THE REMOVAL OF Cd, Cr, Cu, Ni, AND Pb FROM A SYNTHETIC WASTEWATER EFFLUENT BY AN ENVIRONMENTAL BACTERIAL CONSORTIUM

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ABSTRACT

This study describes the removal of heavy metals (Cd, Cr, Cu, Ni, and Pb) from a synthetic wastewater effluent by consortium culture (CC), an environmental bacterial consortium. The metal removal capability of growing (active) and non-growing (inactive) CC cells in heavy metal containing synthetic wastewater (SWW) and deionised distilled water (ddH2O) were examined by determining percentage metal removal (% MR) over a 7 day period. Metal removal capability was tested at both high (1/5) and low (1/10) metal concentration strengths to imitate occurrence of heavy metal pollution in the natural environment due to industrial activities. Growing cells of CC showed higher removal of Pb (49-69%), Cu (45-61%) and Cr (55-67%) in SWW whereas non-growing cells removed more of Cd (100%) and Ni (72-94%) (p < 0.05). It was observed that non-growing cells achieved equilibrium earlier with quicker metal removal (by day 1-3) as opposed to growing cells. However, % MR by growing cells was higher in samples with elevated initial concentration of total metals (p < 0.05). Results of this study indicate the possibility of employing CC in both growing (active) and non-growing (inactive) variety to fulfill varying waste conditions and to cater for in situ and ex situ heavy metal laden waste treatment.

Key Words: Synthetic wastewater, Heavy metal, Consortium culture, Metal removal, Metallurgy

INTRODUCTION

The nature of heavy metals as being persistent and the ability to accumulate in the environment unlike degradable organic pollutants raises many questions that need to be scrutinized extensively in relation to heavy metal laden waste treatment, waste minimization options and approach. Environmentalist and scientist alike agree on the hazardous and detrimental effect of heavy metals towards men and nature.1,2 As industries thrive everywhere, more emphasis...
has to be given towards the by-products of manufacturing which comes in the form of wastes. Manufacturing and processing activities from any metal-based or metallurgical industries, laboratories, petrochemical facilities, amongst others contribute to the release of heavy metal ions into our water bodies. These can be due to the discharge of untreated waste effluent that pose the utmost threat, accidental leakage and spillage of chemicals and chemical waste during transport, storage and disposal. Apart from that, natural origins such as from automobiles, surface runoffs, leachates from dumping grounds or sewage further add to the prevalence of heavy metal contamination in our water bodies. It has been shown that urban runoff pollutants consisting of total suspended solids (TSS), nutrients (phosphorus and nitrogen), heavy metals (Pb, Zn, Cu, Ni, Cr) and hydrocarbons (PAHs, PCBs) pose a major source of water quality problems which leads to public health risk and deteriorating environmental quality. Among the challenges faced by the industry these days is to comply with environmental regulations that requires heavy metal containing wastewater to be treated prior to discharge. The waste treatment options available currently for heavy metal containing waste, i.e. physico-chemical based methods can either be too costly or technically incompatible with the ever-changing conditions and content of any waste effluent. Moreover, the continuous use of chemicals and the chemical sludge resulting from these processes aggravates the issue. A more suitable suggested alternative is to employ biological based materials to sorb heavy metal ions. Microbial-metal interactions have been widely exploited to understand the heavy metal ions removal process from aqueous solutions.

Besides, microbial biomass can be utilized in various forms i.e. active cells, inactive cells or immobilized with or without pretreatment. Process efficiency and effectiveness however will depend upon the metal removal capability of the biomass. Ideally, the proposed system should be able to function at broad concentration ranges, applicable to different and mixed metal species, response to varying environmental conditions, and have metal retaining capability. Bacterial based system as being dynamic will be able to cater to this need. Eventually, this will open up the possibility to use the system to treat other wastes and pollutants as well thus reducing overall waste quantity and operating cost. This paper reports the investigation of consortium culture (CC) an environmental mixed bacterial culture, in both active and inactive forms to remove heavy metals from synthetic wastewater solution. This will be useful to convey further data in the development of a bacterium-based biosorbent for treating industrial waste and wastewater.

AIMS AND OBJECTIVES

1. To evaluate heavy metal removal capability of inactive cells of CC in ddH₂O.
2. To evaluate heavy metal removal capability of active cells of CC in synthetic wastewater.
3. To examine the effect of multi-metals towards individual metal removal in synthetic wastewater.

MATERIAL AND METHODS

Bacterial culture and growth conditions

This study was carried out by using a mixed bacterial culture, collectively known as
consortium culture (CC). The mixed culture was sourced from a pool of bacterial isolates originating from point and non-point sites of areas related to metal-based activities. Consortium culture (CC) was maintained in a basal medium containing yeast extract (0.5 g/L), peptone (0.5 g/L), and NaCl (8.5 g/L) as either a growth culture (without metals) or an acclimatized culture (with 1 mg/L of each Pb(II), Cu(II) and Cr(VI) and subsequently increased to 10 mg/L each), with fortnightly media refreshments. The bacterial cultures were grown at room temperature (28-30°C) at an initial pH of 6.8 ± 0.2. Bacterial growth was monitored by optical density (OD) at 600 nm (spectrophotometer; Hitachi U1100, Japan); plate counts (cfu/mL) and correlated dry weight (g) of biomass.

Inoculum preparation

Starter cell inoculum was prepared by combining aliquots (0.5 % v/v) from growth and metal acclimatized cultures into 10 mL basal media as described previously. Initial pH was set at 6.8 ± 0.2 and incubated under static conditions at room temperature for 48 h. Cell biomass was separated by centrifugation (4000 rpm, 10 min); pellets were washed, re-centrifuged and rinsed twice before re-suspended in saline. An inoculum size of 1% (v/v) standardized to cell density at OD600 of 0.500 is used.

Synthetic wastewater (SWW) preparation

This synthetic wastewater media consisted (in mg/L) of the following: yeast extract (200.0), peptone (300.0), NaCl (15.0), KCl (7.0), CaCl2 (7.0), MgSO4·7H2O (5.0), and NaHCO3 (105.0). The mixture was dissolved with tap water and initial pH was noted before autoclaved for 15 min.

Ionic strength of SWW

The ionic strength (I, M) of the SWW was determined as follows:

\[ I = \frac{1}{2} \sum_{i} C_{\text{ion}} Z_{i}^{2} \]

where, \( C_{\text{ion}} \): concentration of ionic species present in the solution (M), and \( Z_{i} \): charge of species in the solution, (i).

Heavy metal removal study

The basis for the design of this study imitated the concentration of heavy metals (in the range of 0.24-80.50 mg/L) and types of heavy metals (Cd, Cr, Cu, Ni and Pb) found at the respective local sampling sites during the initial phase of the project (unpublished data). The experimental design is presented in Table 1. Metal removal capability is tested at 1/5 (Set A) and 1/10 (Set B) strengths of initial total heavy metals concentration to reflect the high (Set A) and low (Set B) occurrence of heavy metal pollution in the field. Two different solutions were used, i.e. synthetic wastewater (SWW) for growing (active) cells and deionised distilled water (ddH2O) for non-growing (inactive) cells. For tests with growing cells; 5 mL of culture aliquot (1% v/v) was inoculated into the SWW. On the other hand, 50 mL cell suspension of non-growing cells was inoculated into ddH2O. Test media contained the respective concentration of heavy metals to be tested (Cd, Cr, Cu, Ni and Pb). Final volume was made to 500 ml.
The experiment was performed in semi-continuous mode at room temperature, shaken at 150 rpm (orbital shaker, Yihder TS-580) and the percentage removal of metals (MR %) was monitored throughout the 7 days. Initial pH was noted and no subsequent adjustments were made. Initial runs were made at the said metal concentrations to ensure no precipitation would occur.

Table 1: Experimental design and distribution of total heavy metal concentration

<table>
<thead>
<tr>
<th>Set/Concentration (mg/L)</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
<th>Total</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration at site</td>
<td>0.24</td>
<td>22.52</td>
<td>17.62</td>
<td>1.98</td>
<td>80.50</td>
<td>122.8</td>
<td>24.57</td>
</tr>
<tr>
<td>Set A (1/5)</td>
<td>0.05</td>
<td>4.50</td>
<td>3.52</td>
<td>0.40</td>
<td>16.10</td>
<td>24.57</td>
<td>4.91</td>
</tr>
<tr>
<td>Set B (1/10)</td>
<td>0.02</td>
<td>2.25</td>
<td>1.76</td>
<td>0.20</td>
<td>8.05</td>
<td>12.28</td>
<td>2.45</td>
</tr>
</tbody>
</table>

The concentration of total metal in the media was determined daily for 7 days. For analyses purposes, 5 mL aliquots were drawn out daily, centrifuged (4000 rpm, 10 min), and the supernatant separated. The pellet was suspended in 5 mL of ddH₂O and re-centrifuged. This step was repeated twice to clean the cell biomass from media residues and non-sorbed metals. Cell biomass was then digested with concentrated HNO₃ at 60-65 °C for 15-16 h prior to total metal determination. Concentrations of Cr(VI) in the media were determined spectrophotometrically using the diphenylcarbazide method, and total metal concentrations in the media and the cells were measured by atomic absorption spectrophotometer (AAS; Perkin Elmer PE 1100B). The ability of cells to remove metals is reported as percentage metal removal, MR % given as follows:

\[
% \text{ Metal removal} = \frac{C_p}{C_i} \times 100\%
\]

Where, \(C_p\) is the amount of metal (mg/L) contained in the biomass and \(C_i\) is the initial metal concentration (mg/L). All tests were done in triplicates and mean values are reported. Test readings obtained were deducted with values measured in control sets to substantiate for abiotic metal losses.

Heavy metals stock solution

Metals stock solutions were prepared from the following salts with NANOpure ddH₂O: Cd(II) from Cd(NO₃)₂.4H₂O, Cr(VI) from K₂CrO₄, Cu(II) from Cu(NO₃)₂.2.5H₂O, Ni(II) from Ni(NO₃)₂.6H₂O and Pb(II) from Pb(NO₃)₂. Test solutions were prepared by diluting stock solutions (1000 mg/L) to the desired concentrations in ddH₂O.

Statistical analysis

Experimental data were subjected to statistical analysis for mean tests, t-tests, Levene tests and the analysis of variance (one-way ANOVA) by SPSS (SPSS Inc., Chicago, USA). Significant levels were set at \(\alpha = 0.05\).
RESULTS AND DISCUSSION

Synthetic wastewater (SWW) ionic strength
The ionic strength (I, M) of the SWW was in the $9.6669 \times 10^{-4}$ - $9.6648 \times 10^{-4}$ M range at pH 5.7 - 5.8 for Set A (1/5 metal strength) and in the $9.6609 \times 10^{-4}$ - $9.6601 \times 10^{-4}$ M range (pH 6.1 - 6.2) for Set B (1/10 metal strength). The normal ionic strength level for fresh water in the environment falls < 0.01 M (soft water) and < 0.1 M (hard water). Hence the ionic composition and ionic strength (I ~ 0.001 M) of the SWW used can represent real environmental water sample as it was within the reported range.

Heavy metal removal study
The MR % of both cell populations in SWW (for active cells) and ddH$_2$O (inactive cells) was followed over a 7 day period. Mean MR % (over 7 days) and maximum MR % (attained during the 7 day period) were compared at higher metal concentration (in set A, 1/5 strength) and at lower range (set B, 1/10 strength) to ascertain each population’s capability to remove metals. The total heavy metal concentration was in set A was 12.28 mg/L and in set B it was 24.57 mg/L to imitate the occurrence of heavy metal pollution in the natural environment from industrial activities. Levene and t-test showed significant difference ($p < 0.05$) of MR % between the cell populations and types of metals use especially among Cu and Pb.

Cadmium: In set A, over the 7 day period, highest MR % mean was observed with inactive cells (65.52 %) as opposed to active cells (55.62 %). Complete MR % (100%) was achieved with inactive cells and only up to 78.57 % with active cells on day 6. Removal was significantly different between the two cell populations ($p < 0.05$). In set B, significant difference ($p < 0.05$) in mean MR % was seen between inactive cells (71.88 %) and active cells (42.85 %). Also, inactive cells displayed complete removal (100 %) on day 3 and active cells had 95 % removal by day 7 ($p > 0.05$).

Chromium: Active cells showed the highest MR % mean of 34.09 % and inactive cells at 33.09 % ($p > 0.05$) in set A. Active cells also displayed the highest final % MR on day 7 at 67.02 % compared to 40.21 % with inactive cells on day 5 ($p < 0.05$). Highest mean MR % was seen with inactive cells (47.73 %) as opposed to active cells (38.89 %) ($p > 0.05$) in set B. Maximal MR % was also significant ($p < 0.05$) between the active cells (67.02 %; on day 7) and inactive cells (58.02 %; on day 6).

Copper: For set A, highest mean MR % was observed with inactive cells (38.56 %) and active cell removed 31.13 % ($p > 0.05$). However, maximum MR % was insignificant between active cells on day 7 (45.81 %) and inactive cells (46.52 %) on day 2 ($p > 0.05$). A higher mean of MR % was given by inactive cells at 51.32 % in contrast to a low MR % with active cells (35.44 %) ($p < 0.05$) in set B. As in set A, maximum MR % was obtained with active cells on day 7 (61.67 %) and inactive cells on day 5 (60.31 %) ($p > 0.05$).

Nickel: Inactive cells gave a significantly higher ($p < 0.05$) mean MR % (50.31 %) compared to active cells (36.04 %) in set A.
Maximum MR % was also obtained with inactive cells after day 7 (72.50 %) but not significant (p > 0.05) different to and active cells MR % (67.67 %). As with set B, highest mean MR % was seen with inactive cells (73.03 %) and a lower MR % of 30.68 % was given by active cells (p < 0.05). Maximal MR % was significant (p < 0.05) between the two cell populations; 94.74 % (on day 4) and 84.09 % (on day 7) with inactive and active cells, respectively.

**Lead**: No significant difference (p > 0.05) was observed with active and inactive cells displaying a low mean MR % of 26.67 % and 26.05 %, respectively in set A. The maximum MR % was at 49.30 % with active cells and lower with inactive cells (33.03 %) on day 7 (p < 0.05). For set B, the highest mean MR% was observed with active cells (55.78 %) but insignificant (p > 0.05) with inactive cells (42.99 %). Metal removal was significantly (p < 0.05) higher in set B with active cells (69.44 %) and 50.74 % with inactive cells on day 7.

All in all, CC cells exhibited good metal removal ability from SWW media at both high and low heavy metal concentration ranges. The comparison of MR % over the 7 day period demonstrated that growing cells of CC were able to remove higher percentage of Pb (49-69 %), Cu (45-61 %) and Cr (55-67 %) in SWW whereas non-growing cells removed more of Cd (100 %) and Ni (72-94 %) (p < 0.05) (**Table 2**). It was observed that non-growing cells achieved equilibrium earlier with quicker metal removal (by day 1-3) as opposed to growing cells. However, % MR by growing cells was higher in samples with elevated initial concentration of total metals (p < 0.05). Among the tested metals, the removal of Cu was indifferent between the two cell populations at both concentration ranges. This might be linked to the availability and affinity of binding sites present on CC’s biomass towards the Cu ions. Interestingly, the removal of Cr was more pronounced in active cells as opposed to non-growing cells. This suggests that CC may have reduced Cr(VI) to Cr(III) as reduction is one of the cell’s defense mechanism towards heavy metals.

The reduction of Cr(VI) can happen directly as a result of cell metabolism (enzyme dependent) in active cells. For both growing and non-growing populations, the order of removal was Cd > Ni > Cu > Cr > Pb. A study with *T. ceytonica* cells recorded Zn and Cu removal of up to 86.12 % and 93.75 %, respectively from the effluents of a wastewater treatment plant. Dead cells of *Bacillus* sp was shown to remove 44.73 % of Cu, 86.66 % removal of Cd was seen with dead cells of *Pseudomonas* sp. Yet not many studies have been carried out with different forms of mixed bacterial cells in synthetic waste effluent containing mixed metal ions. Our investigation proved the ability of CC to perform in the presence of multi-metals as reflective of the real field situation. The metal removal capability of both cell forms i.e. active and inactive is not hindered emphasizes the advantage of using a mixed bacterial biomass as sorbent source.
The outcome of this study revealed that active growing cells were better when metals were present at a higher initial concentration i.e. Pb (8-16.1 mg/L), Cr (2.2-4.5 mg/L) and Cu (1.7-3.5 mg/L) whereas non-growing cells were better with lower initial metal concentrations as with Cd (0.02-0.05 mg/L) and Ni (0.2-0.4 mg/L). From the time point of view, inactive cells attained equilibrium much earlier as the readily available biomass provided maximal sorbent surface area. On the other hand, active cells will require longer time to allow optimal growth to proceed before being able to accumulate metals actively and passively. The metal containing media darkened over the course of study as have been observed before 8 indicated active accumulation of metals by the cells. Due to the active metal uptake in addition to passive metal sorption expected in CC, eventually a higher removal percentage level will be observed. Based on these findings, we can imply that the use of inactive cells is suitable for waste solutions containing lower metal concentration; also for solutions with higher metal concentration, though these will require the regeneration of the biomass; and importantly for highly toxic waste water which would be inhibitory to cell growth.

The use of active cells will be more appropriate in solutions containing heavy metals below the growth inhibitory level. A clear advantage will be the ability of CC’s active cells to concomitantly utilize the organic pollutants present in any waste effluent as their sole energy and carbon source. This eliminates the need for external nutrient supplement. The use of a mixed bacterial culture as with CC is beneficial as this over time this will lead to a more progressive working culture with good adaptability to treat industrial waste effluent. Application wise, both batch and continuous processes can be applicable. For inactive cells batch process will be preferred as process limitation due to biomass saturation can be overcome by desorbing metals and regenerating the biomass after a specified time. Continuous process is the better choice for active cells, as continuous growth is required and carbon source can be acquired from the supplied waste water as in a continuous bioreactor setup. All of these

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**Table 2: Summary of maximum MR % attained for growing and non-growing cells of CC in set A and set B concentration ranges**

<table>
<thead>
<tr>
<th>Media</th>
<th>Cells</th>
<th>Maximum percentage metal removal (MR %) attained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cd</td>
</tr>
<tr>
<td>Sets</td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>SWW*</td>
<td>Growing</td>
<td>78.57</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Non-growing</td>
<td>100*</td>
</tr>
</tbody>
</table>

* SWW: synthetic wastewater
* denotes significant difference between growing and non-growing cells at α = 0.05
will be beneficial to fulfill and cater for the many different approaches needed for heavy metal laden waste treatment in situ and ex situ.

**CONCLUSION**

In conclusion, although active cells may have higher uptake potential, inactive or dead cells have several other advantages. The fact that it is not subjected to metal or other pollutants toxicity and not affected by adverse or extreme field/process conditions, the absence of specific and extensive nutrient requirements makes it a better choice. Furthermore, it may even be recycled and the metals contained can be recovered. Extended studies are being carried out to explore the potential of immobilization and the capability of CC to treat other environmental pollutants.

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**REFERENCES**


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