Kinetics of heavy metal removal in a suspended and immobilized bioreactors

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Abstract. The capacity of microorganisms to remove heavy metal from wastewater has been a subject of diverse interest. Whereas some heavy metals are essential for effective microbial activity, some heavy metals could be toxic to the microorganisms at concentrations higher than their minimal inhibitory limit. The kinetics of Zn$^{2+}$ removal from aqueous solution was evaluated in terms of substrate removal rate for two identical suspended and immobilized bioreactors. The suspended growth bioreactor was used as a control system (CS) and contains only biomass. The immobilized bioreactor (IB) contains both biomass and microwave incinerated rice husk ash (MIRHA). The bioreactors were operated at a fixed HRT of 29.1 hours, whereas Zn$^{2+}$ influent concentration was varied in the range of 0.5, 1, 2, 5, 10 and 15 mg/L. At steady state conditions, the results show that Zn$^{2+}$ removal was in the range of 72, 75, 72.5, 68.2, 70.3 and 58.7% for CS, whereas it was in the range of 88, 90, 83, 88.6, 86.2 and 83.7% for IB. The substrate removal rate was found as 1.1856 g/L.d for CS and 4.2693 g/L.d for IB. The results clearly show that Zn$^{2+}$ removal was more favorable in IB, indicating that the performance of the bioreactor was enhanced by the addition of MIRHA.

1. Introduction

Biological wastewater treatment system is an age long treatment technology, associated with numerous benefits such as low capital and operational cost, effective organic matter degradation and biogas production. It has been widely used in various manufacturing applications such as food and beverages, pharmaceuticals, refineries and petrochemical industries as secondary treatment. The success of biological treatment technology has largely dependent on the capability of microorganisms to biodegrade wastewater contaminants into acceptable end products. However, the application of biological treatment technologies for the treatment of wastewater from some industries which utilize heavy metals such as tannery and electroplating are greatly hinder by the inability of microorganisms to biodegrade heavy metal. Whereas microorganisms can easily oxidize organic matter to carbon dioxide and other acceptable end products, it can only alter metal speciation and transform it from one chemical state to another. Heavy metals inhibits microbial activities by blocking the essential functional groups on microorganisms, displace essential metal ions or modify the active form of biological molecules [1]. However, while some heavy metals are toxic to microorganisms even at low concentrations, others are essential for microbial composition and activity.

Microorganisms can resist and tolerate metal toxicity. Depending on the intrinsic and detoxification mechanisms of the microorganisms as well as the environmental factors, some microorganisms can grow in the presence of high metal concentration [2]. The intrinsic mechanisms include the ability of microorganisms to tolerate and cope with metal toxicity whereas detoxification mechanisms is the stimulated microbial direct response to resist metal toxicity [2]. The bacteria resistance to heavy metal toxicity can further arise from non-specific mechanisms such as cellular impermeability or by specific resistance transfer factors [3]. The microbial resistance and tolerance to metals are based on the two
significant principles of bioaccumulation and biosorption. In both bioaccumulation and biosorption, heavy metal removal is based on the interaction of the metal ions with the functional groups of the microorganisms [2, 4].

The immobilization of microorganisms has demonstrated higher potential for metal accumulation [5]. The advantage of immobilization is that the immobilization material, howbeit non-toxic, can contribute its properties in various forms for metal accumulation. Microorganisms can grow on insoluble inert material either attach to the material surface or remain suspended in the bulk solution. However, it is well known that those microorganisms which attach on the surface of the inert material have faster growth rate than the suspended counterpart as a result of their exposure to available food resources [6].

The knowledge of the substrate removal rate by biological system is necessary in order to evaluate the performance of the bioreactor. It gives information about the state of the microorganisms, level of bioaccumulation and biosorption, the rate at which metal speciation are altered and a basis for process design and scale up. The objective of this study was to compare the performance of a suspended and an immobilized bioreactor on the basis of their substrate removal rate, treating a heavy metal laden aqueous solution.

2. Materials and Methods
The materials and methods used in this study are presented in this section.

2.1. Bioreactor setup
Two rectangular bioreactors of equal size (8.5L) were made using acrylic glass and have a thickness of about 5 mm. An influent port was made towards the bottom of the bioreactors whereas the effluent port was located towards the top of the bioreactor opposite the influent port. A settling unit was created by means of baffle towards the effluent port to prevent wash-out of biomass into the effluent unit. Horizontal air tube diffusers were installed at the bottom of the bioreactors to provide oxygen in upflow pattern. The bioreactors were then started with a seed sludge from a local domestic wastewater plant with a mixed liquor suspended solid (MLSS) concentrations of about 4000 mg/L. The solid retention time (SRT) was fixed at 30 days through sludge wasting.

2.2 Preparation of immobilized material
The immobilized material was prepared from rice husk. Rice husk was purchased from a local retail shop in Malaysia. Rice husk is an abundant agricultural waste materials. It was washed, dried for 2 hours at 105 °C using a conventional oven and carbonized for 2 hours at 800 °C using a Microwave incinerator. Microwave incinerated rice husk ash (MIRHA) was then produced and stored. No additional modification was carried out on MIRHA.

2.3 Wastewater preparation
Synthetic wastewater was used in this study and was prepared by dissolving appropriate amount of an organic substrate (Purina Alpo) in tap water according to the experimental plan. Synthetic wastewater was used in order to provide consistent loading on the bioreactor. A specific volume (0.15 mL) of phosphate buffer was added to the wastewater for pH stability. The wastewater has a C:N:P ratio of about 100:24:3 which meets the basic domestic wastewater nutrient requirement. The synthetic wastewater was characterized and has an average influent COD, BOD₅ and TSS concentrations of 500, 250, and 300 mg/L, respectively. Various Zn²⁺ concentrations was simulated in the wastewater by the addition of appropriate quantity of Zinc chloride. Zn²⁺ concentrations was varied in the range of 0.5, 1, 2, 5, 10 and 15 mg/L, respectively.

2.4 Bioreactor operation
The experiment was conducted at a fixed influent flowrate of 7 L/d, corresponding to a hydraulic retention time (HRT) of 29.1 hours. For the suspended growth bioreactor (CS), the mixed liquor volatile suspended solids (MLVSS) from a local sewage treatment plant was used only. For the immobilized bioreactor (IB), first, the MLVSS from a local sewage treatment plant was used. Then,
MIRHA was added to the bioreactor (IB) to immobilize the microorganisms. The experiments were conducted in 8 different phases. In phase 1, acclimation of the biomass to the wastewater was carried out in both CS and IB. In phase 2, MIRHA was added to the IB to acclimate with the biomass and initiate the immobilization process and was maintained at 2000 mg/L throughout phase 2 by daily adding MIRHA to IB. The SRT in phases 1 and 2 were controlled through daily recycling and wasting. MIRHA was daily added to IB. In phase 3, MIRHA concentration of 100 mg/L was added daily to IB, taking into consideration the MIRHA wasted daily. Then, 0.5 mg/L of Zn\(^{2+}\) was fed to both the CS and IB in phase 3. Sludge wasting was terminated in this phase and only recycling was conducted. In phases 4, 5, 6, 7 and 8, Zn\(^{2+}\) concentrations of 1, 2, 5, 10 and 15 mg/L was fed to both reactors, respectively. Zn\(^{2+}\) concentration was measured from the effluent port on a daily basis in accordance with Hach colorimetric (Zincon) method.

2.5 Substrate removal rate

The substrate removal rate for both CS and IB was evaluated from the average of the steady state data in each phase using the expression below:

\[
\frac{Q(S_o - S_e)}{V} = K
\]

where, Q is the influent flowrate (L/d), S\(_o\) and S\(_e\) are the influent and effluent Zn\(^{2+}\) concentration, V is the reactor volume (L).

A plot of Q(S\(_o\)-S\(_e\))/V vs S\(_e\) should give a straight line from which the substrate removal rate (K) is obtained from its slope.

3. Results and discussion

The steady state result for all experimental conditions for CS and IB is summarized and presented in Table 1.

![Table 1. Steady state results.](image)

The obtained results clearly show that IB performance was superior to CS at all experimental phases investigated. After the addition of MIRHA to IB in phase 2 (day 13-29), a Zn\(^{2+}\) concentration of 0.5 mg/L was fed to CS and IB in phase 3 (Day 30-47). The steady state result shows that a removal of 72 and 88% was achieved in both CS and IB, respectively. In phase 4 (Day 48-63), the influent Zn\(^{2+}\) concentration was increased to 1 mg/L and the removal efficiency for both CS and IB was 75 and 90%. In phase 5, 6, 7 and 8, the removal efficiency of Zn\(^{2+}\) was in the range of 72.5, 68.2, 70.3 and 58.7% for CS and 83, 88.6, 86.2 and 83.7% for IB, respectively. The IB demonstrated high performance in all phases when compared with CS.
The known principle for heavy metal removal by microorganisms include bioaccumulation and biosorption. Bioaccumulation involves several other mechanisms such as adsorption, precipitation, complexation and active intracellular transport. Several other physicochemical factors such as pH and ionic strength also influence the magnitude of bioaccumulation. Accumulation of heavy metals could specifically vary among microorganisms and the potential formation of minerals within the nucleation sites of the microorganism can occur [2]. It is well known that microorganisms 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. Additionally, some metals can undergo transformation by redox or alkylation processes and form compounds that significantly differ from the original both in mobility and toxicity [2]. It has been reported that the absence of sharp inflections during the titration of bacteria, fungi and yeast suggests that various abundant ligands which have the capacity to bind metals and other elements were present on microorganisms [7]. Additionally, sorption of microorganisms by heavy metals involves several steps depending on the type of microorganisms. The biosorption mechanisms greatly rely on the electrostatic attraction between heavy metals and the reactive groups on the surface of microorganisms which causes these sites to nucleate and accumulate more metals as well as counters ions. The accumulated metals which have nucleated then grow as an aggregate [2]. Thus, the removal of Zn\(^{2+}\) by both CS and IB was achieved through bioaccumulation and biosorption microbial mechanisms.

The superior performance of the IB system clearly shows that the addition of MIRHA enhanced the activities of the microorganisms for heavy metal accumulation and biosorption. In a related study, the performance of a bioreactor immobilized with groundwater treatment plant sludge (GWTPS) was compared with a suspended growth bioreactor for Zn\(^{2+}\) removal. The authors found that the performance of the GWTPS bioreactor was superior to that of the suspended growth bioreactor [8]. Thus, while it is certain that microorganisms can tolerate heavy metals up to their minimum inhibitory limits, the addition or immobilization of microorganisms with specific materials can further improve heavy metal accumulation.

3.1 Substrate removal rate

The substrate removal rate for CS and IB systems are presented in Fig. 1 and Fig. 2, respectively. The results confirm our earlier position that MIRHA enhanced the accumulation of heavy metals in the microorganisms. The substrate removal rate for CS system was found as 1.1856 g/d.L whereas it was 4.2693 g/L.d for the IB system. In a similar study, it was observed that toxic heavy metals effectively accumulated in *Saccharomyces cerevisiae* immobilized in sol-gel matrix. The entrapment of *Saccharomyces cerevisiae* into a sol-gel matrix enhanced the accumulation and overall uptake of some heavy metals investigated [9]. Additionally, *Rhizopus oligosporus* reportedly demonstrated high Cd\(^{2+}\) accumulation capacity when immobilized compared to the suspended system (free cells) [10].

The high performance of immobilized systems for heavy metal accumulation has been attributed to various phenomenon. One of such phenomenon is the composition of the growth medium (immobilized material). The composition of the immobilized material could contribute to heavy metal accumulation through the formation of functional groups on their cell surface. For instance, it is reported that cysteine can insert S- and N-Ligands, ammonium N-Ligands, glucose C-Ligands and phosphate p-ligands on *Saccharomyces Cerevisiae*. A higher accumulation of Cu and Pb was observed when the microorganisms were grown in Cystein-rich media. On the other hand, addition of phosphate improved Zn selectivity [11].
The basic phenomenon in the enhancement of metal accumulation or uptake by immobilized systems could be through the contribution of the properties of the immobilized material for the resistance of metal toxicity, formation of extra ligands on the surface of the microorganisms that helps in the binding of metal, electrostatic attraction between heavy metals and the reactive groups of the microorganisms, electrostatic attraction between the heavy metals and the reactive groups of the immobilized materials and the nucleation and growth of the formed aggregates on the immobilized material. Additionally, chelation of heavy metals to various components of the media as well as the potential formation of various complexes could reduce the activities (toxicity) of free metals and ultimately hinders the capability of the metals to approach the microbial minimal inhibitory concentration [1]. For instance, it is reported that about 30-40% of mercury was found to form complexes with tryptone and yeast extracts of the components of Luria-Bertani broth (LB) during the growth of *Pseudomonas aeruginosa* [12].

Thus, the substrate removal rate for CS and IB systems were in the ratio of 1:3.6 respectively under the same experimental conditions.
4. Conclusion

The performance of two identical bioreactors operated as suspended growth system (CS) and immobilized bioreactor (IB) were evaluated for the removal of Zn$^{2+}$ at various influent concentrations. MIRHA was used as the immobilizing material. The results show that IB demonstrated superior performance compared to CS. At all the influent Zn$^{2+}$ concentration, IB demonstrated high removal efficiency. Additionally, IB also demonstrated higher substrate removal rate of 4.2693 g/L.d compared to the 1.1856 g/L.d for CS. Thus, the superior performance of the immobilized bioreactor in this study in terms of removal efficiency and substrate removal rate clearly shows that MIRHA, a low cost adsorbent produced from abundant agricultural waste material (Rice husk) can effectively enhanced the microbial tolerance towards heavy metal toxicity. The results also serve as a basis and provide kinetic information for the potential upgrading of the laboratory system into a pilot system for extensive use.

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