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Removal of zinc by live, dead, and dried biomass of *Fusarium* spp. isolated from the abandoned-metal mine in South Korea and its perspective of producing nanocrystals

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ABSTRACT

Bioremediation is an innovative and alternative technology to remove heavy metal pollutants from aqueous solution using biomass from various microorganisms like algae, fungi and bacteria. In this study biosorption of zinc onto live, dead and dried biomass of *Fusarium* spp. was investigated as a function of initial zinc(II) concentration, pH, temperature, agitation and inoculum volume. It was observed that dried, dead and live biomass efficiently removed zinc at 60 min at an initial pH of 6.0 ± 0.3. Temperature of 40 °C was optimum at agitation speed of 150 or 200 rpm. The initial metal concentration (10–320 mg L^{-1}) significantly influenced the biosorption of the fungi. Overall, biosorption was high with 30–60% by dried, live and dead biomass. In addition to this, the potential of *Fusarium* spp. to produce zinc nanocrystals was determined by transmission electron microscopy, energy-dispersive spectroscopy, X-ray diffraction and fourier transform infrared spectroscopy, which showed that dead biomass was not significantly involved in production of zinc nanocrystals.

1. Introduction

Mining leads to soil erosion and environmental contamination as a result of waste generation during the extraction, beneficiation, and processing of minerals. According to recent survey, about 900 abandoned-metal mines are disseminated in South Korea, and the waste materials, largely tailings, in most of these abandoned mines have been left uncovered [1]. Under these conditions, it is likely that the mine wastes will quickly be oxidized, and ultimately be eroded away. In fact, many tailing impoundments failed during rainy seasons and contaminated adjacent streams and rivers. According to the acid–base accounting for 50 tailings taken from several abandoned mines, the average neutralization potential (NP) and the acid potential (AP) of the mines have been reported as 20.71 kg $CaCO_3$ t^{-1} and 163.86 kg $CaCO_3$ t^{-1}, respectively [2]. These results indicate that lots of tailings have a strong potential to release acid leachate. Speciation of some metals, such as Fe, Mn, Al, Zn, As, Cd, Cu and Pb in some tailings was in soluble state with high level of concentration and it leads to discharge of acidic leachate with heavy metals and contaminated the environment around. Due to these reasons, abandoned-metal mines have been treated as a pollution source and, in order to prevent further mine pollution, the Government of Korea and local autonomous entities have carried out rehabilitation works for mine wastes from the middle of 1990s. The total budget for prevention of environmental pollution for 936 abandoned-metal mines in South Korea, reached up to 557.3 million dollars from 2007 to 2011, and this project will presumably be continued by Establishment of Mine Damage Prevention Center, South Korea. The major methods for rehabilitation works include construction of dams for restoration of metal contamination by solidification and stabilization technology for abandoned mine tailings, capping, vertical grouting and encapsulation, varied for different situation of abandoned mines. The gross volume of rehabilitated mine wastes ranged mostly from 30,000 to 200,000 m^3 [2]. It is acknowledged that systematic guidelines for restoration works based on characteristics of mine wastes should be provided for more efficient and safer rehabilitation [2]. To conquer this quandary, many chemical processes are earlier tried and being tried like coagulation–sedimentation, membrane process and surface modified substrate like zeolite. Perusal of literature shows the disadvantage of these conventional processes such as sedimentation, generation of secondary wastes and regular renewal [3].

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Alternative methods for heavy metal removal and recovery by bioremediation have received increased attention in recent years due to their potential application in environmental protection of which microbiological processes are of great significance [4–6]. Among the microorganisms studied, fungi have been studied extensively, partly because of the wide range of morphological types they possess and the availability of large quantities of fungal biomass and products derived from industrial processes and fermentations [7,8]. The mechanisms of uptake may include simple physico-chemical binding upon cellular components and extracellular molecules, and metabolism dependent intracellular transport [9]. Although fungi are a large and diverse group of eukaryotic microorganisms, three groups of fungi have major practical importance: the molds, yeasts and mushrooms. Filamentous fungi and yeasts have been observed in many instances to bind metallic elements. Fungal cells, live or dead, possess a remarkable ability for biosorption of toxic and precious metals [10]. *Fusarium* spp. represents an economically important group of fungi e.g., *Fusarium stilboides*, *Fusarium solani*, *Fusarium xylarioides* and *Fusarium oxysporum* [11]. However, information on the impact of heavy metals under different environmental factors on sensitivity, tolerance and biosorption of heavy metals to *Fusarium* spp. was limited. Therefore, in the present investigation, the potential of *Fusarium* biomass for zinc(II) removal as well as the influence of the physico-chemical factors on biosorption were evaluated. There are some earlier reports, which support our present investigations [12–15]. In addition, the potential of *Fusarium* spp. to produce zinc nanocrystals was also investigated.

2. Materials and methods

2.1. Apparatus and glassware

All the experiments were performed in sterile 250-mL Erlenmeyer flasks. Cleaning of all glassware's was done using ultrasonic bath with thermostatic temperature controller (Mujigae, South Korea). Chemicals used in this experiment were of analytical grade. Nanopure purified water (conductivity = 18 μΩ/m, TOC < 3 ppb, Barnstead, Waltham, MA, USA) was used for the preparation of reagents. Stock solutions for zinc were prepared by dissolving 2.085 g L^{-1} of $ZnSO_4 \cdot 7H_2O$ (Junsci Chemicals Co., Ltd., Japan). Working concentrations of zinc (100 mg L^{-1}) were prepared by dilution from the stock solution. Zinc concentration in the samples was measured using inductively coupled plasma (ICP) (Leemans Labs, Inc., USA).

2.2. Sample collection and isolation of fungi

Zinc-contaminated tailing soil samples were collected from 40 abandoned mine areas in South Korea. The fungi used in this study were obtained from a mine named as SKM (Fig. 1). The samples were carefully collected from each mine within a depth of 30 cm from the surface of the tailing-dumping area, and the samples were processed within 18 h. One gram of the soil sample was serially diluted and the appropriate (10^{-4}) dilution of the sample was plated in potato dextrose agar (PDA-Difco, France) media by pour plate technique. The Petri dishes were incubated at 27 °C for 5 d. Morphologically distinct fungi were purified and stored at 4 °C for subsequent studies.

2.3. Minimal inhibitory concentration (MIC)

Tolerance to heavy metals was determined as the minimum inhibitory concentration (MIC) against the test fungi. Potato dextrose agar (PDA) was prepared and amended with various amounts of heavy metals ($CdCl_2$, $CuSO_4$, $NiCl_2$ and $ZnCl_2$) to achieve the desired concentration ranging from 50, 100, 200, 400, 800, 1600, 3200 and 6400 mg L^{-1}. Each plate was subdivided into three equal sectors and an inoculum of test fungi was transferred in triplicate on metal incorporated plate and control plates (plates without metal). The plates were incubated at 29 °C for 5 d to observe the growth of fungi on the spotted area [16].

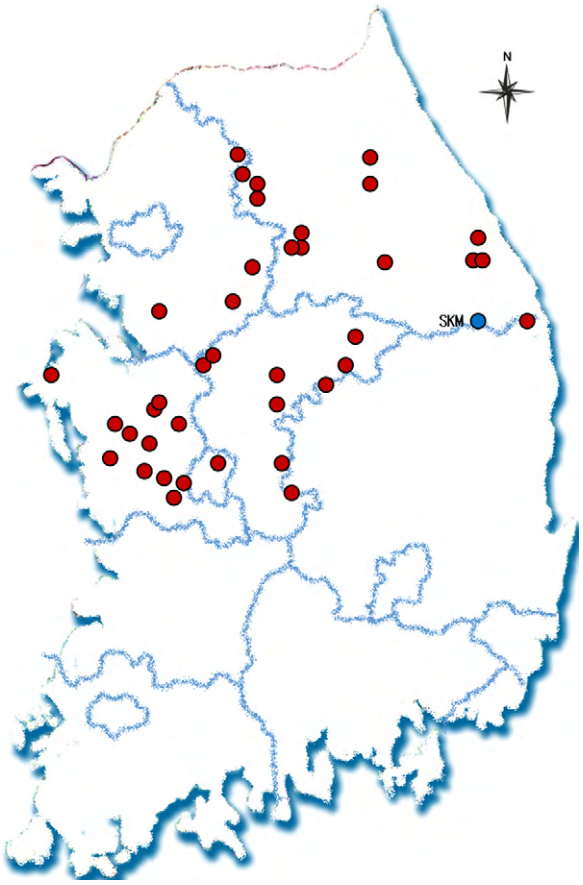

Fig. 1. The 40 mine location and their sampling spot.

2.4. Identification of potential fungi

2.4.1. Identification of the potential isolate

Chromosomal DNA of the potential isolate was extracted using DNeasy Plant Mini Kits (Qiagen, Hilden, Germany). Part of the 18S rDNA fragments was amplified using primers 817f and 1536r (Borneman and Hartin [17]). The PCR was done in a thermocycler (MJ Research) using a thermal cyclic condition at 94 °C (5 min) followed by 35 cycles at 94 °C (1 min), 55 °C (1 min) and 72 °C (1 min) with a final extension temperature at 72 °C for 7 min. The PCR products were purified using the PCR purification kit (Qiagen, USA). The purified amplicons were sequenced in both forward and reverse direction by using an automated sequencer ABI PRISM (Model 3700). The sequences were compared using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) for identification of the isolate [17–19].

2.5. Preparation of biomass

The spore suspension (5×10^5 spores mL^{-1}) was used as inoculum for the preparation of biosorbents. The collected spores were aseptically transferred to the 500-mL flask containing 300 mL of potato dextrose broth (PDB), and the flask was incubated at 29 °C for 5 d, at 150 rpm. After the incubation period, the pellets were

harvested and washed with a copious amount of deionized water; this was referred to as "live biomass". For dead biomass an appropriate amount of washed live biomass was taken and autoclaved at 121 °C in 15 psi for 15 min. Dried biomass was prepared by transferring adequate volume of spores to 500-mL flask with 300 mL of PDB, and the flasks were incubated at 29 °C for 5 d. After the incubation period, the fungal mat was taken and washed several times using copious amount of deionized water to remove the media; the mat was dried at 50 °C until it became crispy. The dried mat was ground and sieved (2 mm mesh) to get the uniform sized particles.

2.6. Batch mode biosorption studies

The effect of contact time (0–240 min), pH (1–7 ± 0.3), temperature (20–60 °C), initial metal ion concentration (10–320 mg L^{-1}), inoculum volume (0.5–5.0 mL) and agitation rate (50–250 rpm) on the removal of zinc was investigated. This experiments were optimized at the desired pH, temperature, metal concentration, contact time, agitation rate and biomass (0.5–5.0 g) dose using 100 mL of 100 mg L^{-1} of Zn(II) test solution in 250-mL Erlenmeyer flask.

2.6.1. Analysis of zinc

Different concentrations of Zn(II) solutions were prepared by appropriate dilution from Zn(II) stock solution. The pH was adjusted using 0.01 M HCl or 0.01 M NaOH, respectively. Desired dose of the biomass was then added and contents in the flask were shaken for the desired contact time in an electrically thermostatic reciprocating shaker at required agitation rate. At the end of the agitation time, the contents of the flask were filtered through whatman No #1 filter paper and the filtrate was analyzed for metal concentration by using ICP. Percent zinc removal was calculated using the following equation:

$$\text{Biosorption}\,(\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where C_i and C_f are the initial and final zinc ion concentrations, respectively.

2.6.2. Analysis of bio conversion of zinc

For analysis of Zn(II) modification, each of the three types of the biomass was resuspended in 1000 mL of 5000 mg L^{-1} Zn(II) solutions in 2000-mL Erlenmeyer flasks. The flasks were then covered with aluminum foil and kept in a shaker at 27 °C (200 rpm), and the reaction was carried out for a period of 48 h. After the incubation period, the mycelia were separated by centrifugation (9000 rpm) and both the supernatant and biomass were separated, dried and powdered. Subsequently, the powder was subjected to different chemical analyses as mentioned below to investigate the biotransformation of the electrolyte salts into the respective minerals. The powder obtained from three types of biomass and supernatants were cast in the form of films onto the different solid substrate for further analysis. The powdered biomasses thus obtained were analyzed by scanning electron microscopy–energy-dispersive spectrophotometric analysis (SEM–EDS), X-ray diffraction (XRD), and fourier transform infrared spectroscopy (FTIR). For SEM analysis, drop-coated films of the biological materials were made on copper substrates. SEM–EDS measurements were performed on a JSM-5410LV scanning electron microscopy (SEM) equipped with energy-dispersive spectroscopy. XRD measurements of drop-coated films of the biomass and supernatant powder on glass substrates were carried out on a Rigaku instrument operated at a voltage of 40 kV and a current of 30 mA with "Cu K-alpha" radiation. FTIR spectroscopy measurements of the purified and dried biological crystal powders taken in KBr pellets were analysed using Perkin-Elmer spectrum one instrument at a resolution of 4 cm^{-1}. To test whether or not zinc exhibits toxic effect on the *Fusarium* spp., the fungi was subjected to grow on different concentrations of zinc incorporated in the PDA media as described earlier. The plates were then incubated for 10 d to observe the toxic effect, and the fungi that could tolerate and grow in high concentration of zinc were selected for further studies [20].

3. Results and discussion

We have isolated 120 isolates from 40 mine samples and the isolates were subjected to minimum inhibitory concentration (MIC) of zinc. Among the isolates, the *Fusarium* spp. obtained from the mine SKM (Fig. 1) exhibited high tolerance to zinc, hence the isolate was selected for further studies. The concentration of the metals in the sample is As 152.23 mg kg^{-1}, Cd 8.185 mg kg^{-1}, Pb 93.58 mg kg^{-1} and Zn 935.43 mg kg^{-1}. Results of the present investigation showed that zinc removal by fungal biomass was influenced by specific surface properties of the fungal cell wall, metal ion concentration, temperature, pH, contact time, rate of agitation and the biomass dosage [21]. The fungal cell wall is mainly composed of polysaccharides, some of which may have associated protein, with other components including lipids and melanins [22]. These biomolecules on the fungal cell wall components have various functional groups (such as, amino, carboxyl, thiol, sulfydryl and phosphate groups) which play an important role in the sorption of various metals [23].

3.1. Effect of biosorbent dose

As shown in Fig. 2(a), the removal of zinc by live and dried biomass recorded an increase with increase in the concentration of biomass and reached saturation at 4.5 g, while in dead biomass saturation was observed at 5.0 g (Fig. 2(a)). The percent removal of zinc by dried biomass varied from 11.22 to 42.21% in the biomass dose ranging 0.5–5.0 g in 100 mL of aqueous solution (C_0 = 100 mg L^{-1}). This was consistent with the findings of Kahraman et al. [24] by *Phanerochaete chrysopsporium* and *Funalia trogii* for copper. Removal of zinc by dead biomass also recorded an increase with increase in biomass dose resulting in percent removal from 6.12 to 22.02% after 12 h of agitation in 150 rpm in 29 °C. With further increase in the biomass concentration beyond (4.5 g L^{-1}) zinc removal by live and dried biomass has recorded a decrease (data not shown) thus implying that dead biomass possess higher affinity for zinc removal compared to live and dried biomass. The increase in removal with increase in the biomass dose can be attributed to increased surface area [24]. A similar study by Garg et al. [25] demonstrated that the adsorption of Cr(VI) was dependent on the agro-industrial waste adsorbent dose. Where increased amount of biomass resulted in higher removal of zinc(II) from the solution as was expected, since the total sites were increased. However, the interference and competition between available binding sites at higher biomass densities caused the decrease in the specific removal capacity of the biosorbent.

3.2. pH effect

pH affects the activity of functional groups, cell surface metal binding sites, property and solution chemistry of the metal ions [12,26]. Maximum removal was recorded at pH 6.0 ± 0.3 after 1 h of incubation, for all three types of biomass with 68.9% for dried biomass, followed by 62.0% for dead biomass and 42.3% in live biomass of *Fusarium* spp. (Fig. 2(b)). Lower removal at low pH value may be attributed to competition of protons (H^+) and metal cations for non-specific removal sites on the biomass, resulting in an increase in protonated sites. This creates a repulsive

ionic environment, resulting in reduced binding of cations, consequently reduced uptake of zinc by the biomass [27]. As the pH increases negative charge groups dominate for e.g., the carboxyl groups become deprotonated and are able to attract the zinc cations [28]. The drop in metal loading beyond pH 6.0 ± 0.3 might be explained by the reduced availability and solubility of the metal ions with the onset of precipitation of metal hydroxides as has been reported by Sannasi et al. [29]. The above findings reveal that metal loading is strongly influenced by the solution's initial pH. Since efficient removal was observed at pH 6.0 ± 0.3 for all the three types of biomass. At this pH range, removal of Zn^{2+} is assumed as resulting both from sorption and surface precipitation of these metallic ions under their free form. Since, further experiments were carried out at pH 6.0 ± 0.3 for comparative removal studies.

3.3. Effect of temperature

Fig. 2(c) shows the effect of temperature on zinc removal. Although temperature dependent biosorption was specific for each of the biomass types, all the three types of biomass recorded maximum removal at 40 °C, with removal capacity of 53.65, 50 and 38.44% for dried, live and dead biomass of *Fusarium* spp., respectively. This was consistent with the findings of Huang and co-

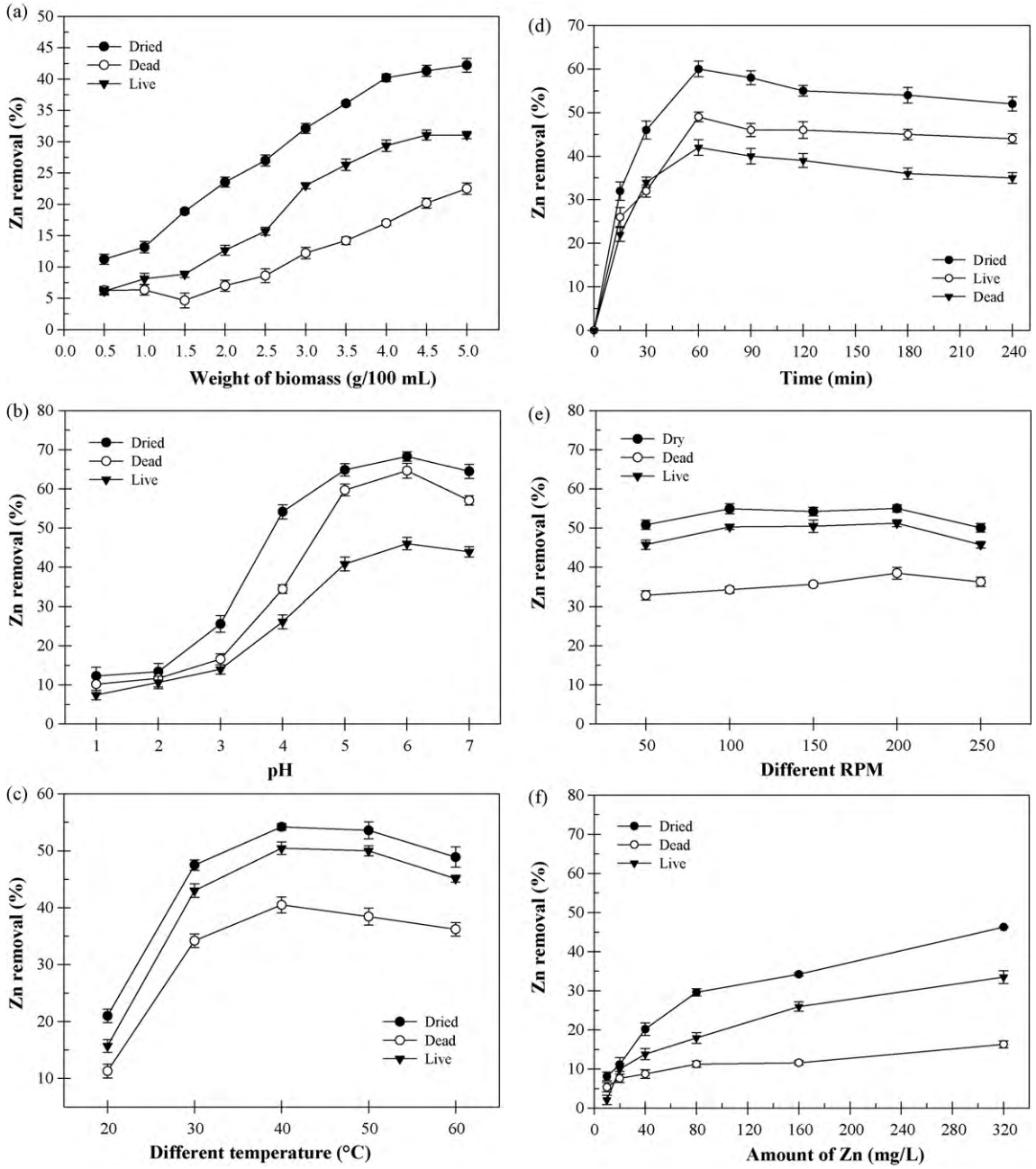

Fig. 2. (a) Effect of the amount of biomass on the removal by live, dead and dried biomass of *Fusarium* spp. (Zn(II) 100 mg L^{-1}; pH = 6.0 ± 0.3; temp = 30 °C; 60 min; 150 rpm). (b) Effect of different initial pH on the removal by live, dead and dried biomass of *Fusarium* spp. (Zn(II) 100 mg L^{-1}; biomass 4.5 g L^{-1}; temp = 30 °C; 60 min; 150 rpm). (c) Effect of temperature for removal by live, dead and dried biomass of *Fusarium* spp. (Zn(II) 100 mg L^{-1}; biomass 4.5 g L^{-1}; pH = 6.0 ± 0.3; 60 min; 150 rpm). (d) Effect of time on the removal by live, dead and dried biomass of *Fusarium* spp. (Zn(II) 100 mg L^{-1}; biomass 4.5 g L^{-1}; pH = 6.0 ± 0.3; temp = 30 °C; 150 rpm). (e) Effect of agitation for removal by live, dead and dried biomass of *Fusarium* spp. (Zn(II) 100 mg L^{-1}; biomass 4.5 g L^{-1}; pH = 6.0 ± 0.3; 60 min; temp = 40 °C). (f) Effect of initial concentration of metal Zn(II) for removal by live, dead and dried biomass of *Fusarium* spp. (biomass 4.5 g L^{-1}; pH = 6.0 ± 0.3; temp = 40 °C; 60 min; 150 and 200 rpm).

workers [14], Srivastava and Thakur [19], for zinc bioaccumulation by *Acinetobacter* spp. PCP3. The higher removal rate with increase in temperature from 20 to 40 °C may be due to either higher affinity of the binding sites for the zinc cations or due to more availability of the binding sites on the relevant cell mass. With further increase in temperature above 40 °C, zinc removal recorded a decrease which may be due to distortion of binding sites available on the cell surface for metal removal [12,30–33].

3.4. Effect of contact time

Zinc uptake increase rapidly during the first 15 min and remained nearly constant after 60 min of removal for all the three types of biomass suggesting that the rate of removal was fast and reached saturation at 60 min, beyond which it recorded a decrease (Fig. 2(d)). The metal uptake was rapid for all concentrations in the first 60 min of contact time, reaching 60, 48 and 40% removal for dried, live and dead biomass, respectively. The initial contact time was 30 min and it reached a maximum at 60 min for all the three types of biomass and started to decrease from next 30 to 240 min for all the three types of biomass. This indicates that removal occurs in two stages: the first rapid surface binding and the second slow intracellular diffusion [33]. There are several parameters that determine the removal rate such as the stirring rate of the aqueous phase, structural properties both of the supports and the biomass, the amount of biomass, properties of the ion under study, initial concentration of ionic species and, of course, existence of other metal ions, which may compete with the ionic species of interest for the active removal sites [13]. Therefore, it is very difficult to compare the zinc removal times reported [34].

3.5. Effect of agitation rate

The effect of agitation rate (50–250 rpm) on zinc removal by *Fusarium* spp. was studied. Optimum zinc removal was recorded when agitation speed was 100 and 200 rpm as shown in Fig. 2(e). The results indicate that the agitation rate ensures maximum availability of binding sites for zinc uptake. This is to say; a speed of 150 rpm gives the best homogeneity of suspension in dried biomass (55%), whereas for the live and dead biomass a speed of 200 rpm was optimum with 52.2 and 39.4% biosorption, respectively. At high agitation speed, vortex phenomena occur where the suspension is no longer homogenous, thus making removal is difficult [34]. While in the high speed (above 200 rpm) of agitation the zinc may not be available due to the inadequacy of contact time for zinc and sorbent interactions in dried biomass.

3.6. Effect of initial zinc concentration

The removal of zinc by all three types of biomass recorded an increase with increase in the concentration of zinc in the solution (Fig. 2(f)). Perusal of literatures shows such an increase in several studies [12,24,31,32]. The zinc removal capacity of the live, dead and dried *Fusarium* spp. biomass increases first with increase in the initial concentration and reached at maximum concentration and reached saturation [31]. The removal rate of zinc ions is exemplified for dried and live fungal biomass at 40 °C, 4.5 g biomass weight, 200 rpm agitation, 60 min and at pH 6.0 ± 0.3. Increase in removal with increase in initial zinc concentration may be due to an increase in electrostatic interactions, involving sites of progressively lower affinity for metal ions [34]. Fig. 2(f), shows that maximum removal rate was at 320 mg L^{-1} of zinc for all the three types of biomass, whereas the minimum removal rate was observed at low (10 and 20 mg L^{-1}) Zn(II) concentrations in all the three types of biomass.

Zinc is considered to be toxic to various organisms [34,35], and hence it is important to establish whether the fungus *Fusarium* spp. survives after exposure to the high Zn(II) concentration (5000 mg L^{-1}). On evaluating the toxicity of zinc for *Fusarium* spp. showed high tolerance to zinc up to 5000 ppm implying that zinc was not toxic to *Fusarium* spp. Fig. 3(a) shows representative TEM images recorded from solution-cast films of the zinc ion after treated with live, dead and dried biomass of *Fusarium* spp. It was observed from the TEM image that the sample predominantly consisted of large, highly irregular particles of size >100 nm (Fig. 3(a)). At progressively higher magnifications 200 nm, it was seemed to be different for each type of biomass treated with zinc. It is to say that, in dried biomass (100 nm), these structures are seen to be aggregates of zinc nanocrystals that are uniform in size and highly encircling in morphology. However, in higher magnification (200 nm) it is seemed too irregular in shape as the individual crystal [14]. In live biomass, an accurate shape of the crystals as triangle crystals was seen in 100 nm and 200 nm. The reason behind this crystal formation might be that live biomass act as a bearer of protein and secrete the protein by utilizing zinc as a micronutrient. However, in killed biomass crystals appeared as aggregates at 100 nm and in 200 nm it was little bit different from the others, appearing square in shape. These results indicate that each type of biomass possess some individual affinity towards the zinc to pro-

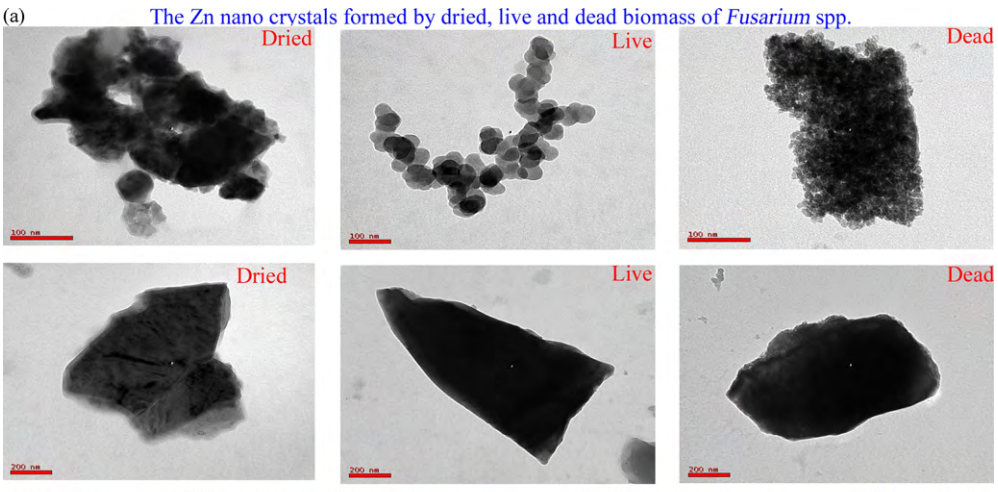

Fig. 3. (a–d) The Zn nanocrystals formed by dried, live and dead biomass of *Fusarium* spp.

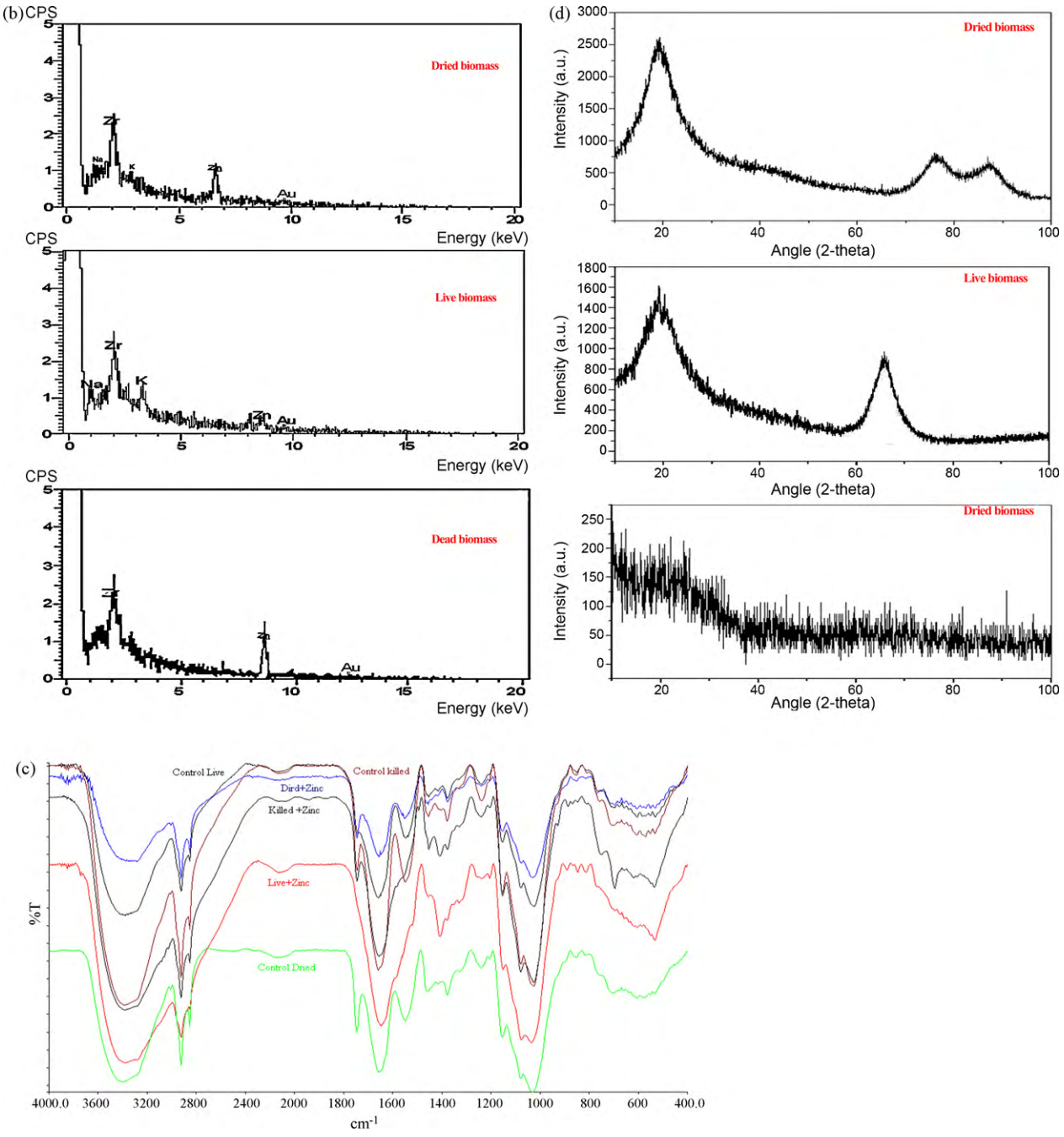

Fig. 3. (*Continued*).

duce different morphology of crystal. It would be interesting to understand the chemical composition of each type of crystallites synthesized using *Fusarium* spp.

Presence of zinc nanocrystals was confirmed by spot-profile SEM-EDS measurement. In addition to the expected zinc signals, Zr, Au, Na and K signals from zinc crystals were observed in dried and live biomass whereas dead biomass showed only signals for Zr (Fig. 3(b)). Hence it can be confirmed that it is the role of protein secreted by the fungi, which has the potentiality to covert the zinc to nanocrystals. There are no 'O' signals obtained in the EDS spectrum, subsequently there is no possibility of obtain ZnO crystals [36] (Zr and Au signals are from the substrate). The Na and K signals in the EDS spectrum indicate the presence of proteins within and on the zinc crystallites. That these signals are likely to be due to proteins secreted by the fungi is supported by the FTIR measurement for the formation of zinc crystals (Fig. 3(c)). The peaks clearly indicate the presence of protein and amide, I and II bands at 1100, 1400, 1650, 2900 and 3000 cm^{-1}, respectively [14,15,20,37]. This was observed only in dried and live biomass, since live biomass is holding the protein up to its live state and in dried biomass the protein from the cell is liberated during the drying process and bound on the surface of cell. This observation indicates that the zinc crystals in the different morphology are present with proteins that are possibly occluded into the crystals or are bound to the surface of the crystals thereby acting as a sort of glue holding the spherical crystallites together in the super-structure. Consequently, the proteins do play an important role in directing the morphology of the zinc crystals can also be attributed to the fact that the irregular morphology obtained for each type of biomass is different from earlier reports [38].

XRD analysis of the biological zinc crystals formed by the reaction of aqueous Zn(II) ions with *Fusarium* spp. was performed and the diffraction pattern obtained is shown in Fig. 3(d). The broad Bragg reflections in the XRD spectrum indicate that the zinc crystals are rather small and are consistent with the TEM analysis that revealed the crystallites to be of 120–200 nm dimensions [12,14]. Fig. 3(a) shows representative TEM images recorded from supernatant zinc crystals after reaction of aqueous Zn(II) ions with the fungus *Fusarium* spp. The zinc crystals exhibit an inconsistent morphology for each type of biomass that is completely different from that reported earlier developed for the synthesis of zinc sulfide (ZnS) nanoparticles using immobilized *Rhodobacter spheroides* [39]. Bai et al. [39] reported variation in the average diameter and shape of ZnS nanoparticles as a result of variation in culture time has also been reported [39]. A large number of very uniform shaped crystals of zinc are observed in the TEM micrograph (Fig. 3(a)).

The above experiments present some important facts. First, zinc was not toxic to the fungus, at least up to 5000 mg L^{-1} concentration of the metal ions. Second, the zinc crystals are formed by reaction of the corresponding metal ions with Na and K produced by the fungus during metabolism; this is an important point of deviation from earlier biomimetic approaches to metal crystal growth where Na and K were converted. In the biological synthetic process for ZnS nanoparticles, soluble sulfate acts as the source of sulfur. First, soluble sulfate enters into biomass via diffusion, and later carried to the interior membrane of cell facilitated by the sulfate permease. Then, the sulfate is reduced to sulfite by the ATP sulfurylase and phosphoadenosine phosphosulfate reductase with further reduction to sulfide by sulfite reductase. The sulfide reacts with O-acetylserine to synthesize cysteine via O-acetylserine thiolyase [39–41], and then cysteine produces S^{2-} by a cysteine desulfhydrase in the presence of zinc. After this process, S^{2-} reacts with the soluble zinc salt and the ZnS nanoparticles are synthesized [14,39,42]. Finally, ZnS nanoparticles are discharged from the biomass to the solution. Although the detailed study of this mechanism is in progress, it suggests that many other high grades binary metal sulfides can also be produced using this method. That a non-calcareous microorganism such as a fungus should be capable of crystal growth and engineering at a high level of sophistication opens up the exciting possibility that other microorganisms when challenged with metal ions may lead to similar exciting results. Third, the morphology of the zinc crystals is different from that reported in other studies suggesting that the proteins secreted by the fungus during mineralization play a crucial role in determining the morphology. It is assured that this crystal formation is fully directed by the protein present in the biomass. Further studies have to be carried out to determine the nature of proteins responsible for controlling the crystal morphology.

4. Conclusion

Three types of biomass were used in this study to determine its ability for removal of zinc. In this regard, the dried fungal biomass showed high potential to removal of zinc from aqueous solution compared to live and dead biomass. Removal and zinc nanocrystals formation using different biomass were separately studied. In addition to this, it has been observed that the fungi possess the ability of producing zinc nanocrystals. These zinc nanocrystals are useful byproducts for various applications [43] and possess morphologies that are directed by proteins secreted by the fungi. The metal ions are not toxic to the fungus under the experimental conditions of this study and thus highlight the potential of this process in bioremediation and large-scale mineral growth.

Acknowledgments

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References

[1] M.C. Jung, Contamination by Cd, Cu, Pb, and Zn in mine wastes from abandoned metal mines classified as mineralization types in Korea, Environ. Geochem. Health 30 (2008) 205–217.
[2] M.J. Sik, C.Y. Wook, Rehabilitation works of mine wastes in abandoned metal mines in Korea, in: 2003 Seattle Annual Meeting, November 2–5, 2003.
[3] G. Borbely, E. Nagy, Removal of zinc and nickel ions by complexation–membrane filtration process from industrial wastewater, Desalination 240 (2009) 218–226.
[4] E.W. Wilde, J.R. Beneman, Bioremoval of heavy metals by the use of microalgae, Biotechnol. Adv. 11 (1993) 781–812.
[5] B. Volesky, Z.R. Holan, Biosorption of heavy metals, Biotechnol. Prog. 11 (1998) 235–250.
[6] D. Inthorn, Removal of heavy metal by using microalgae, in: K. Hiroyuki, Y.K. Lee (Eds.), Photosynthetic Organisms in Environmental Biotechnology, Springer-Verlag, Hong Kong, 2000, pp. 111–135.
[7] C. White, G.M. Gadd, Biosorption of radionuclides by fungal biomass, J. Chem. Technol. Biotechnol. 49 (1990) 331–343.
[8] G.M. Gadd, Role of microorganisms in the environmental fate of radionuclides, Ciba Found. Symp. 203 (1997) 94–104.
[9] A. Kapoor, T. Viraraghavan, D.R. Cullimore, Removal of heavy metal using the fungus *Aspergillus niger*, Bioresour. Technol. 70 (1999) 95–104.
[10] W. Jianlong, C. Chen, Biosorbents for heavy metals removal and their future, Biotechnol. Adv. 27 (2009) 195–226.
[11] J.M. Waller, M. Holdeness, Fusarium Diseases on Coffee, International Mycological Institute, Surrey, UK, 1997.
[12] M.R. Huang, Q.Y. Peng, X.G. Li, Rapid and effective adsorption of lead ions on fine poly(phenylenediamine) microparticles, Chem. Eur. J. 12 (2006) 4341–4350.
[13] X.G. Li, R. Liu, M.R. Huang, Facile synthesis and highly reactive silver ion adsorption of novel microparticles of sulfodiphenylamine and diaminonaphthalene copolymers, Chem. Mater. 17 (2005) 5411–5419.
[14] X.G. Li, X.L. Ma, J. Sun, M.R. Huang, Powerful reactive sorption of silver(I) and mercury(II) onto poly(*o*-phenylenediamine) microparticles, Langmuir 20 (2009) 1675–1684.
[15] X.G. Li, H. Feng, M.R. Huang, Strong adsorbability of mercury ions on aniline/sulfoanisidine copolymer nanosorbents, Chem. Eur. J. 15 (2009) 4573–4581.
[16] S. Zafar, F. Aqil, I. Ahmad, Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil, Bioresour. Technol. 98 (2007) 2557–2561.
[17] J. Borneman, R.J. Hartin, Primers that amplify fungal rRNA genes from environmental samples, Appl. Environ. Microbiol. 66 (2000) 4356–4360.
[18] J.D. Thompson, D.G. Higgins, T.J. Gibson, W. Clustal, Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[19] J. Felsenstein, Confidence limits on phylogenies: an approach using the bootstrap, Evolution 39 (1985) 783–791.
[20] A. Sanyal, D. Rautaray, V. Bansal, A. Ahmad, M. Sastry, Heavy-metal remediation by a fungus as a means of production of lead and cadmium carbonate crystals, Langmuir 21 (2005) 7220–7224.
[21] G. Donmez, Z. Aksu, The effect of copper (II) ions on the growth and bioaccumulation property of some yeasts, Process Biochem. 35 (2002) 135–142.
[22] G.M. Gadd, Interaction of fungi with toxic metals, New Phytol. 124 (1993) 25–60.
[23] S. Srivastava, I.S. Thakur, Isolation and process parameter optimization of *Aspergillus* sp. for removal of chromium from tannery effluent, Bioresour. Technol. 97 (2006) 1167–1173.
[24] S. Kahraman, D. Asma, S. Erdemoglu, O. Yesilada, Biosorption of copper by live and dried biomass of the white rot fungi *Phanerochaetye chrysosporium* and *Funalia trogii*, Eng. Life Sci. 5 (2005) 72–77.
[25] V.K. Garg, R. Gupta, R. Kumar, R.K. Gupta, Adsorption of chromium from aqueous solution on treated sawdust, Bioresour. Technol. 92 (2004) 79–81.
[26] P. Sannasi, J. Kader, B.S. Ismail, S. Salmijah, Sorption of Cr(VI), Cu(II) and Pb(II) by growing and non-growing cells of a bacterial consortium, Bioresour. Technol. 97 (2006) 740–747.
[27] J.S. Chang, R. Law, C.C. Chang, Biosorption of lead, copper and cadmium by biomass of *P. aeruginosa* PU21, Water Res. 31 (1997) 1651–1658.
[28] K.C. Sekhar, S. Subramaniam, J.M. Modak, K.A. Natarajan, Removal of metal ions using an industrial biomass with reference to environmental control, Int. J. Miner. Process. 53 (1998) 107–120.
[29] P. Sannasi, S. Salmijah, J. Kader, Isolation and selection of mixed cultures (environmental isolates) from metal contaminated areas, in: Proceedings of the 12th National Biotechnology Seminar, Damai Laut Country Resort, Lumut, November 12–15, 2000, 2000, pp. 116–120.

[30] A. Mellah, S. Chegrouche, The removal of zinc from aqueous solutions by natural bentonite, Water Res. 31 (1997) 621–629.
[31] P.R. Puranik, K.M. Paknmar, Biosorption of lead, cadmium and zinc by *Citrobacter* strain MCM B-181: characterization studies, Biotechnol. Prog. 15 (1999) 228–237.
[32] N. Goyal, S.C. Jain, U.C. Banerjee, Comparative studies on the microbial adsorption of heavy metals, Adv. Environ. Res. 7 (2003) 311–319.
[33] U. Yetis, A. Dolek, F.B. Dilek, G. Ozcengiz, The removal of Pb(II) by *Phanerochaete chrysopsporium*, Water Res. 34 (2000) 4090–4100.
[34] Y.G. Liu, T. Fan, G.M. Zeng, X. Li, Q. Tong, F. Ye, M. Zhou, W.H. Xu, Y.E. Huang, Removal of cadmium and zinc ions from aqueous solution by living *Aspergillus niger*, Trans. Nonferrous Met. Soc. China 16 (2006) 681–686.
[35] H. Babich, G. Stotzky, Toxicity of zinc to fungi, bacteria, and coliphages: influence of chloride ions, Appl. Environ. Microbiol. 36 (1978) 906–914.
[36] K.Y. Ng, A. Muley, Y.F. Chan, A.C.M. Ng, A.B. Djurisic, A.H.W. Ngan, Highly facetted metallic zinc nanocrystals fabricated by thermal evaporation, Mater. Lett. 60 (2006) 2423–2427.
[37] A. Dong, P. Huang, W.S. Caughey, Redox-dependent changes in beta-extended chain and turn structures of cytochrome c in water solution determined by second derivative amide I infrared spectra, Biochemistry 31 (1992) 182–189.
[38] Z.L. Wang, ZnO nanowire and nanobelt platform for nanotechnology, Mater. Sci. Eng. Rep. 64 (2009) 33–71.
[39] H.J. Bai, Z.M. Zhang, J. Gong, Biological synthesis of semiconductor zinc sulfide nanoparticles by immobilized *Rhodobacter sphaeroides*, Biotechnol. Lett. 28 (2006) 1135–1139.
[40] J.D. Holmes, D.J. Richardson, S. Saed, R.E. Gowing, D.A. Russell, J.R. Sodeau, Cadmium-specific formation of metal sulfide "Q-particle" by *Klebsiella pneumoniae*, Microbiology 143 (1997) 2521–2530.
[41] S. Auger, M.P. Gomez, A. Danchin, I.M. Verstraete, The PatB protein of *Bacillus subtilis* is a C-S-lyase, Biochimie 87 (2005) 231–238.
[42] J.L. Li, W.X. Zhang, H.C. Xu, Q.R. Yang, L.Q. Zhao, Microorganism Physiology, vol. 14, 1st ed., University of Agriculture Press, China Beijing, 1993, pp. 246–248.
[43] A.V. Kachynski, A.N. Kuzmin, M. Nyk, I. Roy, N.P. Prasad, Zinc oxide nanocrystals for nonresonant nonlinear optical microscopy in biology and medicine, J. Phys. Chem. C 112 (2008) 10721–10724.