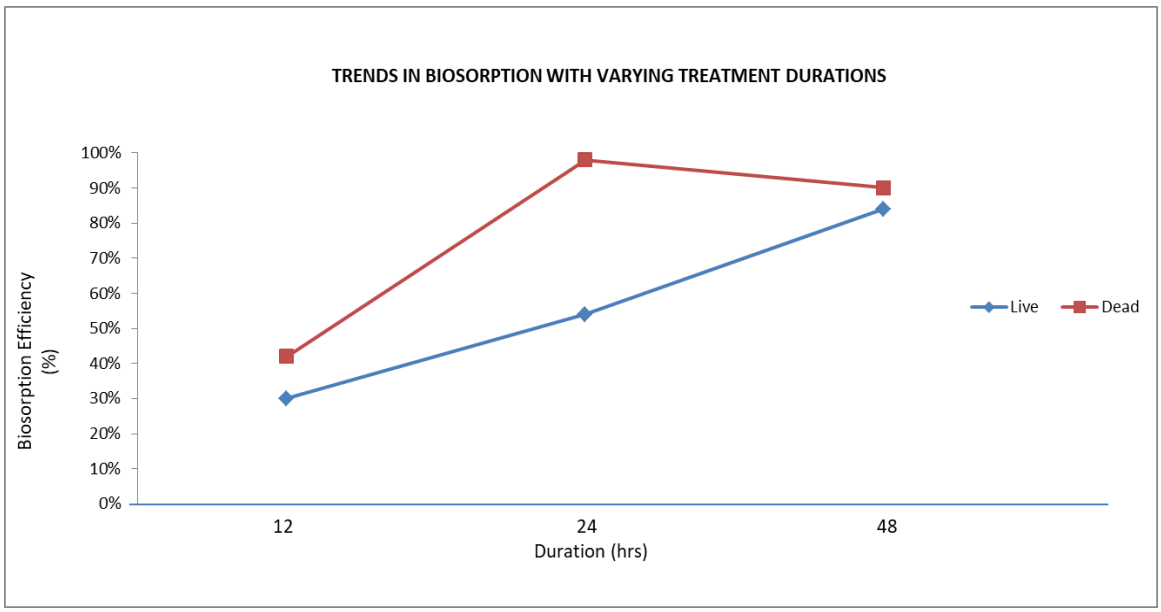

Figure 04. Trends in biosorption with varying treatment durations

5.5 Effect of biosorbent dosage (determined with both dead and live biomass)

The biosorbent dosage is also one of the significant factors to be considered for effective biosorption. It determines the sorbent/sorbate equilibrium of the system. Chromium biosorption with varying biosorbent loads is shown in Figure 05. From the figure it is observed that as the concentration of biosorbent increases from 0.5 g to 2 g there is increase in the percentage removal, which may be due to the increase in the number of binding sites on the biomass, with a maximum biosorption of 86% using 2 g live biomass. Santhi and Guru reported similar results where they used *A. niger* biomass as the biosorbent (Santhi and Guru, 2014).For the dead biomass, maximum biosorption efficiency was observed with 0.5 g. In many instances, lower biosorbent dosages yield higher uptake (Vijayaraghavan et al., 2006).

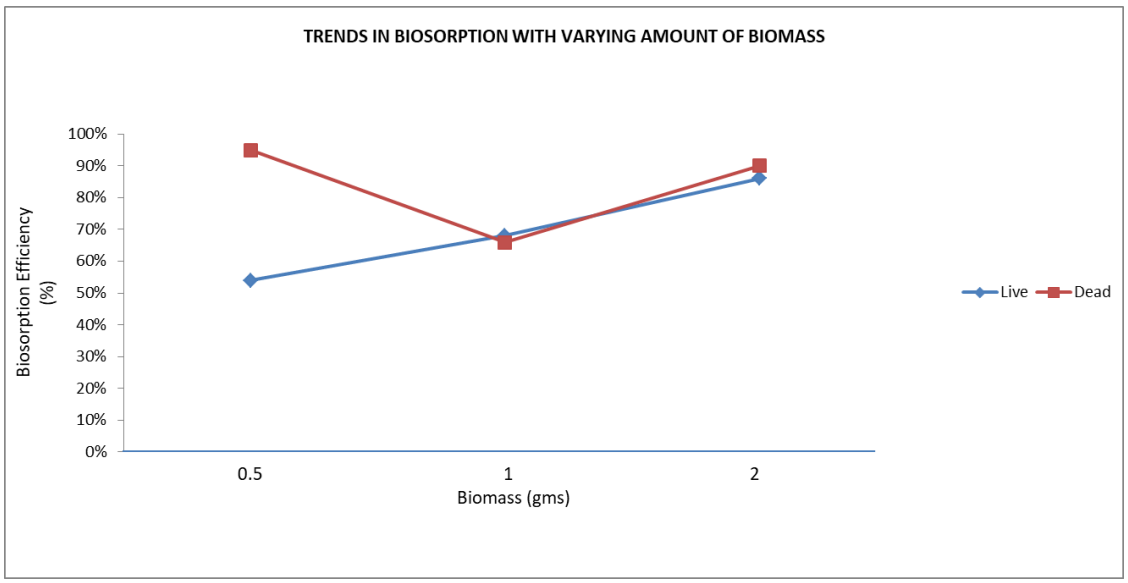

Figure 05. Trends in biosorption with varying amount of biomass

5.6 Effect of temperature (determined with both dead and live biomass)

Metal adsorption to biomass maybe manipulated by temperature variations. Results are shown in Figure 06. The percentage removal of Chromium was maximum at 25° C (96%) and gradually decreased with increasing temperature, as for exothermic nature of the adsorption process, with a minimum at 45° C (Mamaeri et al., 1999; El-Gendy et al., 2017).Temperature affects the cell wall stability components, configuration, and ionization of chemical moieties and energy-independent mechanisms are likely to be affected due to temperature changes since the process responsible for removal is largely dependent on the physiochemical characteristic of the medium (Hassouna et al., 2018). Santhi and Guru (2014) reported similar results where they achieved maximum biosorption at 27° C, using *A. niger* biomass as the biosorbent. For live biomass, initially, there was a gradual increase in the adsorption efficiency with increasing temperature and the highest biosorption efficiency of 84% was recorded at 35° C.

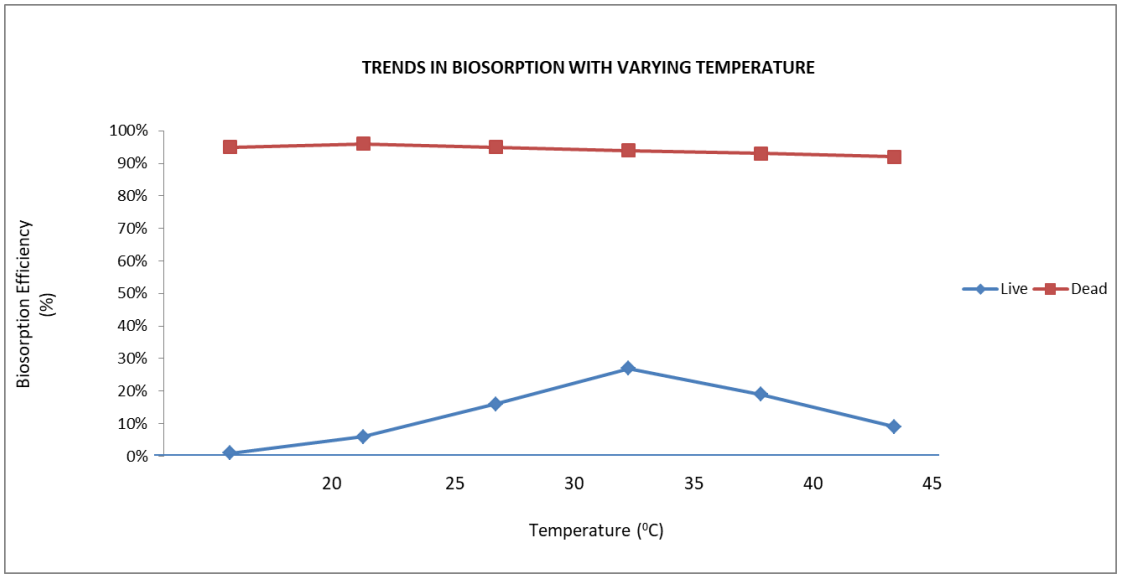

Figure 06. Trends in biosorption with varying temperature

5.7 Effect of initial metal ion concentration (determined with both dead and live biomass)

The effect of initial metal ion concentration on biosorption of Cr (+6) is shown in Figure 07. The percentage removal of Chromium was around 95% to 72.8% with initial metal ion concentrations from 1 mg/L to 5 mg/L respectively. The percentage removal decreased as the concentration increased and this may be due to saturation of live sites (Saleh et al., 2010). After attaining the optimum concentration, the efficiency decreased due to an increase in the metal dose which may be beyond the toxic threshold of the fungus. Similar result was obtained by Jaglan et al. (2020). Santhi and Guru (2014) also reported synonymous results with *A. niger* biomass where they also achieved maximum biosorption at 1 mg/L Cr (VI) concentration which gradually decreased with increase in the metal concentrations tested. Similarly, when live biomass was used there was a gradual decrease in the adsorption efficiency with increasing metal concentration. The highest efficiency was noted at Chromium concentration of 1 mg/L.

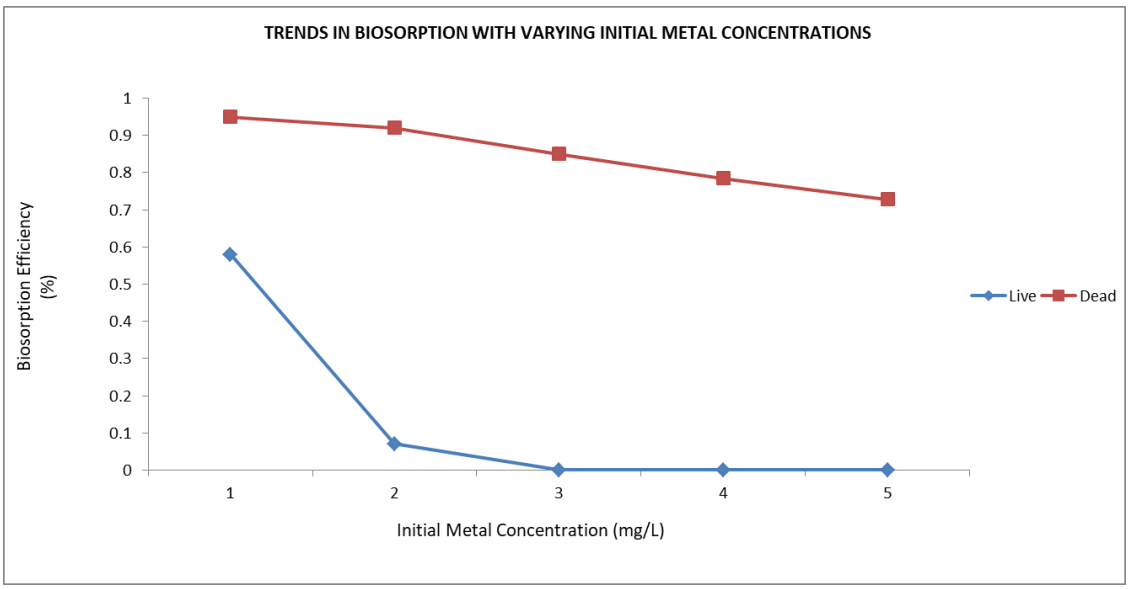

Figure 07. Trends in biosorption with varying initial metal concentrations

5.8 Effect of volume differences (keeping metal concentration constant) (determined with both dead and live biomass)

Metal adsorption to biomass maybe manipulated by variations in solution volumes. Results are shown in Figure 8. The percentage removal of Chromium was maximum when 250 ml of solution volume was taken (95%) and gradually decreased with decreasing volumes. This may be due to the unavailability of space for optimal interactions with the biomass. Similarly, for live biomass, the percentage removal of Chromium was maximum when 250 ml of solution volume was taken (69%).

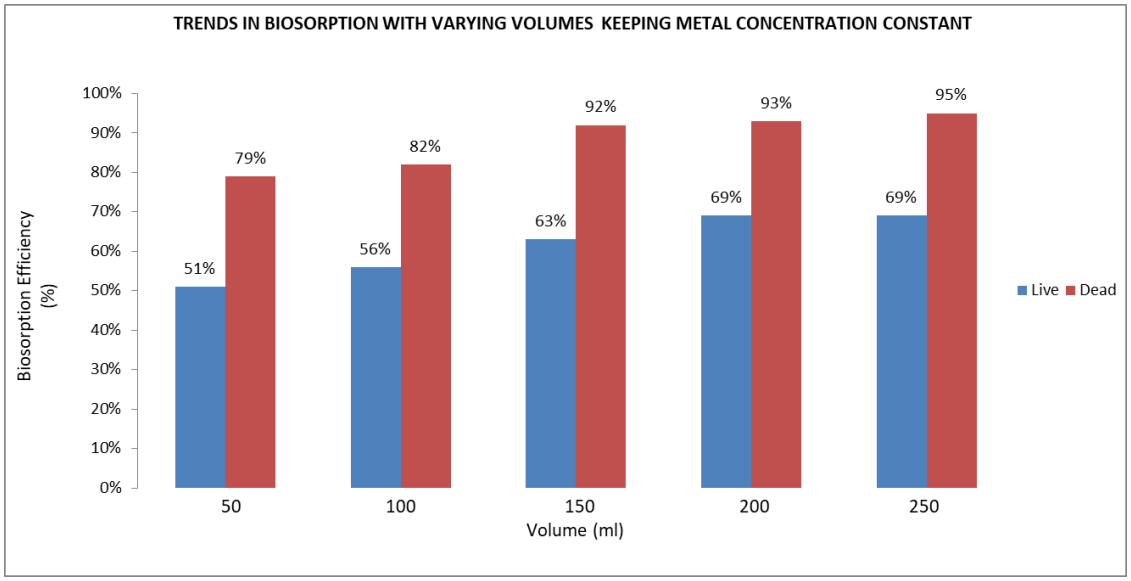

Figure 08. Trends in biosorption with varying volumes keeping metal concentration constant

5.9 Effect of varying combinations of biomass load and volume (determined with both dead and live biomass)

The combined effect of biomass loading and volume differences were studied for Chromium removal and results are shown in Figure 09. It was observed that the most effective combination was 0.5 g of biomass in 100 ml aqueous solution with an efficiency of 98%. For live biomass, the most effective combination was 1.5 g of biomass in 300 ml aqueous solution with an efficiency of 63%.

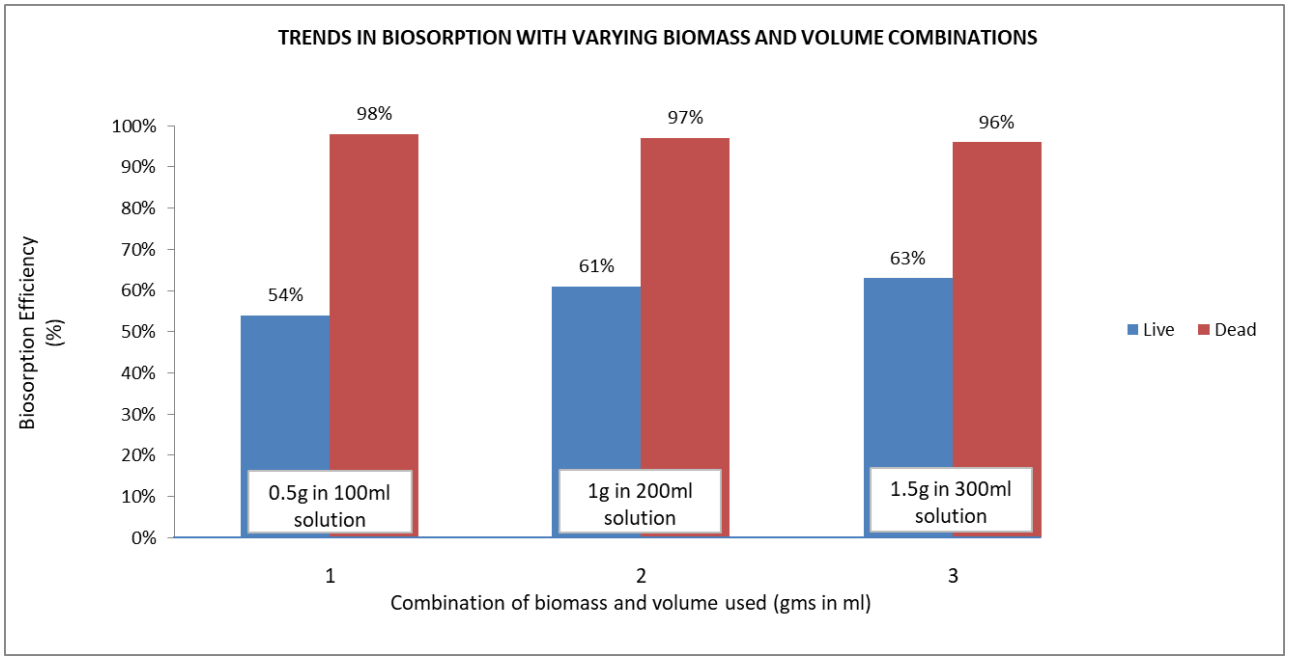

Figure 09. Trends in biosorption with varying biomass and volume combinations

6. Discussion

The results show that the hexavalent Chromium can be effectively removed up to 82% from an aqueous solution using sulfuric acid as the pre-treatment/inactivating agent using *A. nomius* biomass at 37° C. The results also showed that as the concentration of the sulfuric acid was increased, the percent removal was also found to increase linearly with a maximum of 96.5% at 1N. The effect of pH on biosorption efficiency was different between the dead and live biomass. For the dead biomass the pH optimum of the Chromium solution was 6 while it was 2 for the live biomass. This may be, since treatment of the dead biomass with sulphuric acid might had already exposed or modified the cell wall structure which did not require any further modification for efficient biosorption and maximum efficiency was obtained near the neutral range. However, the live biomass which did not undergo any such treatment might have undergone some modification in its cell wall structure at acidic pH thereby showing optimum activity at pH 2. Maximum Chromium removal of 86% was obtained using 2 g live *A. nomius* biomass whereas 0.5 g of live biomass was enough to obtain the maximum efficiency. 96% Chromium was removed at 25° C using dead biomass, but, it declined gradually at higher temperatures, whereas, maximum removal of about 84% was obtained when live biomass was used for biosorption and it took place at 35° C. Maximum Cr (VI) removal of about 95% was obtained when dead biomass was used and initially the concentration of Cr (VI) was 1 mg/L and declined gradually at higher concentrations. For the live biomass also the maximum Cr (VI) removal was obtained at 1 mg/L concentration. It was also found that 0.5 g biomass (dead) in 100 ml, 1 mg/L solution, was optimum for Cr (VI) removal at pH 6.0 and 37° C. While for live biomass, maximum Cr (VI) biosorption of 63% was obtained when 1.5 g of it was used in 300 ml at pH 2 and 1 mg/L of initial Chromium concentration. The mechanism of cell surface sorption of heavy metal by dead biomass includes many processes like physical adsorption, ion exchange, chelation, electrostatic interactions, and metal ion complexation (Sharma et al., 2016) whereas biosorption by live biomass is an active process whereby uptake of heavy

metals requires the metabolic activity of living organisms such as biomineralization, biotransformation, bioprecipitation, and bioaccumulation (Khan et al., 2019). High environmental resistance, greater toxicity tolerance, no need for nutrition, maintenance, and storage of the biosorbents for long periods without any adverse effects are some definite bonus advantages of using dead biomass (Javanbakht et al., 2014). Metal uptake level of dead cell fungal has been observed to be greater than living cell, based on pretreatment methods that favorably exposes or modifies the functional groups on the cell surface (Kapoor, 1998).

7. Conclusion

From the above observations it can be easily concluded, that dead biomass although is certainly superior to live biomass in biosorbing Cr (VI) in many aspects as for the different parameters used but the live biomass is also moderately capable of removing hexavalent Chromium from solutions. Similar observations have been reported by Dhankhar and Hooda (2011), Kapoor (1998). So, this study shows the application of biosorption using natural, abundant and cheap microbial biomass in the removal of metal ions from waste water and offers a high potential for large-scale exploitation.

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