Screening and isolation of heavy metal tolerant bacteria in industrial effluent

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\textbf{Abstract}

Heavy metals tolerant bacteria were isolated from electroplating industry. The activities of electroplating and metal processing industries are regarded as one of the major source of heavy metal pollution. This paper present about the role of microorganisms in heavy metal removal from industrial waste water. In this study 21 bacterial colonies were isolated and tested in the presence of different metals. After screening five best isolates which showed high resistances were selected. The result reveals that all isolates MH1, MH4, MH6 MH15 and MH21 were able to tolerate 50 mg/l of Cr, Cu, Pb and Cd. Isolates MH1 and MH21 tolerate 200 mg/l of cadmium while isolate MH4 also showed high degree of resistance to copper. These indicated that the isolates can be used efficiently in removal of heavy metals in contaminated industrial effluents.

\textbf{Keywords:} Screening; isolation; heavy metals; bioremediation; bacteria

\section{Introduction}

The presence of heavy metals in industrial effluents is known to have major hazard to natural water, animal and human health. High concentrations all of heavy metals have deleterious effect on the environment [4]. Toxic heavy metals like Hg, Cr, Cu, Zn, Pb and Cd have no biological role they are well known for their toxicities, mutagenic, and carcinogenic impact on human beings and other living system especially those metals classified under priority

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list of pollutants. [1]. Untreated industrial waste water discharged to the nearby water bodies may cause severe ground water pollution [7].

The advance towards strict environmental regulation and water quality monitoring has improved public awareness on environmental quality and management. The Conventional methods used for the removal of heavy metals in contaminated effluent includes electrochemical treatment, ion exchange, evaporation, chemical precipitation, reverse osmosis, and sorption, however all these physical and chemical methods were observed to have technical and economical constrains like high cost of operation and release of chemical and huge sludge to the environment as by-product therefore the need to replace them with cost effective and environmental friendly biological method of treatments [17].

Microbes, has the capability of consuming organic waste. When the microorganisms consume waste, they convert the waste into nontoxic by products and in the process of this conversion they actually produce many metabolites to degrade the complex waste into simple compounds this is because microorganisms have developed many resistance mechanisms to survive the presence of toxic heavy metals in their environment [19]. Among the mechanisms developed by microbes include, metal sorption, extracellular precipitation uptake and accumulation, mineralization and enzymatic oxidation or reduction to a non-toxic form, and efflux of heavy metals from the cell were reported by many literatures [6,10,12].

2. Materials and methods

2.1 Wastewater sampling

Effluent samples were collected from outlet of Landchard Electroplating Industry in Klang, Selangor Malaysia. The sampling was carried out at about 12 noon which was the peak production time for the industries observed. Samples were collected in a screw cap sterilized sampling bottles acidified and aseptically transported to the laboratory in an ice-bucket the sample temperature was maintained at approximately 4°C to prevent from contamination and allow the sample to stay longer [5,8].

2.2. Measurement of physicochemical parameters

Physicochemical parameters like temperature, pH, turbidity, conductivity, COD, TSS, DO and BOD of the wastewater sample was also determined [2].

2.3. Sterilization of glassware and other materials

All glassware used were thoroughly washed with deionized water and detergent, rinsed and allowed to dry. The glassware were then enfolded with aluminium foil and sterilized. 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 and all experiments were conducted in three replicate and the results were statistically analysed using mean, standard deviation and student t test to examine the significance differences.

2.4. Screening and isolation of bacteria

Heavy metals tolerant bacteria were isolated on nutrient agar supplemented with 50 mg/l of Cd, Cr, Pb and Cu. The nutrient agar was sterilized at 121°C for 15 min and allowed to cool 40-45 °C. Then the metals were added to the nutrient agar and transfer into petri plates. The waste water sample was serially diluted in which 9ml of sterile
saline water in 6 test tubes and then 1ml of sample was added to the first test tube to have $10^1$ repeated up to $10^{-6}$ then 0.1 ml of the dilution was spread on the surface of the agar plates and incubated at 36-37°C for 2 days colonies differing in morphological appearance were selected for further studies and sub cultured on the same media [11,14].

3 Results and discussion

3.1. Physicochemical characteristic of industrial effluent

The physicochemical parameters were measured using standard methods. The physicochemical parameters of the effluents sample were shown in Table 1. The effluent was reddish brown in colour, temperature, dissolve oxygen, turbidity, pH and conductivity were measured in-situ using YSI 52, portable DO meter, turbidity meter. The temperature of the effluent was 35.2 °C at pH of 4.9. This indicated that the effluent sample were slightly acidic in nature. If water becomes very acidic or alkaline, the concentrations of microorganism slowly reduce. Most microorganisms cannot tolerate pH levels above 9.5 or lower than 4.0 [15].

Generally, the optimum pH for bacterial growth is between 6.5 and 7.5. Turbidity of the effluent was found to be 9.43 ntu. Dissolved oxygen is an essential factor used for water quality control, effect of waste release on a surface water source is mainly determined by the oxygen balance of the system and its existence is essential in maintaining biological life within the water body (Barman et al., 2001) Dissolve oxygen was found to be 8.3 mg/l. while the Total Dissolved solids (TDS) and Total suspended solids (TSS) in the effluent samples were measured and found to be (20.1) mg/l and (28.7) mg/l. Other important parameter like BOD and COD were similarly measured BOD and COD tests shows the measure of relative oxygen depletion in waste water. Both have been extensively adopted as a measure of pollution effect. The BOD test measures the oxygen demand of biodegradable pollutants while the COD test measures the oxygen demand of oxidizable pollutants the COD of the waste water is generally 2 to 3 times than that of BOD [9]. In the same way in this work the COD and BOD were found to be (65.30) mg/l and (34.3) mg/l respectively. Similar results reported by other researchers [13, 16, 18].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>35.2°C</td>
</tr>
<tr>
<td>pH</td>
<td>4.9</td>
</tr>
<tr>
<td>Turbidity</td>
<td>9.43 ntu</td>
</tr>
<tr>
<td>TDS</td>
<td>20.1 mg/l</td>
</tr>
<tr>
<td>TSS</td>
<td>28.7 mg/l</td>
</tr>
<tr>
<td>DO</td>
<td>8.3 mg/l</td>
</tr>
<tr>
<td>COD</td>
<td>65.30 mg/l</td>
</tr>
<tr>
<td>BOD</td>
<td>34.3 mg/l</td>
</tr>
</tbody>
</table>

3.2 Isolation of Bacteria from industrial effluents

In this study, the population of bacterial present in the sample was range from 200 – 300 colonies per 100 ml at different sample location. A total of 21 bacterial single colonies were isolated which can tolerate 50 mg/l of Cd, Cr, Pb and Cu in nutrient agar. Five best isolates MH1, MH4, MH6, MH15 and MH21 were selected based on degree of resistance to heavy metals. Bacteria have metal binding abilities and they are known to exhibit not only tolerance to metals but also their detoxification [11].
3.3. Colony Morphology

Copper resistant isolate (MH4) was found to be round in shape and milky in colour, whereas the elevation and margin of the colony were entire margin and raised. Also the isolate that show high tolerant to Cadmium (MH1) showed a similar colony morphology with the copper resistant isolate (MH4). Chromium resistant isolate (MH21) and lead resistant isolate (MH6) have the same colony morphology and characteristics in which colony colour, colony margin and colony elevation were found to be milky white, convex and undulate, respectively. The colonies differ in terms of their shape. Where (MH6) has round shape while (MH21) was irregular. While isolate (MH15) have distinct characteristics because of its colour, which observed as yellowish with round shape and acquires entire margin and flat colony elevation as shown in table 2.

Table 2 The details colony morphology of each isolates.

<table>
<thead>
<tr>
<th>Isolate name</th>
<th>Shape</th>
<th>colour</th>
<th>Margin</th>
<th>Elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH1</td>
<td>Round</td>
<td>milky</td>
<td>Entire</td>
<td>Raised</td>
</tr>
<tr>
<td>MH4</td>
<td>Round</td>
<td>milky</td>
<td>Entire</td>
<td>Raised</td>
</tr>
<tr>
<td>MH6</td>
<td>Round</td>
<td>Milky white</td>
<td>Undulate</td>
<td>convex</td>
</tr>
<tr>
<td>MH15</td>
<td>Round</td>
<td>yellowish</td>
<td>Entire</td>
<td>flat</td>
</tr>
<tr>
<td>MH21</td>
<td>Irregular</td>
<td>Milky white</td>
<td>Entire</td>
<td>convex</td>
</tr>
</tbody>
</table>

4. Conclusion

The capability of bacteria resistance against different heavy metals may offer a beneficial tool for the simultaneous monitoring of many contaminants and pollutants in the environment. It is noticeably evident that domestic and industrial wastes toxic effects are the reason why bacteria developed resistance mechanisms along with the hazard of human health and environment. Therefore, the study is very useful to suggest that the possible impact of metal contaminated locations in human life may be greater than the direct consequence of the pollution. Based on the result obtained all isolates MH1, MH4, MH6 MH15 and MH21 were able to tolerate 50 mg/l of Cd, Cr, Pb and Cu. For MIC the isolate MH1 and MH21 tolerate 200 mg/l of cadmium while isolate MH4 also showed high degree of resistance to copper up to 200 mg/l and MH6 showed good tolerance against the metals used in the study. These shows that the bacterial species isolated can be used as a bioremediation tool for the treatment of effluent containing metals like cadmium, copper and lead.

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