

Biosorption an innovative tool for bioremediation of metal-contaminated municipal solid waste leachate: optimization and mechanisms exploration

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Abstract Municipal solid waste poses a risk on surrounding environment and public health, mainly because of unscientific disposal and shortage of facilities for proper handling and recycling of leachate. This research article objective is to pinpoint the indigenous fungal isolates of waste leachate samples. Therefore, we carried out biosorption of Cd²⁺ tested the applicability by applying indigenous fungal isolates. The limited number of fungal isolates was found based on their ability for biosorption of Cd²⁺ metal. The fungal strains *Trichoderma* sp., *Aspergillus niger* and *Aspergillus flavus* were reported as potential strains for metal exclusion ability from the leachate. Among them, the *Trichoderma* sp. was found as excellent fungal agent for Cd²⁺ absorption. The optimum pH was 5.5 ± 1, temperature 45 °C, and spore concentration 10⁻⁵ to achieve the maximum biosorption, and 35 days of incubation period were required by three strains. The maximum metal biosorption achieved was comparable

for the three isolates: 56.34% by *Trichoderma* sp., 44.74 and 42.04% by *A. niger* and *A. flavus*, respectively. Concluding, the further intending application to identified potentially fungal isolates is able to improve the efficiency of metal biosorption. These strains are recommended for development of consortia could become a best technique for MSW leachate treatment if its reliability and applicability should be verified prior to technology acceptance.

Keywords Solid waste · Leachate · Cadmium metal · Biosorption · Fungi

Introduction

The pure water, clean air and uncontaminated soil are three natural resources that we cannot live without. However, today the growing population with its increased needs has enlarged several industrial expansions (Saravanan et al. 2014a, 2015a, b, c). This has led to the rise in environmental pollution. The results are an increase in toxic elements in air, soil and water resources. Once these contaminants especially heavy metals enter the environment, they are very hard to eliminate (Gupta et al. 2012; Saravanan et al. 2013a, 2014b).

Presently, management of municipal solid waste (MSW) is a critical issue in worldwide and be able to risky both the environment and human health in both developed and less developed countries (Worell and Vesilind 2012; Cheng and Hu 2010; Li et al. 2016). Particularly, in most developing countries, the open disposal practices are still carried out for solid wastes generated from municipal cities, commercial waste, sometime hospitals waste and hazardous waste (Shekdar 2009; Gupta et al. 2015; Tang and Steenari 2016). Owing to its profitable benefits, open dumping is still the best leading handling method used in these less

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developed countries for MSW. The global waste generation estimated 1.7–1.9 billion metric tons (Mt) of MSW/year. According to the World Bank, it reported that the common practices for MSW management are composting or recycling, landfilling, incineration, open dumping. The generation rate of MSW was 0.34 per capita (kg/capita/day) and 109,589 tonnes/day in 2005, and it is expected to rise up to 0.7 per capita (kg/capita/day) and 376,639 tonnes/day in 2025 (Hoorweg and Tata 2012).

The MSW leachate is generated due to microbial degradation of MSW, including chemical reaction and physical changes inside the solid waste (Renou et al. 2008; Akgul et al. 2013; Li et al. 2016). MSW leachate is a highly polluted effluent that contains a complex mixture of both inorganic and organic compounds, heavy metals, ammonia, inorganic salts, etc (Cecen and Aktas 2004). Cadmium (Cd) is a non-essential toxic heavy metal with extensively identified environmental and health risks. Many researchers highlighted that the risk caused by Cd could rise as a consequence of its anthropogenic activities. Spreading of Cd emissions from municipal solid waste treatment of Cd-containing products could lead to a rise in its environmental concentration, and hence the decrease in Cd concentration is a main issue (Ono 2013). However, the composition of leachate can be different and depend on type of waste, climatic condition, pattern of rainfall, hydrological factors and age of disposed waste (Akgul et al. 2013; Ghosh et al. 2015; Hassan et al. 2016). Therefore, untreated and discharge of leachate from MSW may possibly pollute the receiving medium both soil and water systems.

The conventional approach is effective, but not eco-friendly, for the treatment of MSW leachate and, in the same time, technologically expensive (Dursun 2006; Fan et al. 2008; Hermosilla et al. 2009). Although several chemical and physical methods have been already applied for the leachate treatment (Akgul et al. 2013; Gotvajn and Pavko 2015). Many authors have confirmed the ability of carbons as adsorbents to exclude different pollutants both inorganic and organic species (Saleh and Gupta 2014; Al-Saadi et al. 2013). In this sense, the adsorption is a widely considered approach for the separation of pollutants from effluents. However, still detailed advanced research should be essential on the improvement in efficiency, cost-effectiveness and environmental friendly method (Gupta et al. 2013; Saleh 2011; 2015a, b). However, for reducing the harmful effect of leachate, numerous investigators have carried out different experiments to efficiently treat MSWL.

One of the best ways to exclude the contamination of leachate is by microbial treatment. Researchers have investigated many microbes that utilize waste material to degrade them into smaller forms. Recently, the biosorption

has become the most promising technology owing to their efficiency, comparatively cost-effective and eco-friendly prospective (Das 2010; Razarinah et al. 2014; Saetang and Babel 2010, 2012; Vijayaraghavan and Balasubramanian 2015). Microorganisms are able to survive in adverse environmental conditions because of their potential capability to catch up the contaminants as nutrients through absorptive/adsorptive/accumulative mechanism. Indigenous fungi are promising agent to play an important role in the exclusion of metals. Both fungi and bacteria like, *Aspergillus*, *Bacillus*, *Penicillium*, *Phanerochaete*, *Pseudomonas* and *Sporophyticus* are reported as very suitable for the biosorption of heavy metals, e.g., chromium and nickel (Abd El Hameed et al. 2015; Munoz et al. 2012). Fungal cell walls principally comprised of polysaccharides, proteins and lipids with several functional groups that are acting in metals binding action. Many researchers reported different fungal biosorbents, e.g., *Penicillium*, *Trametes versicolor*, *Lentinus sajorajuc*, *Rhizopus arrhizus*, *Rhizopus oryzae*, *Aspergillus oryzae*, *Aspergillus niger* and *Mucor rouxii* have been applied for the heavy metal removal from sample (Yan and Viraraghavan 2000, 2003). For instance, *T. versicolor* is a basidiomycete fungus has various functional groups responsible for the heavy metals biosorption. In addition, this biosorbent is economically, environment friendly and easily available (Subbaiah et al. 2011).

Dhankhar and Hooda (2011) and Zafar et al. (2007) had suggested that the use of fungi is more convenient, such as smooth handling and maintenance, higher metal uptake capacity with high treatment rate, minimal sludge production and selectivity, needs very less technical support, and highest capabilities for reusability. Hence, if we employ the indigenous fungus for removing heavy metals from MSW leachate, this approach will be eco-friendly and cost-effective. Although the mechanism of metal removal through microorganisms is quite complex process due to competition for surface-binding site (Sag and Kutsal 1996; Tunali and Akar 2006). The metal biosorption mechanism depends on combination of chelation, ion exchange, complexation, adsorption, absorption and micro-precipitation (Volesky and Holan 1995; Wang and Chen 2006, 2009; Volesky 2007; Abdolali et al. 2014; Vijayaraghavan and Yun 2008). Ahalya et al. (2003) stated that transportation of metal from outside of cellular membrane into the intracellular accumulation is a metabolism-dependent process by living microbial system. The process is as active defense system by microbes which have higher metal tolerance ability (González-Guerrero et al. 2009). Many authors suggested that these microorganisms, including fungi, yeast, bacteria, are previously reported from metal-contaminated sites due to their continuous enrichment and



extremely adaption ability (Srivastava and Thakur 2006; Parvathi et al. 2007; Gabr et al. 2008; Ansari et al. 2011; Wysocki and Tamas 2010). Ahmad et al. (2006) suggested that the active and inactive biomass of different filamentous fungi, such as *Penicillium*, *Rhizopus*, *Aspergillus* and *Mucor*, may be used in biosorption (Gadd 1990; Kurniati et al. 2014).

Therefore, we have evaluated indigenous fungi from a polluted MSW sites to assess their metal tolerance level and metal exclusion potential from MSW leachate. The present work has been carried between 2013 and 2014 in India.

Materials and methods

Isolation, identification and optimization of conditions for indigenous fungal strains

The MSW leachate samples were collected from dumping site Municipal Corporation, Chhatarpur, MP (Madhya Pradesh) India. All the collected leachate samples were filtered by Whatman filter paper (Grade 42), prior to use in further study. The both (pre-treated and post-treated) leachates were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). The metal concentration founds before and after treatment is presented in Table 1.

The indigenous fungal strains were isolated on potato dextrose agar (PDA) medium, and spore suspensions were prepared as described by Gautam et al. (2012). After desired incubation period, the fungal species were further purified by single spore culture method. These isolates were identified by performing the slide culture technique with the help of standard manuals (Ellis 1976; Domsch et al. 1980; Barnett and Hunter 1998; Gillespie and Pearson 2001). All the fungal isolates were deposited in the Fungal Germplasm Culture Collection Center (FGCC), MP Council of Science and Technology, Department of Biological Sciences, RD University, Jabalpur, and obtained accession number as mentioned serially from FGCC#CH1 to FGCC#CH10 is as listed in supplementary information (SI).

The effects of environmental parameters (e.g., temperature, pH, inoculum concentration, incubation period) on

Table 1 Effect of fungal strains (*Trichoderma* sp. and *Aspergillus* sp.) as bio-remediating agent for cadmium metal

Tank	Before	After
Tank-1	1.16 ± 0.07	0.43 ± 0.03
Tank-2*	1.16 ± 0.07	0.51 ± 0.02

Tank-1: *Trichoderma* sp. and *Tank: *Aspergillus* sp. Unit: mg/L

the metal removal efficiency by potential fungal isolates were considered. The experiments were carried out in a 250-mL sterilized Erlenmeyer flask containing 150 mL broth medium in triplicate. Batch experiments were executed at different pH ranges from 3.0 to 8.0 and also at different temperature range 28 °C ± 1–60 °C ± 1, from 7 days to 49 days of incubation period. The Cd metal concentration in leachate (before and after) was analyzed by using ICP-MS and determined against the control (the leachate sample was not inoculated any fungal spore concentration). The metal removal potential, i.e., the amount of cadmium metal ion mg/g⁻¹ was calculated by using following equation:

$$Q = \frac{[C_i - C_f]v}{s} \quad (1)$$

where Q , mg of metal ion biosorbed per g, C_i , initial metal ions concentration (mg/l), C_f , final metal ion concentration (mg/l), s , wet biomass, and v , volume of reaction mixture.

Statistical analysis

Data presented on the average of three replicates (± SE) were obtained from the independent experiments by using SPSS 15 software.

Results and discussion

Determination of fungal tolerance potential against Cd²⁺ metal

The data revealed that different responses of isolates in terms of Cd²⁺ biosorption are shown in Fig. 1. Out of ten fungal strains, only three isolates have shown highest tolerance to Cd²⁺ metal. The screening test showed that *Trichoderma* sp. (FGCC#CH9), *A. niger* (FGCC#CH1) and *Aspergillus flavus* (FGCC#CH3) have potential biosorption ability for Cd²⁺ (Fig. 1). Similarly, many researchers have investigated about heavy metal biosorption (Blaudez et al. 2000; Massaccesi et al. 2002; Dursun et al. 2003; Liu et al. 2006; Tsekova et al. 2010; George et al. 2012), but it was noted that indigenous fungal strains have more tolerance ability than other isolates (Pandey et al. 2013; Zafar et al. 2007). Additionally, the indigenous fungal strains are well known for their ability to survive in highly metal-contaminated site (Maheswari and Murugesan 2011; Joo and Hussein 2012). On the above concern, similar findings were reported by many authors (Fazil et al. 2015). In addition, Fazil et al. (2015) also advocated that *A. versicolor* is able to accumulate (Cd: 7 mg/1gm) mycelium of *Paecilomyces* sp. (5.878), *Microsporium* (5.07), *Trichoderma* sp. (4.55), respectively. Similarly, several

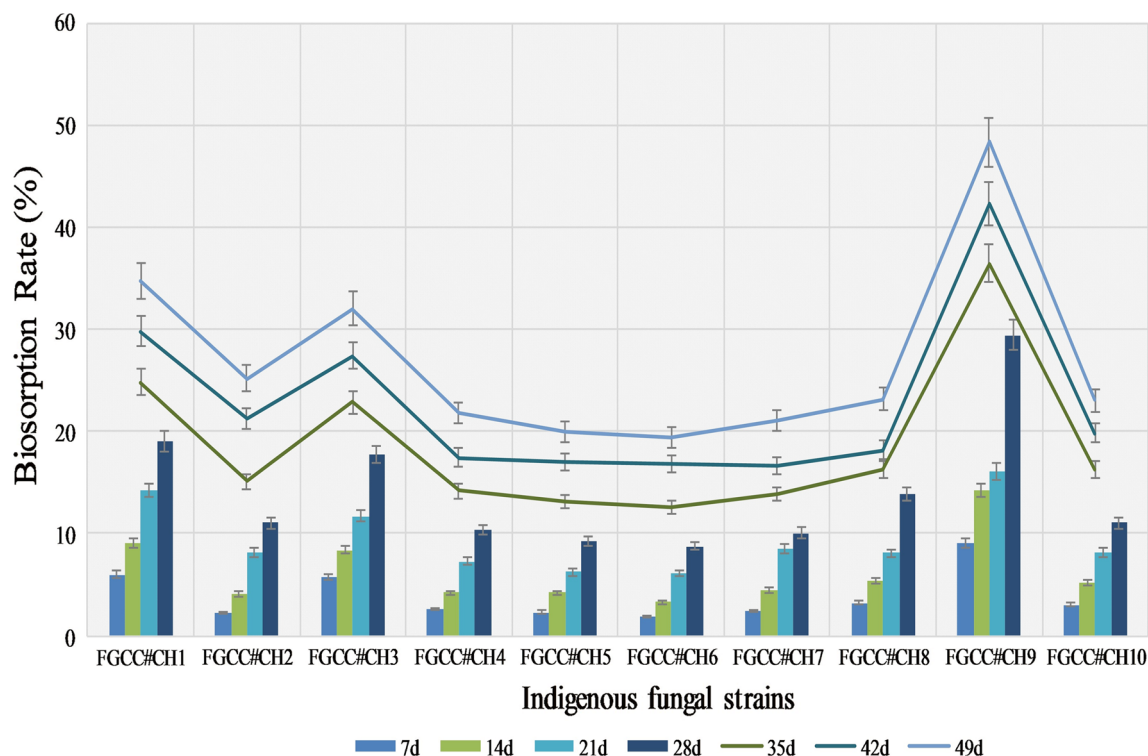


Fig. 1 Metal removal potential for cadmium metal by various indigenous fungal isolates

researchers have also proved the fungal tolerance ability against the different heavy metals (Salinas et al. 2000; Graz et al. 2011; Carrillo et al. 2012).

Fungal exposure to heavy metals can lead to physiological adaptation (Gadd, 1993), and these alterations possibly will be increased the metal sorption capability. Fungi able to grow in the occurrence of heavy metals ($50 \mu\text{g ml}^{-1}$) were recovered. Particularly, fungus belongs to the genera *Alternaria*, *Aspergillus*, *Fusarium*, *Geotrichum*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Monilia* the Mycelia sterilia group, etc. Zafar et al. (2007) stated that the minimum inhibitory concentration (MIC) values advise that the tolerance level against specific metals was dependent on the related fungal stains. The fungal genera *Monilia* and *Geotrichum* exhibited comparatively low tolerance to every metal in contrast to other fungal strains, while the genus *Aspergillus* (two isolates *Aspergillus* sp.1 and *Aspergillus* sp.2) presented a noticeable metal tolerance levels. Among the tolerance level by filamentous fungi was detected in following pattern $\text{Cu} > \text{Cr} > \text{Cd} > \text{Co} > \text{Ni}$. The existence of different fungi—*Aspergillus*, *Chaetomium*, *Fusarium*, *Geomyces*, *Rhizopus*, *Penicillium*, *Paecilomyces*—species in land polluted with heavy metals (Cd, As, Cu, Pb and Zn) has conveyed by many researchers from worldwide (Babich and Stotzky 1985; Gadd 1993). The deviation in the metal resistance might be owing to the occurrence of either one

or more kinds of resistance mechanisms showed by various fungi. In this context, the fungi *Aspergillus* (Deuteromycetes) and *Rhizopus* (Zygomycetes) exhibit different metal tolerance.

Optimization of biosorption conditions for individual fungal isolates

Several microbial agents are able to bind heavy metal; few of them are sufficient for high metal binding ability. In addition, maximum metal biosorption can be obtained through optimization of favorable environmental growth condition (Murugesan et al. 2009).

Effect of pH on metal biosorption

The pH is an important factor in biosorption process because it affects the equilibrium by disturbing the metal ion(s) speciation in solution, the normal chemistry of the active binding sites on the fungal biomass and the concentration of competing hydrogen ions. The fungal cell wall comprises of carboxyl, sulfhydryl, amino, thiol and phosphate functional reactive groups. The phosphate groups and carboxyl groups bring negative charges that permit the fungal cell wall constituents to be potential scavengers of metal ions. The pH factor has been known as one of the most important parameter that is active on metal

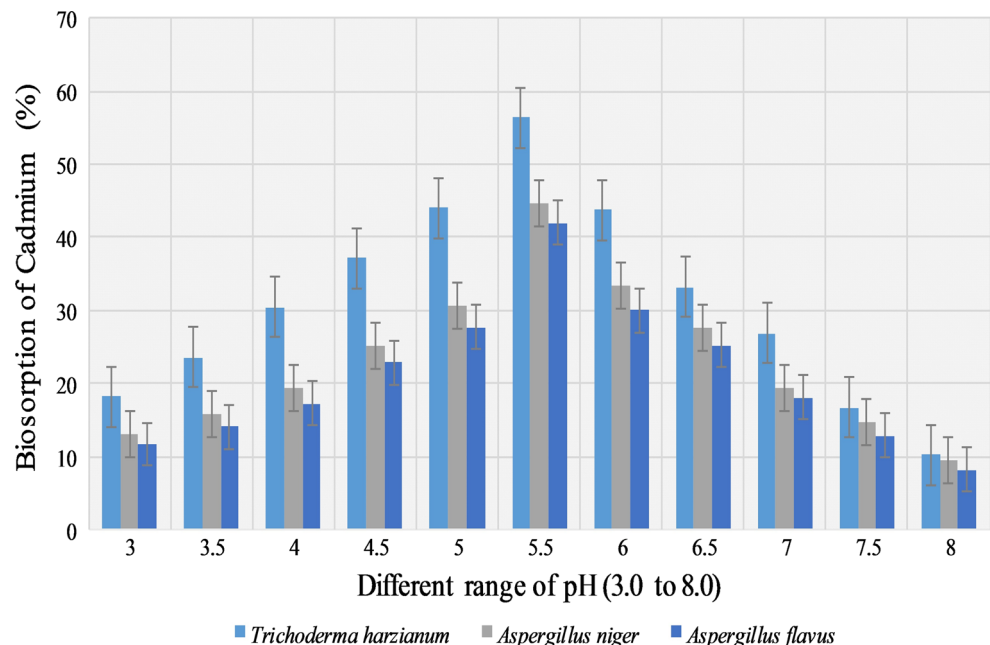
sorption. It is directly associated with competition capability of hydrogen ions with metal ions to active sites on the fungal biosorbent. The effect of pH on the biosorption of Cd metal onto three fungal strains (*Trichoderma* sp.; *A. niger* and *A. flavus*) were studied at pH 3–8, and the results are given in Fig. 2. The biosorption efficiency was better from 18.23 to 56.34% for Cd(II) ion at pH level of 3–5.5. The highest biosorption was observed to be 56.34% for Cd metal at pH 5.5. Thus, the all biosorption tests were carried out at different pH level. Amini et al. (2008) sated that the biosorption mechanisms on the biomass surface reveal the nature of the physicochemical interaction of the species in solution and the biosorptive sites of fungal sorbent. Iqbal and Edyvean (2004) said that at very acidic pH level (pH < 2.0), the surface charge on the active biosorbent sites became positive and metal cations and protons compete for binding sites on cell wall, which results in lower uptake of metal. In another hand, the biosorbent surface was more negatively charged as the pH increased up to 5.5. Dursun (2006) explained the functional groups of the fungal biomass were highly deprotonated and as a result accessible for the metal ions. Mainly, carboxyl, phosphate and amine groups are the key functional groups participated in biosorption of heavy metals (Kapoor and Viraraghavan 1995, 1997). Yan and Viraraghavan (2001) added that during reduction in biosorption rate at higher pH (pH > 5.5) is not individually associated with the formation of soluble hydroxylated complexes of the cadmium ions in form of Cd(OH)₂. In addition, a number of

researches have already described the almost closely related pH effect on biosorption of Cd through diverse fungus (Akar and Tunalı 2005).

The metal biosorption potential can be affected by different environmental parameters, such as pH, which is shown in Fig. 2. The results indicating that the Cd²⁺ removal gradually increased with pH when fungus, such as *Trichoderma* sp. (FGCC#CH9), *A. niger* (FGCC#CH1), and *A. flavus* (FGCC#CH3), are employed, and maximum biosorption reported at pH 5.5 and reduced from 6.0 to 8.0 pH, although, the lower pH (pH < 4) can affect the heavy metal removal efficiency (Fan et al. 2014). Additionally, the metal removal rate also reduces at pH > 6, which might be due to complexation with soluble organic ligands.

The biosorption of metal is extremely related to solution pH because it might cause impact on metals chemistry, activity of functional groups (carboxylate, phosphate and amino groups) on the cell wall, because of competition among metal ions and binding sites (Baldrian 2003). Studies conducted by Liu et al. (2006) suggested that biosorption of metal capacity is too much low at lower pH owing to hydrogen ions competition with metal ions at cell wall site. When the pH increases, subsequently the negative cell surface numbers will increase. On the other hand, those metals involved in higher pH level could cause the inhibition of metal contact between biomass of metals. However, metal precipitates at a higher pH, inhibiting the contact of metal with most fungal biomass (George et al. 2012).

Fig. 2 Influence of diverse pH on cadmium metal removal by potential fungal strains



Effect of temperature on metal biosorption

Temperature is also very important parameter which can amend the whole biosorption development. The data revealed from present study that the Cd^{2+} biosorption rises within temperature range from 28 to 45 °C and afterward rapidly falls from 45 to 60 °C. The highest Cd^{2+} removal (56.34%) was reported at 45 °C when employing *Trichoderma* sp. (FGCC#CH9), as shown in Fig. 3. This study established that temperature plays an important role in the metal removal practice. Similarly, previous reports that deal with microorganism (bacteria: *Bacillus jeotgali* and *E. coli*) suggested that the optimum temperature for Cd^{2+} is not similar to our findings (Green-Ruiz et al. 2008; Kao et al. 2009; Pan et al. 2009). Liu et al. (2006) advocated that metal uptake activity is related to rise in temperature level, owing to higher affinity of sites for metal binding on available cell wall. Sun et al. (2010) estimated that the highest biosorption activity by *Aspergillus terreus* for cadmium metal at temperatures ranges between 25 and 28 °C. Vijayaraghavan and Yeoung (2008) stated that the room temperature is suitable for conducting the biosorption process. Our findings are not getting support from studies conducted by other researchers, indicating that biosorption ability is not very clearly defined, which means needed more detail investigation.

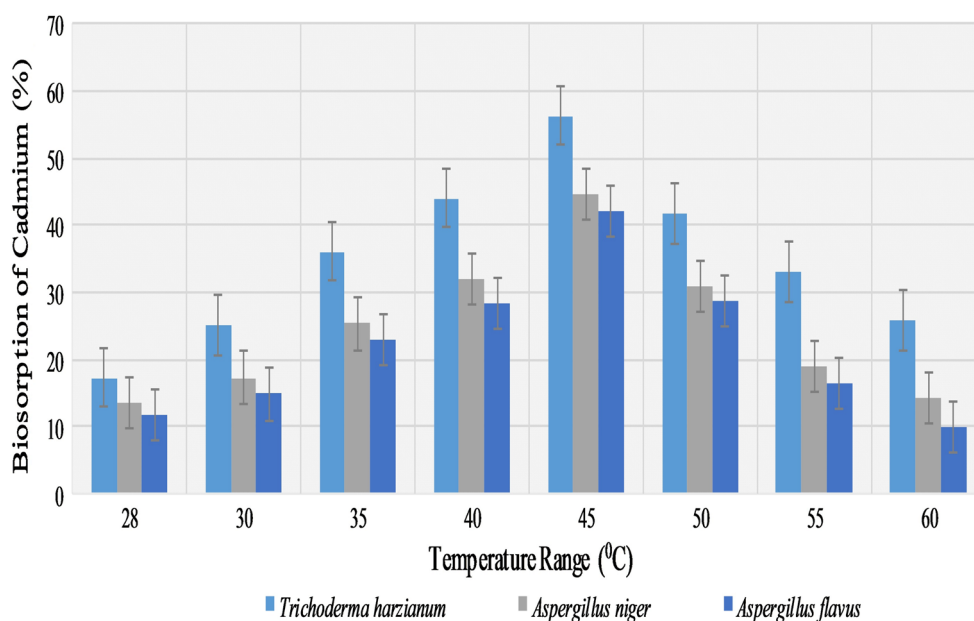
This is well known that the energy cannot be gained or lost under thermodynamically in isolated system; the entropy change is the driving force. In addition to the practice of environmental engineering, both entropy and energy factors ought to be considered in order to define the procedures that occur spontaneously (Fan et al. 2008; Liu

and Liu 2008; Anayurt et al. 2009; Subbaiah et al. 2011). The magnitude of Gibbs free energy change of adsorption (ΔG°) also increased with rise in temperature level which shows that the biosorption was more favorable at higher temperatures. The positive values of enthalpy (ΔH°) indicate the endothermic nature of the biosorption of $\text{Cd}(\text{II})$ ions onto fungus (*T. versicolor*) between temperature ranges of 303–323 K (Subbaiah et al. 2011). These findings are quite similar and also supported by other researchers reports such as *Phanerochaete chrysosporium*—23.0 mg g^{-1} (Say et al. 2001), *Mucor rouxii*—20.31 mg g^{-1} (Yan and Viraraghavan 2003), *Phanerochaete chrysosporium*—15.2 mg g^{-1} (Li et al. 2004). Furthermore, the study conducted by (Fan et al. 2008) suggested that biosorption of $\text{Cd}(\text{II})$ can reach up to 61.35 mg g^{-1} employing *Penicillium simplicissimum*. Zaki et al. (2000) stated that the negative value of ΔG° with a proliferation in temperature shows the $\text{Cd}(\text{II})$ ion adsorption on fungus (*Trichoderma*), which turns into more promising at higher temperature level. Bazrafshan et al. (2016) classified the adsorption mechanism can be possible, either physical adsorption (less than 84 kJ/mol) or chemisorption (between 84 and 420 kJ/mol), through the degree of enthalpy change. Gupta (1998) said that if ΔS° value is positive, few structural alterations occur on the adsorbent.

Effect of inoculum concentration on metal biosorption

The most often recovered fungal isolates are *Aspergillus* sp. followed by *Alternaria* sp., *Curvularia* sp., *Fusarium*

Fig. 3 Influence of different temperature on cadmium metal removal through diverse potential fungal strains



sp., *Mucor* sp., *Rhizopus* sp. and other fungal strains from the leachate sample (SI). The maximum cadmium metal uptake by potential fungus can be represented in following order *Trichoderma* sp. (FGCC#CH9), *A. niger* (FGCC#CH1) and *A. flavus* (FGCC#CH3) which are shown in Fig. 4.

Several authors have reported different fungi, such as *Ganoderma austral*, *A. niger*, *Aspergillus terreus*, *A. lentulus* and *Rhizopus oryzae*, from MSW leachate (George et al. 2012; Kapoor et al. 1999; Mishra and Malik 2014a; 2014b; Razarinah et al. 2014). In this study, the potential fungal isolate belongs to deuteromycetes (*Trichoderma* sp., *A. niger* and *A. flavus*).

Furthermore, during standardization stage, the maximum biosorption of Cd^{2+} metal was achieved by *Trichoderma* sp. (FGCC#CH9) (56.34%) followed by *A. niger* (FGCC#A1), (44.74%) and *A. flavus* (FGCC#CH3) (42.04%) (Figs. 4, 5).

The promising use of fungi in biosorption of Cd^{2+} metal has been reported previously (Fazil et al. 2015; Kumar et al. 2015; Shakya et al. 2015). Several studies showed that heavy metals biosorption based on fungi has great capability owing to their cell wall composed of proteins, lipids, polysaccharides and diverse functional groups that are responsible for the metal binding (Akar and Tunali 2006; Pacheco et al. 2011). Skorik et al. (2010) added that chitin and chitosan (mixed polysaccharides) play important role in metals binding. In this sense, fungi such as *A. niger*

remove metals by spores that act as an excellent biosorbent for metals (Dursun et al. 2003; Yang et al. 2004). The level of Cd^{2+} metal biosorption employing different fungi have been well reported (Veglio and Beolchini 1997; Kapoor and Viraraghavan 1998; Kapoor et al. 1999). However, the findings might be varied in different investigations owing to type of microbes used, inoculum concentration, temperature, pH and type of method used. Yazdani et al. (2010) observed that metal exposure by heavy metal (e.g., Cd) can cause alteration in fungal morphological in all the indigenous fungal strains. Shakya et al. (2015) claimed that fungus forming colorful mycelia owing to admits of heavy metal on media. Jarosz-Wilkolazka et al. (2006) previously argued that supplementation of Cd^{2+} is in growth media able to form an orange brown pigment which is directed to the coloration of mycelium of *Abortiporus biennis*, accompanied with the cell-free culture medium (Jarosz-Wilkolazka et al. 2006).

The interaction of fungi with heavy metals possesses some alterations in the physiological developments and some conditions it can even damage the mycelium. Thus, fungi evolved dynamic defense mechanisms that alleviate the toxicity of metals. The protection is usually based on immobilization of heavy metals expending intracellular and extracellular chelating compounds. In diverse taxonomic groups of fungi, the heavy metals are intracellularly chelated by peptidic low molecular weight compounds—

Fig. 4 Influence of inoculum concentration level on cadmium metal removal by three potential fungal isolates (C1–C10 means is 10^{-1} to 10^{-10})

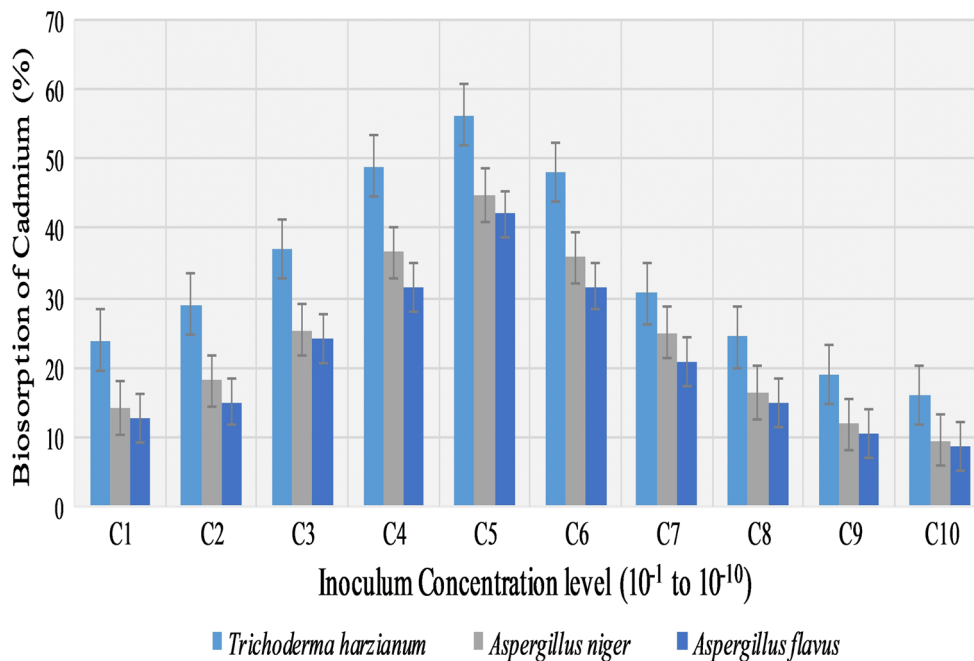
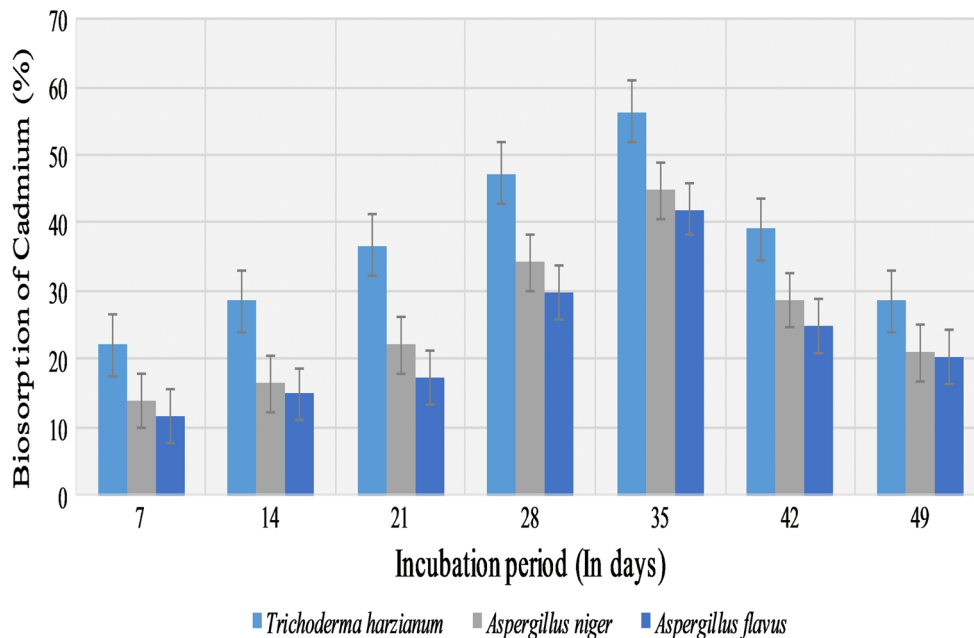


Fig. 5 Influence of incubation period on removal of cadmium metal employ potential fungal strains



phytochelatins or metallothioneins (Tomsett 1993). Even though the production of these low molecular weight chelators was identified in basidiomycetes group of fungi (Munger and Lerch 1985), it appears that their part in wood-rotting fungi is restricted and their production by these fungi was never proven. While in brown-rot and white-rot fungi, the extracellular metal chelation is perhaps significant. Though it is not very clear, this is an active defense method. The metal chelators produced by both brown-rot and white-rot fungi are oxalate. The oxalic acid production by fungi offers a soluble metal ions or complexes as insoluble oxalates, as a result reducing bioavailability and growing tolerance to metals (Sayer and Gadd 1997). Similarly, metal oxalates may be also formed with Cd. Since the oxalate production is more usual by brown-rot fungi, it has a significant role in the white-rot fungi with lignin peroxidase. Mn-peroxidase of *P. chrysosporium* or a low molecular weight compound contributes to the Fenton-based breakdown of wood components. Among white-rot fungi, higher amounts of oxalate are produced by *P. ostreatus*, *P. chrysosporium* and *T. versicolor* (Shimada et al. 1997; Machuca et al. 2001). It is consequently not unexpected that diverse fungal species can change in the degree of their heavy metal tolerance. In this context, Sanglimsuwan et al. (1993) investigated 21 fungal strains of 16 species of wood-rotting fungi for their tolerance to metals and found that minimum inhibitory

concentrations (MIC) were lowest in case of Cd (0.5–5 mM). Hence, the tolerance level varied from one species to other species with *P. cystidosus* and *P. ostreatus* showing the maximum resistant.

Many authors have been discussed biosorption potential of fungi and other biological materials (He and Chen 2014; Kumar et al. 2015). Vijayaraghavan and Balasubramanian (2015) underlined that most of the studies those carried out previously had some drawbacks for metal removal (Al-Garni et al. 2009). In addition, some authors explained that biosorption of metal is related to ion exchange which is one of the main mechanisms for metal exclusion. Fan et al. (2014) stated that the microbes can vary based on type of membrane lipid composition, cell wall composition and cell membrane in different growth phases which may be directly affect their susceptibility to toxic substances.

The application of microorganisms for the biosorption of metals from municipal waste water has been suggested as a promising unconventional way to make heavy metal management strategies. While the clear mechanism of metal uptake and sorption by microorganisms is still not fully understood, the sorption to polysaccharides, proteins or other molecules happening in the outer layer of the cell wall undoubtedly plays the most significant role. The tests with chemically altered cell walls confirmed that different functional groups may contribute to cation binding. The heavy metals adsorption on the fungal mycelia fits the

Langmuir adsorption isotherm. From the kinetic viewpoint, it is a two-stage process with a rapid surface adsorption with a slow intracellular diffusion. At the starting time of interaction, pH declines owing to the release of protons. The ion exchange mechanism also plays an important role in metal binding. The fungal cell wall has the significant role in sorption of heavy metals and accounted between 38 and 77% of metal uptake. In addition, the heavy metal binding ability is also dependent on the age of fungal mycelia, composition of culture media. The overall characters are possibly owing to the changes in composition of fungal cell wall. Furthermore, the metal binding may be affected by physical or chemical treatment of mycelia (Yetis et al. 2000; Wu and Li 2002).

Saleh et al. (2015) evaluated the efficiency of activated carbon loaded with zinc oxide nanoparticles (AC/ZnO) for eliminating carbon tetrachloride, dichloromethane, trichloro methane from aqueous solutions. For this experiment, the thermo-chemical process was used for the production of activated carbon (AC) from the waste tires as a raw material. The removal of these pollutants by AC/ZnO was defined well by the pseudo-second-order model, and the hydrophilic fraction adsorption fitted the intra particle diffusion model. The reusability of the composite was evidenced when no substantial reduction in its adsorption ability was witnessed even subsequently numerous times of regeneration. In similar context, Saleh (2016) tested nanocomposite of carbon nanotubes/silica nanoparticles and their application sorption mechanism for adsorption of metal Pb(II) from surface properties. Saleh and Gupta (2012a) said that the MnO₂/CNT nanocomposite is utilizable as a fixed into bed in a column system. The experimental situations were examined and also the optimized pH range among 3–7 was studied and the best results was reported at pH was identical to 6 and 7. Saleh and Gupta (2012b) stated a composite of multi-walled carbon nanotube/titanium dioxide (MWCNT/TiO₂) has been produced in order to hybridize photocatalytic activity of TiO₂ with adsorptivity of MWCNTs. This catalytic composite was used by degradation of methyl orange taken as a model compound, under UV irradiation. The outcomes determined appearance that the application of carbon nanotube

as a backing for TiO₂ is good for higher degradation level of methyl orange dye in aqueous solutions (Saravanan et al. 2011, 2013b, c, d, e; Joicy et al. 2014). Saleh and Gupta (2011) added that the use of sunlight-induced photocatalytic degradation process is simple way with easy safe handling, and cost-effective, consequently has the higher potential to be an advantageous technology for environmental decontamination. The complex of MWCNT/MnO₂ was effectively produced that the material has an exceptional characteristic that syndicates the oxidation properties of MnO₂ with adsorption features of MWCNTs. The findings suggested that the composite has shown high effectiveness for exclusion of As(III) and As(V) and it was observed that the produced composite of MWCNT/MnO₂ is an useful oxidizing agent with adsorbent for the decontaminating of sample containing As(III) and As(V) (Saleh et al. 2011). Saravanan et al. (2015d) stated that the developed nanocomposites are between the supreme efficient catalysts in effluent treatment and in the degradation of industrial pollutants. In addition, the low-cost synthesis, maximum efficiency and sensitivity create the catalysts an appropriate material for the pollutant degradation as well as allow us to transfer a step additional toward a greener environment (Saravanan et al. 2014b, c). Saravanan et al. (2011) suggested that the photocatalytic activity of ZnO/CdO was verified through the decomposition of methylene blue (MB) in aqueous medium under visible light as well as the effectiveness of the catalyst has been evaluated in fact. In addition, the used method is very simple, rapid and cost-effective, while other methods are not.

It is understandable that the interference of heavy metals with enzymatic, physiological and reproductive processes of fungi has its ecological significances. The restrictions in growth in the existence of metals lead to the alterations of community structure and the heavy metals effects on their enzymatic actions effects the energy change in the environment. The amino, hydroxyl amide and carboxyl groups of the biosorbent played as the adsorption sites for metal (Fig. 6). This inference also clarified the effect of solution pH on biosorption ability. Low pH was favorable for protonation of the biosorbent's functional groups and hydrogen bond formation which resulting in the observed

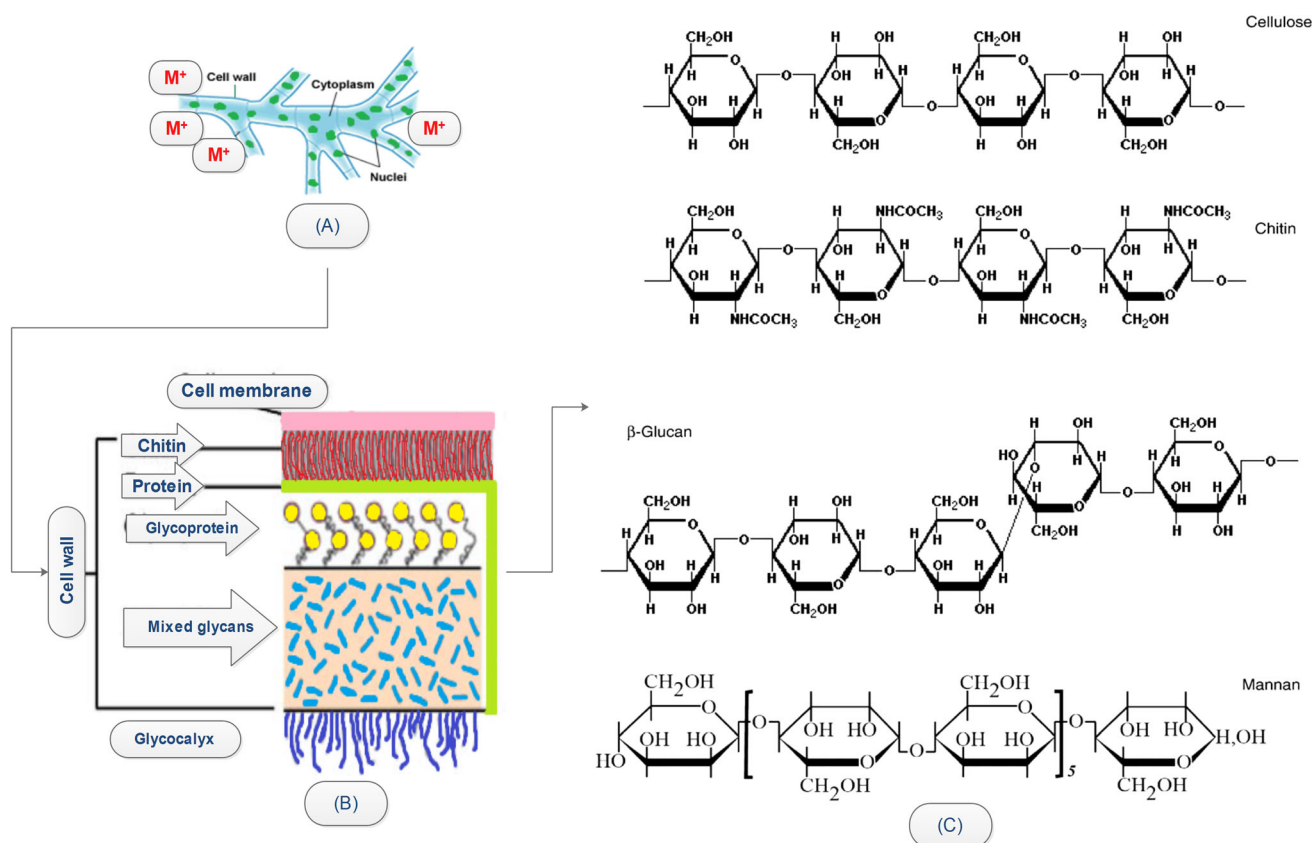


Fig. 6 Systematic structure Glycocalyx. (A + B): Cross section through the tip of a fungal cell shows the general structure of the cell wall and other features; (C): Structures of cellulose, chitin, glucan and manna (Wang and Chen 2009)

increase in metal biosorption. In addition, other feeble intermolecular forces via hydrophobic–hydrophobic and van der Waals interactions may also take part in the metal biosorption.

Conclusion

In this research, we have validated that some of the indigenous fungal strains grown well and also have an excellent Cd^{2+} biosorption. Cadmium tolerant isolates, especially *Trichoderma* sp., *A. niger* and *A. flavus*, can survive well. According to the literature discussed in this article, we can have assumed that intracellular compartmentalization is critical to the detoxification of Cd^{2+} . Most of the cadmium is stored in the cytoplasm, either in cytosol or vacuoles. Our work has demonstrated that the indigenous fungal isolates have a great potential for biosorption of metal from MSW leachate. The further work is to see how this investigated work can be scale up to a larger scale.

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