COPPER REMOVAL USING OCHROBACTRUM SPECIE ISOLATED FROM CONTAMINATED WASTEWATER

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ABSTRACT
Bacterial and metal removal have received considerable attention recently, due to the potential use of microorganisms for treatment of contaminated wastewater. Removal and recovery of heavy metal ions from polluted wastewaters has appeared to be the potential alternative method for conventional treatment of contaminated wastewater. The purpose of the present work was to study the removal capacity of toxic metal from contaminated wastewater by using ochrobactrum sp. isolated from contaminated wastewater using different pH and temperature. Bacteria species have a high surface area to volume ratio, due to their small size and thus, they can offer a large contact interface that would interact with metals ion. The results indicated that bacterial isolate Ochrobactrum sp. is a suitable biosorbent for the removal of Cu (II) ion from metal polluted wastewater and might be applicable to the development of possibly cheap biosorbent for removing and recovering copper from effluents.

KEYWORDS: Bioremoval; Copper; Ochrobactrum; Metal-polluted effluent

1. INTRODUCTION

Anthropogenic activities, such as mining processes and the discharge of untreated industrial wastewater, have resulted in the accumulation of metals in the environment (Chipasa, 2003). The presence of heavy metals such as copper ions in water poses serious ecological and human health related hazards due to their toxicity and tendency to bio accumulate (El Baz *et al.,* 2015). Heavy metals usually form compounds that can be toxic, mutagenic and carcinogenic even in trace concentrations. Copper ions, are one of the most widely used heavy metals, largely used by electroplating industries and is extremely toxic to living organisms (Wang and Chen, 2009). Conventional methods of heavy metals from industrial effluents, such as activated carbon, reverse osmosis, chemical precipitation, electrochemical treatment, evaporation, ion exchange and membrane technologies were not cost-effective and have many other limitations (Volesky and Holan, 1995; Volesky, 1994; Gil *et al.,* 2018). Therefore the need for applying biological materials to serves as an alternative methods of metal removal and recovery system (Zhang *et al.* 2013; Guevara *et al.* 2012). High metal-binding capacities of several biological materials including bacteria, fungi and other microbial biomass have already been identified by many researchers (Davis *et al.* 2003; Minamisawa *et al.* 2004; Bruins *et al.* 2000). Microorganisms have evolved various mechanisms to respond to increasing heavy-metal stress through several mechanisms such as absorbing the metals to their cell walls, transport across the cell membrane, precipitation, complexation and oxidation reduction reactions (Kamika and Momba 2013; Batta *et al.* 2013). Bacteria cells are excellent bio-sorbent materials, because of their high surface to volume ratio and high content of potentially active chemisorption's sites such as teichoic acid in their cell wall (Joutey *et al.* 2015; Garg *et al.* 2012; Jadhav *et al.* 2010). The present study was conducted to characterize metal resistant bacteria, ochrobactrum sp. which was isolated from electroplating wastewater. The bacteria were found to have high copper removal capacity. The ultimate objectives of the present study were to develop a novel and economical methods for removing and recovering heavy metals ions from aqueous wastes using bacterial species as biosorbents (Girardeau *et al.* 2019; Chen *et al.* 2019).

2. MATERIALS AND METHODS

2.1 Sampling: Wastewater sample were collected from the electroplating industry (at Port Klang district, Selangor, Malaysia) that uses copper (Cu), cadmium (Cd) and lead (Pb) for plating. The sample was transported immediately to the laboratory in a sterile container and maintained at 4 °C (why?) for further studies (Gongora-Echeverria *et al.* 2018).

2.2 Sterilization of glassware and other materials

All glassware used in the experiment were thoroughly cleaned to sterile and get ready for use.. The distilled water used for serial dilutions was autoclaved at 121 °C for 15 minutes. The workbench was cleansed with 75 % alcohol prior and after every experiment (Kaminski *et al.* 2018).

2.3 Screening and isolation of bacteria

The wastewater sample was serially diluted and heavy metals resistant bacteria were isolated in nutrient agar (NA) yeast extract 3 g, beef extract 3 g, peptone 5 g, sodium chloride 5 g, agar 18 g, pH 7 and distilled water 1,000 ml supplemented with 50 mg/L of heavy metals. Sterilized at 121 °C for 15 minutes and allowed to cooled 40 °C (Pandit *et al.,* 2013; Kirmizakis *et al.,* 2019). Spread plate method was used and incubated at 37 ºC for 24-48 hours (Rajkumar *et al.* 2012; Sinha *et al.* 2013). Pure colonies differing in morphological structures were selected for further studies and sub cultured. All of the experiments were carried out in triplicate.

2.4 Metal removal studies

Heavy metals removal were performed using methods recommended by (Ahemad and Malik, 2012) with some little modifications. The metal tolerant bacterial culture was kept in agitation at 150 rpm. The removal studies of Cu were carried out under various temperatures of 27 ºC, 32 ºC and 37 ºC; and pH of 5, 7 and 9. The metals removal study was carried out with living bacteria cells in nutrient broth (NB) supplemented with 100 mg/L of Copper ion. The media was inoculated with an overnight culture of bacterial isolates *Ochrobactrum sp.* The flasks containing metal and bacterial isolate were incubated at 37 ºC for 96 hours in a constant shaking condition at 150 rpm. With flasks having no bacterial biomass serves as control running in concert. For every 24 hour 5 ml of the sample was taken from each of the experimental flask and centrifuged to separate the biomass suspended to have the supernatant. Atomic absorption spectrophotometer (AAS) was used for the analysis of the heavy metals and the bacterial growth absorbance was measured at 600 nm against the blank (Malik, 2004; Costa and Duta, 2001).

3. RESULTS AND DISCUSSION

Removal study of copper, using the bacteria strain *Ochrobactrum sp. KT964694* was conducted using initial copper concentration of 100 mg/L. Since metal-containing water and wastewater has variation in pH values from one location to another, it is essential to use solutions of diverse pH values to examine the influence of heavy metal bioaccumulation. In the experiment, initial pH was adjusted to the range (5, 7, and 9) and temperature (27 ºC, 32 ºC and 37 ºC) with the biomass free solution used as control. All experiments were in triplicate. The mean values for the percentage of heavy metal bioaccumulation, the optical density of copper removed by *Ochrobactrum sp.* against time interval at different pH and temperatures were shown in the Figure (1,2 and 3) respectively.

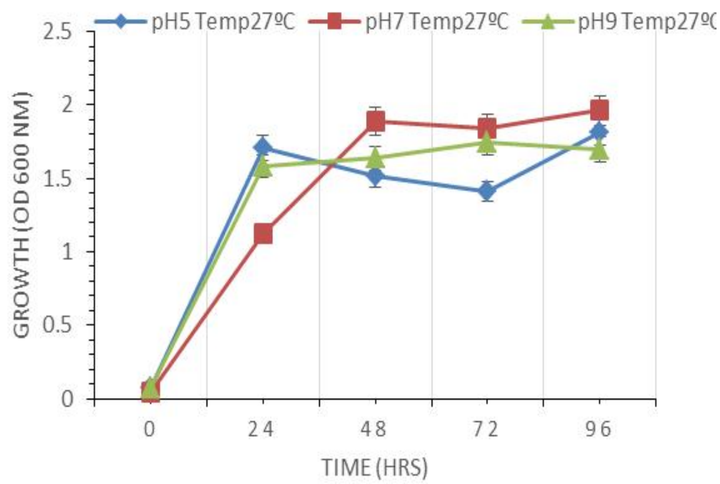

Figure1: Growth responses of (MH6) strain *Ochrobactrum sp.* in removal of copper at OD600 nm and temperature 27ºC and pH 5, 7 and 9

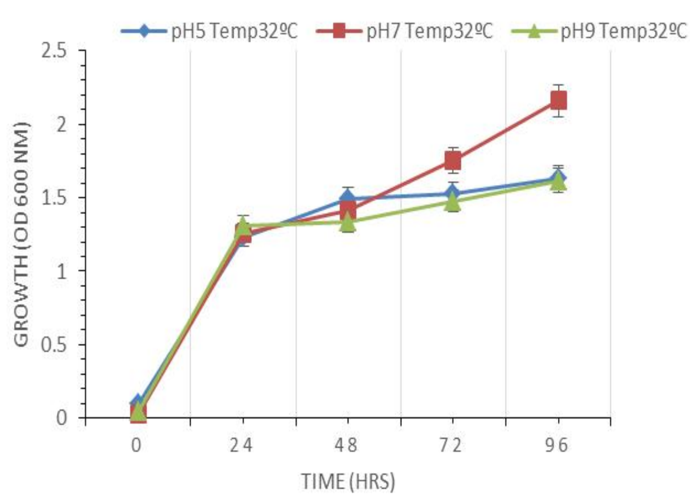

Figure 2: Growth responses of strain *Ochrobactrum sp.* in removal of copper at OD_{600} nm and temperature 32 °C and pH 5, 7 and 9.

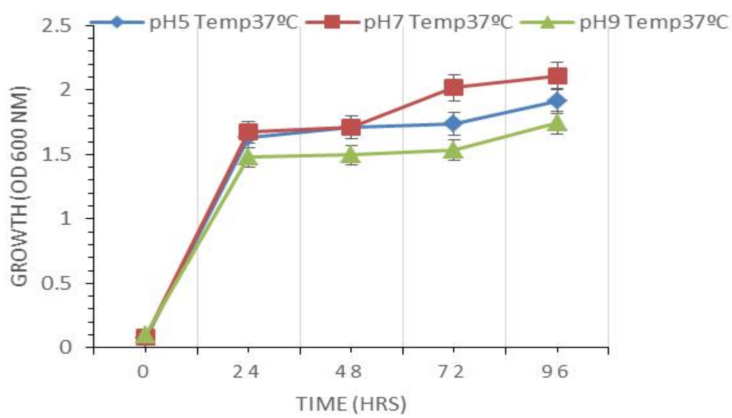

Figure 3: Growth responses of strain *Ochrobactrum sp.* in removal of copper at OD600 nm and temperature 37 ºC and pH 5, 7 and 9

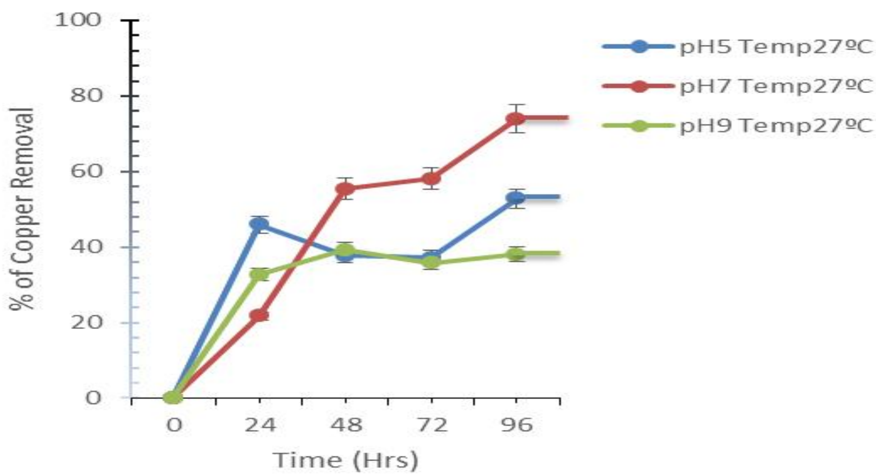

Figure 4: Copper ion removal by bacterial strain *Ochrobactrum sp.* at pH 5, 7 and 9 as well as temperature 27 °C and different time intervals.

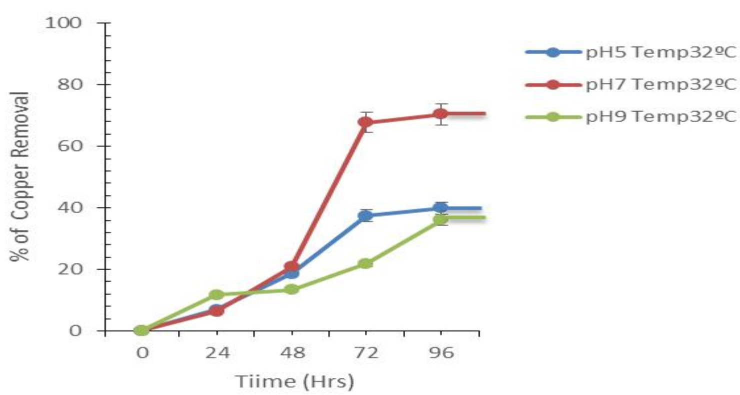

Figure 5: Copper ion removal by bacterial strain *Ochrobactrum sp.* at pH 5, 7 and 9 as well as temperature 32 ºC and different time intervals.

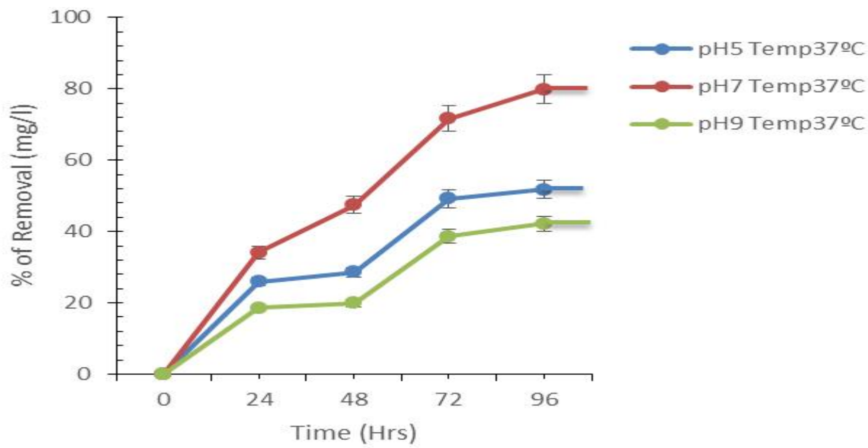

Figure 6: Copper ion removal by bacterial strain *Ochrobactrum sp.* at pH 5, 7 and 9 as well as temperature 37 °C and different time intervals

The presented data in the figures 4, 5 and 6 showed the percentage of copper removed by strain *Ochrobactrum sp.* At pH 5, 7, and 9 and temperatures 27, 32 and 37 ºC. The removal efficiency of the strain, an *Ochrobactrum sp*. at temperature 27 ºC was found to be maximum at pH 7 (73.9 %) and minimum at pH 9 (38.1 %) Figure 4. Likewise, at temperature 32 ºC copper removal was lower at pH 7 (6.4 %) after the first 24 hours but become higher (70.4 %) in 96 hours shown in Figure 5. Meanwhile, at 37 ºC the lowest was 18.6 % and 79.9 % (Figure 6). Analysis of variance revealed that there was a significant difference between temperature, pH and time interval $P \leq 0.05$. Based on the analysis, it can be concluded that there is the maximum percentage for copper bioaccumulation at pH 7 and 37°C temperature. Heavy metal accumulation by microbes basically involves using nonliving microbial biomass or other products called the biosorption process or using living bacterial cells an energy-dependent process called the bioaccumulation (Ozdemir *et al.* 2012). Bioaccumulation is the process by which living bacterial cells actively accumulate metal ions. The metal removal ability of gram positive bacterial cells might be due to carboxyl groups of peptide that supply negatively charged binding sites in gram positive cell wall. Andreazza *et al.,* 2010 reported that copper bioaccumulation by *Bacillus sp.* was maximum at temperature 35 ºC and pH 6 and the strain they isolated were able to remove more than 100 mg/L of copper within 24 hours. Similarly, (Kartic 2011) also reported maximum copper removal of about 88 % by *Bacillus* sp. at temperature of 35°C and pH 8.0. The ability of living bacterial cells to accumulate metal ions in a contaminated area is significantly affected by physical and other environmental factors like pH, temperature and concentrations of biomass (Virender Singh, 2010). Therefore, due to variation in the physicochemical properties of wastewater performing bioaccumulation studies under different pH and temperature is very essential because these factors affect the biological removal of contaminants and also influence microbial metabolisms and specifically stimulates or inhibit enzymatic activity in the microbial cells (Ozdemir *et al.* 2012; Perez Silva *et al.* 2009).

4. CONCLUSION

The strain *Ochrobactrum sp*., was a gram negative bacteria isolated from metal contaminated wastewater and was tested for its metal removal potential. It has been observed that the living cells of ochrobactrum sp. could adsorb high concentration of copper from synthetically wastewater. the capacity of the organism was optimum at pH 7, initial copper concentration of 100 mg/L was used. The obtained results showed that *Ochrobactrum* sp. is good adsorbing medium for metal ions and had high adsorption yields for the treatment of wastewater containing and may be applied to the development of potentially cost-effective biosorbents for the removal and recovery of copper from wastewater.

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