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# Bio-physical removal of heavy metal from aqueous solution

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ABSTRACT

The aim of this study was to compare the performance of a suspended growth bioreactor and a combined bio-physical bioreactor for $Zn^{2+}$ removal from aqueous solution. Two identical bioreactors (8.5 L each) were operated at a fixed HRT of 29.1 h. The suspended growth bioreactor was used as the control reactor and contained only sludge. The bio-physical bioreactor contains sludge and a low-cost adsorbent derived from groundwater treatment plant sludge. The influent $Zn^{2+}$ concentration was varied from 0.5 to 15 mg/L in 8 experimental phases. Influent and effluent $Zn^{2+}$ concentration was monitored daily. Phase 1 (day 1–15) and phase 2 (day 16–29) were used as acclimation period for both bioreactors, respectively. Results show that $Zn^{2+}$ removal increased with increasing influent $Zn^{2+}$ concentration from 0.5 to 1.0 mg/L (phases 3–4) but fluctuated thereafter for the suspended growth bioreactor. $Zn^{2+}$ removal in the bio-physical bioreactor increased with increasing influent $Zn^{2+}$ concentration from 0.5 to 10 mg/L (phases 3–7) and decreased with further increase in influent $Zn^{2+}$ concentration to 15 mg/L (phase 8). The effluent $Zn^{2+}$ concentration in phase 8 for the suspended growth and the bio-physical bioreactors were 58.7 and 90%, respectively. The higher removal of $Zn^{2+}$ in the bio-physical bioreactor was due to heavy metal tolerance and the resistance in heavy metal toxicity on the microbial community of the combined system.

*Keywords:* Zinc; Activated sludge; Groundwater treatment plant sludge; Bioreactor; Heavy metal

## 1. Introduction

Wastewater from various industries such as pigment, galvanizing plants, fertilizer plants, tanneries, batteries, and mine drainage are associated with high zinc concentrations [1]. Improper discharge of zinc-contaminated wastewater constitutes severe health and environmental problems. Health disorders such as irritability, lung disorder, gastrointestinal distress, metal fume fever, growth retardation, and even cancer has been associated with zinc toxicity [2]. Heavy metals including zinc are commonly classified as priority pollutants due to their mobility and toxic characteristics in natural water streams [3]. Thus, the concern and interest for heavy metal removal from wastewater has intensified over the past decades.

The removal of zinc and other heavy metals from wastewater has been investigated using various methods such as ion exchange [4], adsorption [5,6], solvent extraction [7], ultrafiltration [8], electrodialysis [9], and

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physico-chemical precipitation [10]. However, several demerits such as operational cost and environmental concerns have been major constraint [11]. Thus, it is pertinent to develop relatively simple, low-cost, and effective treatment method to enhance zinc removal from wastewater.

Biological treatment methods are considered as cheap technologies in terms of capital and operational cost. Depending on the objective of the process, biological treatment and bioreactor configuration can be achieved in different approaches such as aerobic, anoxic, and anaerobic processes. Micro-organisms are used to remove water pollutants from wastewater. However, biological treatment methods are not commonly used for heavy metal removal from wastewater due to the inhibitory effects and toxicity of heavy metals on the microbial community. In order to overcome this constraint and at relatively low-cost, low-cost physical processes have been adopted as a means to enhance microbial tolerance and resistance toward heavy metal toxicity and inhibition. The addition of powdered activated carbon derived from agricultural waste and solid waste materials in biological treatment processes have been explored but the application of precursors such as microwave incinerated rice husk ash [12] and groundwater treatment plant sludge (GWTPS) in biological treatment process are still at infancy [13,14]. In addition to their beneficial use in biological processes, it is also a waste minimization approach.

The objective of this study was to examine the performance of two identical bioreactors treating zinc-contaminated synthetic wastewater, operated as suspended growth and bio-physical modes at various zinc concentrations. The influent and effluent $Zn^{2+}$ concentrations as well as the mixed liquor volatile suspended solids (MLVSS) were daily monitored.

## 2. Methodology

### 2.1. Wastewater preparation

Synthetic wastewater was prepared by daily dissolving an appropriate amount of $ZnCl_3$ in tap water according to the experimental plan. Purina Alpo, a high organic substrate was daily added to the wastewater to give a C:N:P ratio of about 100:24:3, which meets the required minimum 100:5:1 ratio for domestic wastewater. Synthetic wastewater was used in order to provide consistent organic loading for a better assessment of the bioreactors. The synthetic wastewater was characterized as follows COD 500 mg/L, BOD 220 mg/L, and TSS 500 mg/L. The pH of the wastewater was kept between 7 and 7.5 by the daily addition of 0.15 mL/L of phosphate buffer.

### 2.2. GWTPS preparation

GWTPS was collected from Air Kelantan Sdn. Bhd., Malaysia, in muddy form. It was then dried at 105˚C for 24 h and grounded into powder. No further modification was done to the GWTPS. The powdered GWTPS biosorbent was then stored in a tight container prior to use. Elemental composition analysis shows that GWTPS contains high amount of $Fe_2O_3$.

### 2.3. Bioreactor setup

Two rectangular biological reactors of equal size were made from acrylic glass (5-mm thick). The dimension of the bioreactors were 37 × 17 × 25 cm (length × width × height) and have an individual total volume of 8.5 L. A settling compartment was made in the reactors by means of baffle to retain biomass (7 cm from the length) near the effluent port. The influent port was located 4 cm from the bottom and was operated in upflow pattern. The effluent port was located 17 cm from the bottom, opposite the influent port. The bottom of the reactors were equipped with six horizontal long air tube diffusers to circulate fine air bubble in upflow pattern, throughout the reactor. The bioreactors were operated at constant aeration (DO concentration of about 3–4 mg/L). Seed sludge with a mixed liquor suspended solid (MLSS) concentration of about 4 g/L was collected from the domestic wastewater treatment plant in Universiti Teknologi PETRONAS (UTP), Malaysia. The influent flow to the bioreactors was fixed at 7 L/d (HRT 29.1 h) using a Masterflex Precision Pump. The study was carried out in eight experimental phases consisting of different $Zn^{2+}$ concentrations as shown in Table 1.

### 2.4. Bioreactor operation

The experiment was conducted in eight different phases. In phase 1 (day 1–12), the seed sludge and the

Table 1
Experimental plan

| Phase | Concentration | Days |
|---|---|---|
| 1 | Acclimation | 1–12 |
| 2 | Addition of GWTPS | 13–29 |
| 3 | $Zn^{2+}$ dosage 0.5 mg/L | 30–47 |
| 4 | $Zn^{2+}$ dosage 1.0 mg/L | 48–64 |
| 5 | $Zn^{2+}$ dosage 2.0 mg/L | 65–79 |
| 6 | $Zn^{2+}$ dosage 5.0 mg/L | 80–96 |
| 7 | $Zn^{2+}$ dosage 10.0 mg/L | 97–112 |
| 8 | $Zn^{2+}$ dosage15.0 mg/L | 113–125 |

wastewater (without the addition of heavy metal) were allowed to acclimate in both the suspended growth and bio-physical bioreactors, respectively. In phase 2 (day 13–29), 2,000 mg of GWTPS was added to the bio-physical bioreactor and further acclimation was given to both bioreactors until day 29. In phase 1 and 2, the sludge age was controlled through wasting. GWTPS (2,000 mg) was daily added to the bio-physical bioreactor throughout phase 2 (taking into consideration, GWTPS wasted daily). In phase 3 (day 30–47), influent $Zn^{2+}$ concentration of 0.5 mg/L was applied to both bioreactors. In phase 4 (day 48–64), influent $Zn^{2+}$ concentration was increased to 1.0 mg/L in both bioreactors. Henceforth, 100 mg of GWTPS was added daily into the bio-physical bioreactor. The purpose was to prevent GWTPS from reaching the exhaustion point, where all GWTPS becomes saturated with heavy metal. In phase 5 (day 65–79), influent $Zn^{2+}$ concentration was increased to 2.0 mg/L. In phases 6 (day 80–96), 7 (day 97–112) and 8 (day 113–125), influent $Zn^{2+}$ concentration was increased to 5, 10, and 15 mg/L, respectively, for both reactors. The influent and effluent samples were collected daily and the performance of both reactors was monitored for more than 4 months. $Zn^{2+}$ concentration was measured using Zincon method (HACH 8009) while the solids (MLSS and MLVSS) were analyzed according to standard methods Number 2540 E.

## 3. Results and discussion

The time course profile for $Zn^{2+}$ removal from the suspended growth and bio-physical bioreactors is presented in Fig. 1 whereas the percentage removal is depicted in Fig. 2. The impact of $Zn^{2+}$ on the microbial community in both bioreactors is shown in Fig. 3. Phase 1 of the experiment was acclimation phase. The MLSS on day 12 were about 6.3 and 6.0 g/L (figure not shown) whereas the MLVSS were 3.78 and 3.79 g/L for the suspended and bio-physical bioreactors, respectively. There was no notable difference in the concentration of the MLSS and MLVSS for both bioreactors at this phase. No $Zn^{2+}$ was applied to the bioreactors at this stage (day 1–12). In phase 2 (day 13–29), the acclimation period was extended by the addition of GWTPS into the bio-physical bioreactor. It was observed at the end of this phase that the MLSS increased to 6.7 and 7.1 g/L for the suspended growth and bio-physical bioreactors, respectively. The notable increase in the concentration of the solids in the bio-physical bioreactor was as a result of the addition of the powdered biosorbents (GWTPS) into the system. However, the MLVSS concentration in the suspended growth bioreactor was higher (4.4 g/L) when compared with the bio-physical bioreactor (4.03 g/L). Phase 3 started on day 30 with an influent $Zn^{2+}$ concentration of 0.5 mg/L in both bioreactors. A steady state condition was observed on day 47 for both bioreactors. $Zn^{2+}$ removal was more prominent in the bio-physical bioreactor in this phase. The $Zn^{2+}$ removal for the suspended growth and bio-physical bioreactors was 72% (0.14 mg/L) and 92% (0.04 mg/L), respectively. The MLSS increased from 6.7 to 6.8 g/L in the suspended growth bioreactor and 7.1 to 7.7 g/L in the bio-physical bioreactor. The MLVSS also increased from 4.4 to 4.6 g/L for the suspended growth bioreactor and 4.03 to 4.8 g/L for the bio-physical bioreactor. The suspended growth and bio-physical bioreactors demonstrated remarkable stability to $Zn^{2+}$ concentration in this phase, which resulted in the increase in the MLVSS concentration. However, the rate of increase in the MLVSS concentration in the suspended growth bioreactor (0.2 g/L) was markedly inferior to the bio-physical bioreactor (0.77 g/L). In phase 4 (day 48), influent $Zn^{2+}$ concentration was increased to

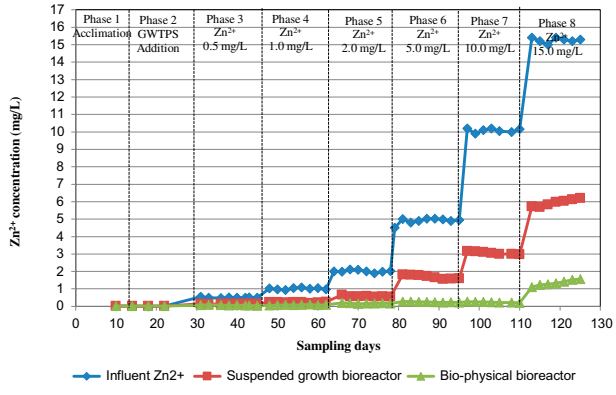


Fig. 1. $Zn^{2+}$ removal from suspended growth and bio-physical bioreactors.

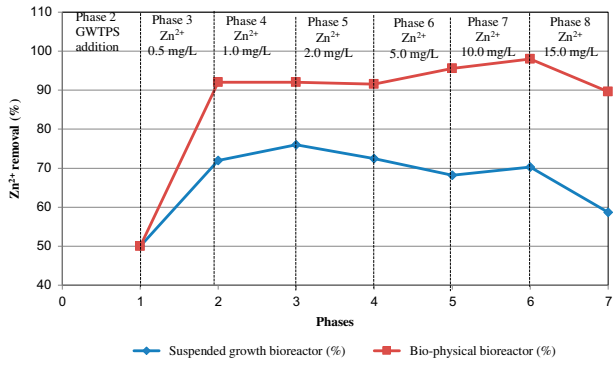


Fig. 2. Percent removal of $Zn^{2+}$ in suspended growth and bio-physical bioreactors at steady state.

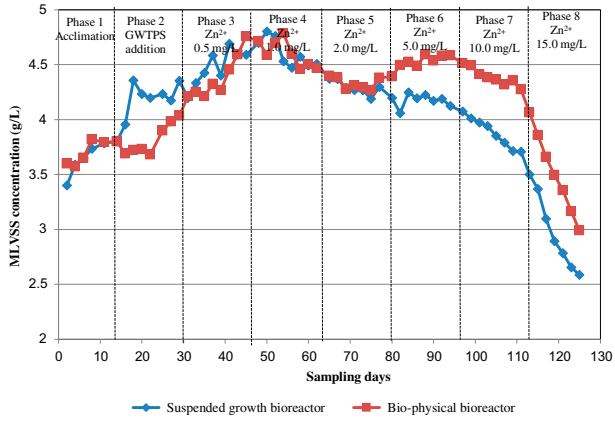


Fig. 3. MLVSS Concentration in the suspended growth and bio-physical bioreactors.

1 mg/L. An increase in $Zn^{2+}$ effluent concentration was observed in the suspended growth bioreactor and reached about 0.24 mg/L on day 64. On the contrary, no significant increase in the effluent $Zn^{2+}$ concentration of the bio-physical bioreactor was observed. The percentage removal of $Zn^{2+}$ in the suspended growth and bio-physical bioreactors in this phase was 76 and 92%, respectively. The MLSS and MLVSS for both bioreactors decreased. In the suspended growth bioreactor, the MLSS decreased from 6.8 g/L in phase 3 to 6.5 g/L in phase 4 whereas the MLVSS decreased from 4.6 g/L in phase 3 to 4.5 g/L in phase 4. In the bio-physical bioreactor, the MLSS also decreased from 7.7 g/L in phase 3 to 7.1 g/L in phase 4 whereas the MLVSS decreased from 4.8 g/L in phase 3 to 4.5 g/L in phase 4. In phase 5 (day 65), influent $Zn^{2+}$ concentration was increased to 2 mg/L. In line with earlier observation, the effluent $Zn^{2+}$ concentration increased in the suspended growth (0.55 mg/L) and bio-physical bioreactors (0.17 mg/L) on day 78, respectively. The removal of $Zn^{2+}$ for the suspended growth bioreactor decreased from 76% in phase 4 to 72.5% in phase 5. However, no change (91.5%) was observed in the removal efficiency of $Zn^{2+}$ in the bio-physical bioreactor in phase 5. The MLSS of the suspended growth and bio-physical bioreactors further decreased to 6.1 and 6.5 g/L with a slight decrease in the MLVSS to 4.3 and 4.4 g/L, respectively. In phase 6 (day 79), influent $Zn^{2+}$ concentration was increased to 5 mg/L. The effluent $Zn^{2+}$ concentration was remarkably high for the suspended growth bioreactor (1.59 mg/L) compared to the bio-physical bioreactor (0.22 mg/L) at steady state (day 95). The $Zn^{2+}$ removal efficiency further decreased to 68.2% for the suspended growth bioreactor but increased to 95.6% for the bio-physical bioreactor. The MLSS and MLVSS of the suspended growth bioreactor decreased to 5.7 and 4.1 g/L, respectively. In the bio-physical bioreactor, the MLSS and MLVSS increased to 7 and 4.6 g/L, respectively. In phase 7 (day 96), influent $Zn^{2+}$ concentration was increased to 10 mg/L. The effluent $Zn^{2+}$ concentration of the suspended growth bioreactor increased and reached about 2.97 mg/L on day 112 with a removal efficiency of about 70.3%. In the bio-physical bioreactor, the effluent $Zn^{2+}$ concentration was stable (0.2 mg/L) on day 112 with a removal efficiency of about 98%. The MLSS and MLVSS in the suspended growth bioreactor decreased to 5.3 and 3.5 g/L, respectively. In the bio-physical bioreactor, the MLSS and MLVSS also decreased to 6.6 and 4.1 g/L, respectively. In phase 8 (day 113), influent $Zn^{2+}$ concentration was further raised to 15 mg/L. The effluent $Zn^{2+}$ concentration in the suspended growth bioreactor was very high (6.2 mg/L) at steady state on day 125 but lower for the bio-physical bioreactor (1.56 mg/L). The suspended growth and bio-physical bioreactors achieved $Zn^{2+}$ removal of 59 and 90%, respectively. The MLSS and MLVSS for both bioreactors decreased (4.6 and 2.6 g/L for the suspended growth bioreactor) and (5.9 and 3 g/L for the bio-physical bioreactor). With a remarkable decrease in the performance of the suspended growth bioreactor in phase 8, the experiments were terminated.

### 3.1. Discussion

It is well known that micro-organisms can oxidize organic contaminants to carbon dioxide but cannot oxidize heavy metals because metals are not biodegradable [15]. However, micro-organisms can alter the metal speciation and transform metals from one chemical form to another, which are easily precipitated or volatized from solution [15,16].

Micro-organisms are typically small in size and possess a high surface area to volume ratio, which provide a considerable contact area for interaction with metals in activated sludge process [16]. Micro-organisms possess a number of functional sites such as imidazole, carboxyl, amino, sulphydryl, sulfate, phosphate, carbonyl, thioether, phenol, hydroxyl moieties, and amides. These functional sites have different affinities for metals due to their cell wall compositions [17]. The cell wall of micro-organisms is also relatively abundant with macromolecules such as polysaccharide and glycoproteins (glucans, mannans, chitin, and phosphomannans) which contains metal binding ligands [18].

The basic principles for the removal of heavy metal by micro-organisms are bioaccumulation, biosorption,

and bioreduction. Different mechanisms such as precipitation, adsorption, complexation, and active intracellular transport are involved in bioaccumulation [16]. Heavy metal accumulation could occur through passive sorption (independent of metabolism) or by intracellular active uptake (metabolism dependent). Passive heavy metal accumulation could be affected by the surface properties of the micro-organisms such as charge, cellular functional groups, metal speciation, and chemistry [16]. For instance, the surfaces of the isolated cell wall of *Rhodococcus erythropolis* possess about three groups of metal binding sites [19] whereas numerous metal binding sites were detected on the cell surface of *Pseudomonas atlantica* [20]. Biosorption of heavy metal by micro-organisms involves various physico-chemical processes through which metal species are removed from aqueous solution and usually occur at the cell wall of micro-organisms [21]. The sorption of heavy metals by micro-organisms depends on the basic principle of electrostatic interaction between metal ions and the exposed functional groups on the microbial cell surface. Such interaction can cause the nucleation and build-up of more metal ions and counter ions at the reactive sites and also enable the aggregates to grow. Furthermore, micro-organisms can produce extracellular macromolecules such as protein, DNA, and RNA which have high metal binding capacity [16].

The surface characteristics of micro-organisms play a significant role for metal accumulation and the capacity of these functional groups to accumulate metals varies [16]. For instance, the bacteria strains which produced the LamB hybrids with the "His" chain in the outer membrane protein of *Escherichia coli* have a higher accumulating capacity (11-fold more $Cd^{2+}$) than cells without the "His" ligand on the protein molecule [22]. Micro-organisms can produce extracellular melanins in response to metal toxicity. Furthermore, substances within a growth medium can result to the formation of various functional groups on the cell surface of micro-organisms. For instance, cysteine, glucose, ammonium, and phosphate can insert the S and N-ligands, C-ligands, N-ligands, and P-ligands on the cell surface of *Saccharomyces cerevisiae*, respectively [23]. Cell density can also influence metal accumulation. At higher biomass concentrations, microbial cells in suspension can affix to each other and lower the contact between the cell surface area and the solution [16]. Several other physico-chemical factors such as pH and ionic strength also influence the magnitude of bioaccumulation.

Heavy metal can be toxic to micro-organisms at concentrations higher than the minimal inhibitory concentration [24]. However, some heavy metals are essential for microbial composition and activity [16]. Heavy metal tolerance could be expressed as the capacity to withstand metal toxicity through the intrinsic properties of the micro-organisms whereas resistance represents the capacity to survive metal toxicity through detoxification mechanisms. Micro-organisms can response to toxic metals by transporting the metals outside the cells through alkylation or efflux pumps, or by transformation into a harmless form by producing ligands [16,25]. The bacteria resistance to heavy metal toxicity can arise from non-specific mechanisms such as cellular impermeability or by specific resistance transfer factors that result in the uptake of free metal cations [25,26]. Micro-organisms can also respond to metal toxicity by producing metallothioneins (MTs) which are cysteine-rich protein molecules that can bind metal ions and sequester them into biologically innocuous forms. However, intracellular metal uptake by MTs has proved less effective than the extracellular uptake [27].

A removal trend was established for both the suspended growth and bio-physical bioreactors, respectively. In the suspended growth bioreactor, $Zn^{2+}$ removal efficiency increased with increasing influent $Zn^{2+}$ concentration from 0.5 to 1.0 mg/L (phase 3–4) but fluctuated with further increase in influent $Zn^{2+}$ concentration (phase 5–8). Similarly, effluent $Zn^{2+}$ concentration increased with increasing $Zn^{2+}$ concentration from phase 2 to phase 8. In the bio-physical bioreactor, $Zn^{2+}$ removal efficiency was stable from phase 2 to phase 6, increased in phase 7, and decreased in phase 8. The effluent $Zn^{2+}$ concentration increased from phase 2 to phase 6 but decreased from phase 7 to 8. Several factors such as pH, concentration, sludge concentration, metal species, and solubility influence metal toxicity to micro-organisms [28,29]. It is also well known that micro-organisms are sensitive to a wide range of organic and inorganic toxic compounds. At concentrations above their threshold, metabolic activities significantly reduce [30].

The performance of the suspended growth bioreactor decreased from phase 5 until phase 8. This indicated that the microbial threshold limit could have been attained. The addition of small concentration of heavy metals into a microbial environment results in cell growth until the threshold concentration is surpassed and a relative decrease in the stimulation effect is noticed [31]. Further increase in heavy metal concentration will adversely affect the cell growth until the complete decrease in microbial activity and system failure [31]. Thus, from phase 5 to phase 8, the suspended growth bioreactor was inhibited by toxicity and a relative decrease in the growth rate (MLVSS) was observed. Similar observation was made elsewhere [13]. As the

MLVSS concentration decreases in the suspended growth bioreactor, $Zn^{2+}$ removal efficiency decreased and the effluent concentration increased. The critical point for the suspended growth bioreactor was phase 4 (1 mg/L). Heavy metal inhibitory effect at higher concentration (phase 5) was observed and resulted to significant decrease in the removal of $Zn^{2+}$. A similar observation is reported elsewhere [32].

For the bio-physical bioreactor, no significant $Zn^{2+}$ impact was noticed throughout the study. Although $Zn^{2+}$ removal in phase 8 was high (90%), the effluent concentration increased (1.56 mg/L) and the MLVSS concentration decreased to 3 g/L. A slight decrease in biosorption was observed in the bio-physical bioreactor in phase 8. At the initial phases, $Zn^{2+}$ could build up in both bioreactors and cause damage on the living cells, resulting to partial loss of sorption abilities and the release of accumulated metal into the solution in the later phases [32]. Heavy metals can damage surfaces of living cells and leach accumulated compounds into the solution [33]. However, the bio-physical bioreactor demonstrated that immobilized biomass has higher potential for metal accumulation. GWTPS provided an immobile inert medium for biomass attachment. The higher $Zn^{2+}$ removal in the bio-physical bioreactor could be attributed to direct metal binding to the micro-organisms, chelation to GWTPS, and complexation. $Zn^{2+}$ removal of 90% from the bio-physical bioreactor in phase 8 indicated that the bioreactor was yet to reach its critical point and can tolerate higher $Zn^{2+}$ concentrations. For instance, it is reported that the accumulation of $Cd^{2+}$ on the *fungus "Rhizopus oligosporus"* was higher in the cells immobilized with polyurethane foams (34.25 mg/g) than suspended cells (17.09 mg/g) [34]. Furthermore, in the case of GWTPS, the immobilized material (matrix) can contribute its own properties ($Fe_2O_3$) to enhance metal accumulation and precipitation.

## 4. Conclusion

Heavy metal was successfully removed from aqueous solution in this study. Two identical bioreactors were operated in the suspended growth and bio-physical modes, respectively. It was observed that the removal capacity of both bioreactors differed based on the concentration of heavy metal applied. While the performance of the suspended growth bioreactor decreased in phase 5 due to heavy metal inhibitory effect and toxicity, the bio-physical bioreactor was stable throughout the study. In phase 8, the removal efficiency of the suspended growth and bio-physical bioreactors were 58.7 and 90%, respectively. Micro-organisms offer high potential for metal removal with appreciable operational flexibility *in situ* or *ex situ* using various bioreactor configurations. Biological processes are therefore a viable option for heavy metal removal because micro-organisms can accommodate a wide variety of contaminants including both organic and inorganic compounds. It is important to note that micro-organisms do not destroy metals but transform them into more innocuous forms. This study, therefore demonstrates that solid waste materials from groundwater treatment plants could be put to beneficial use in biological systems, to improve microbial resistance and tolerance toward heavy metal toxicity.

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