Removal of Heavy Metal Compounds from Industrial Wastes Using a Novel Locally-Isolated Vanrija sp. HMAT2

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A novel yeast, Vanrija sp. HMAT2 was isolated from the PHILEX mine site, which was capable of adsorbing heavy metals (chromium, copper, manganese, nickel, and zinc); neutralizing acidic wastewaters and was effective as either free or immobilized cells for laboratory bioreactor for bioremediation. Using the novel strain, its efficiency to treat different industrial wastewater streams including simulated acid mine tailings, actual untreated electroplating waste effluents, and untreated or treated tannery effluents were evaluated. Heavy metal removal efficiencies after 48 h were for Cu (97.29%) and Mn (94.22%) from simulated acid mine tailings; after five days for Zn (97.55%), Cr (68.65%), Cu (87.28%) and Ni (82.69%) from actual electroplating effluents. Highly efficient chromium removal rates of 99.15% (for untreated tannery) and 100% (for pre-treated tannery wastewater) were obtained using corncob-immobilized HMAT2 after five days and 20 h, respectively. The change in pH was greatest for simulated acid mine tailings from 3.7 to 8.9; intermediate for electroplating effluents from 3.0 to 6.6 and for untreated tannery effluents from 3.0 to 6.8; least for the treated tannery effluents from 7.2 to 7.3. These results suggested that the HMAT2 strain could be used for treatment of chromium-laden tannery waste effluents and help mitigate the source of heavy metal pollution of the Meycauayan River.

Key words: acid mine tailings, exhaust, heavy metals and yeast strain (Vanrija sp. HMAT2)

INTRODUCTION

The Philippine river systems have been suffering from heavy metal pollution due to unabated discharges of wastewater from industrial plants. Government regulations prohibit this practice and required industrial plants to install wastewater treatment facilities for compliance prior to discharge. The small-scale tannery plants along the Meycauayan River (Bulacan Province) practically failed to comply (Coronado FF, personal communication with DOST Region III) with the wastewater quality standards of the Department of Environment and Natural Resources (DENR) due to the inadequately installed technologies based on the conventional removal of chromium and had no means to provide the advanced treatment technologies. Thus, a microbial based technology would be necessary as an alternative and affordable mode for the local tanning industry.

The Marilao-Meycauayan-Obando River system (MMORS) is one of the hot spots for water quality and heavy metal pollution, and was branded to be one of the heavily polluted rivers. Heavy metal pollution for lead and hexavalent chromium had accounted for 99.2% of disease burden from toxic exposure among Asians in India, Indonesia and the Philippines (Chatham-Stephens et al. 2013).
In biological wastewater treatment processes, microbes actively function in the accumulation and deposition of metals by removing them from their dissolved states in water and fixing them in the underlying soils in an insoluble state, thereby returning them to the ore cycle. The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption or bioaccumulation, bioreduction (Focardi et al., 2013), and biofilm formation (Batool et al. 2015). Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through physico-chemical pathways of uptake (Fourest and Roux 1992; Ahemad and Kibret 2013) independent of metabolic processes. Algae, bacteria, fungi and yeast have proved to be potential metal biosorbents (Volesky 1986). Some yeasts (de María Guillén-Jiménez et al. 2008; Villegas et al. 2008) and bacterial genera capable of reducing Cr(VI) to Cr(III) had been reviewed including Escherichia, Pseudomonas, Bacillus, Enterobacter, Desulfovibrio, Serratia, Rhodobacter, and Arthrobacter (Reviewed in Contreras et al. 2011).

Some examples of strains used in tannery wastewater treatment for removal of chromium were Acinetobacter sp. (Srivastava et al. 2008), Acidithiobacillus thiooxidans (Wang et al. 2007), Rhizoclonium hieroglyphicum (Onyancha et al. 2008), unidentified bacterial strain (Shakoori et al. 2000), and a Cd-, Pb-, Zn-, Cu-resistant Cryptococcus sp. CBSB78 (Deng et al. 2012).

Metal tolerant fungi were described and shown to have selectivity for metal ions (Herrera-Estrella and Guevara-Garcia 2009). The predominant genera were Geomyces and Paecilomyces that subsist in soil with toxic levels of Cu and Zn, while Penicillium and Oidiodendron were less common. Strobilurus tenacellus, Mycena ammoniaca and Armillaria lutea were the most common species of basidiomycetes present in soil contaminated with cadmium dust. Some Penicillium species thrived in mine drainage and may account to 23% (P. lilacinum), while Penicillium ochrochloron was commonly found in industrial effluents. Halotolerant Penicillium spp. have been isolated from mangroves, that exhibit Pb, Cu and Cd tolerance. Some species were also isolated frequently from organomercurial-treated golf green, such as Trichocladium asperum, Trichoderma hamatum, Zygorrhynchus moelleri and Chrysosporium pannorum, whereas the genera Chaetomium, Fusarium, Penicillium and Paecilomyces were less encountered.

The chemical structure (e.g., chitin, chitosan) of the hyphal wall (Holan & Volesky 1995) contributes to the high metal uptake systems of fungi (Gadd 1986) with metabolism-independent biosorption as being the most efficient.

Environmental biotechnology and bioremediation make use of biological material such as microorganisms to improve the environment and reduce or eliminate environmental hazards resulting from the accumulation of toxic chemicals and other hazardous wastes. This technology is based on the utilization of naturally occurring microorganisms to transform organic and inorganic compounds. Biological methods compete with physical and chemical technologies. For an economical biological process of remediation and elimination of toxic metal contaminants, the following features are essential: inexpensive biomass, high metal binding capacity, selective metal binding, effective desorption methods, recycling and regeneration of biosorbents (Ahemad and Kibret 2013), and repeated use of biomass. Conventional techniques to remove toxic metals, e.g., ion exchange and precipitation, are considered expensive and are ineffective at low concentrations of metal ions and could require further processing of wastewater.

A recent report (Casado Canque et al. 2015 DEMOWARE. eu) cited leading technologies for wastewater treatment such as: Microfiltration – Reverse Osmosis/ Ultrafiltration – Reverse Osmosis (RO MF/UF); Anaerobic Membrane Bioreactor Technology (AnMBR); Forward Osmosis Membrane Bioreactor (FO MBR); Modular Wastewater Treatment (MWWT); Soil Aquifer Treatment (SAT); Advanced Oxidation for pre-treatment in SAT (AOP-SAT); and the most recent Struvite Crystallization, which rely on a metastable undissolved magnesium ammonium phosphate (with a repeating unit of [NH4MgPO4·6H2O]n, also known as struvite kidney stone) recovered as slow-release fertilizer or industrial chemical. These technologies were mature except for FO MBR and SAT’s, which were in pilot scales. Environmental concerns were noted for SAT’s due to the possibility of introducing pollutants (such as halogenated and oxygenated radical products) into groundwater aquifers that have long-term negative impacts. These have incorporated biological treatment and have been successfully employed in some industries around the world.

Some pilot scale technologies such as ChromeBacTM (Ahmad et al. 2010), biofilm bioreactor (Quintelas et al. 2010), biofilters (Leles et al. 2012), and activated sludge (Orozco et al. 2010; Contreras et al. 2011) have
been utilized in treating tannery derived wastewater. The activated sludge presented a promising technology based on the use of non-sterile conditions in contrast to the use of pure cultures (Contreras et al. 2011).

This study was aimed to utilize the novel isolate Vanrija sp. HMAT2 for wastewater treatment and determine the efficiency in removing heavy metals from various industrial effluents. This could help solve the problems of small tannery plants producing high-Biological Oxygen Demand (BOD) and acidic wastewater effluents contaminated with heavy metals. Moreover, the isolates can be adapted in existing wastewater treatment facilities as free cells or as immobilized cells.

MATERIALS AND METHODS

Culture Media
All reagents were analytical grade. Hydroxylamine and glucose were obtained from Merck (Darmstadt, Germany). O-phenanthroline was purchased from Sigma ((Missouri, USA). Sodium acetate, acetic acid, FeSO$_4$$\cdot$7H$_2$O, CuSO$_4$$\cdot$5H$_2$O and MnSO$_4$$\cdot$H$_2$O were from J.T. Baker (New Jersey, USA). Hydrochloric acid and nitric acid were from Univar (New South Wales, Australia). Silver nitrate and potassium nitrate were from Ajax (New South Wales, Australia). Nutrient agar (NA) and nutrient broth (NB) were from Difco (Becton, Dickinson and Company, Maryland USA). Corncobs were obtained as refuse material from vendor of “binatog” (Tagalog term for boiled corn) from a market in Bicutan, Taguig City. Corncobs were dried and cut into 2 to 2.5 cm sections before use as immobilizing agent in column bioreactors.

Wastewater Effluents
Acidic wastewater simulating that of the PHILEX mines containing heavy metals used in the study was prepared with synthetic mine water (SMW) using reagent grade chemicals dissolved in distilled water. Actual effluents were used to validate the results in the study using wastewaters of tannery (Valenzuela Tannery, Meycauyan, Bulacan), and electroplating industry (Robles Heritage Inc.).

Isolation of Organisms from Soil
Microbial samples were sourced from soil taken randomly from various locations at the PHILEX mining site in Benguet Province. One gram each, from five wet soil samples were suspended in 10 ml NB with different pH values (3, 4, 5, and 6.5), and incubated for one week at room temperature. Serial dilutions of 10-1 to 10-5 were prepared from each soil suspension. A 0.1 ml from each suspension was then spread plated over the NA petri dish. The plates were then incubated at room temperature and 48-hour colonies were picked and streaked on NA slants. The different isolates were then stored in the refrigerator for screening. Out of the total one hundred and fifty (150) isolates, forty (40) were selected randomly for subsequent screening.

Screening of Microorganisms
The 40 selected strains were screened in 10 ml liquid culture media containing a set concentration of NB diluted in synthetic mine water (SMW). Please see the specific formulations below. The strains were screened for their ability to grow at a low pH and their ability to neutralize the culture pH. The resulting four acid-neutralizing strains were cultured under stationary and shake flask conditions using various concentrations of NB. A 100% NB corresponds to 8.0 g nutrient broth/L solution, 50% NB contained 4.0 g/L, 30% NB contained 2.0 g/L nutrient broth, 15% NB contained 0.1 g/L and 5% NB contained 0.5 g/L nutrient broth. Instead of distilled water, SMW (containing FeSO$_4$$\cdot$7H$_2$O (0.0186%), CuSO$_4$$\cdot$5H$_2$O (0.0031%), and MnSO$_4$$\cdot$H$_2$O (0.00341%)) was used as diluent. Then, a 200 ml of synthetic mine water (SMW) was cultured with 10% inoculant of a particular selected organisms. After three days of incubation and subsequent screening, the four isolates were finally reduced to two promising isolates.

Inoculum Propagation and Immobilization
Inoculum propagation for both screening of microorganisms or for neutralization and adsorption of heavy metals were generally the same in media concentration. Nutrient agar slants were streaked with stock culture of each chosen test organism and incubated for 24 hours at room temperature. The organism was further grown in nutrient broth for another 24 h. For screening of microorganisms, the inoculum equivalent to 10% of culture media was used directly. On the other hand, for neutralization and removal of heavy metals, the selected microorganism, which was later identified as a yeast strain, was grown in a column bioreactor and immobilized in corncobs inside the column. The residual inoculum retained 50% of its original optical density (OD) that was drained out after a period of 17 hours.

Neutralization and Removal of Heavy Metals from Industrial Wastewater
Microbial-assisted removal of heavy metals using free cells of the yeast isolate required only one-tank bioreactor as shown in Figure 1-A. On the other hand, for immobilized yeast cells, treatment was done in one or two-tank bioreactor system depending on the volume of wastewater involved, as shown in Figure 1-B and Figure 1-C.
Analytical Methods
The pH was determined using a portable pH meter with a glass electrode. Heavy metals like copper, zinc, chromium, nickel, and manganese were determined using Shimadzu 680 ICL 24 by Flame Atomic Absorption Spectrometer (Flame-AAS) and optical densities (ODs) were determined at 660 nm in a Spectronic 20 spectrophotometer. Data presented were average raw values based on the submitted report from the testing laboratory at the STD (Standard and Testing Division of ITDI). Standard error bars were calculated from the data and plotted using Microsoft Excel chart.

Molecular Identification
Identification of the strains were performed at the Natural Science Research Institute (NSRI, UP Diliman) and at the Philippine Genome Center (University of the Philippines Diliman, Quezon City). The internal transcribed spacer (ITS) region was amplified using the universal fungal primer ITS4. Resulting sequences were analysed and compared with GenBank database for ITS RefSeq fungi using the National Center for Biotechnology Information USA, Basic Alignment Search Tool (NCBI BLAST) algorithm.

RESULTS AND DISCUSSION
Isolation and Screening of Microorganisms
A total of 150 potential microbial strains were isolated from five soil samples collected from the PHILEX Mine site. After a three to four day incubation period of soil samples in NA petri dishes, growth of 40 isolates was observed. The forty isolates were then grown in a NB medium at pH three for another three to four day incubation period. The resulting four promising isolates, which survived in the acidic medium, were further screened and isolate #94 and #116 were selected for their acid-neutralizing capability at pH 3 in synthetic mine water (SMW), which resulted in a change of pH to 7.8 or 7.3 respectively (data not shown). Out of the original 150 potential isolates, #116, which grew well in the acidic medium was finally selected in this study and its acid-neutralizing capability was shown in Figure 2. This isolate was morphologically identified as a yeast strain by the Natural Science Research Institute (NSRI). The ITS region sequencing at Philippine Genome Center (UP Diliman) with accession number ICC 118 118.1 and subsequent ITS BLAST resulted to 90% sequence similarity, which we designated as Vanrija sp. HMAT2 (upon the advice of the GenBank USA); and since the genera Cryptococcus, is undergoing taxonomic revision (Schoch et al. 2014). In a recent report (Garnica et al. 2016) involving more than 3000 collections of...
basidiomycetes fungi a threshold value of 99% within a range of 97% to 99.5% sequence similarity for the ITS region was found suitable for distinguishing species at a single genus lineage. The ITS region was recently proposed as a universal DNA barcode marker for fungi (Begerow et al. 2010; Schoch et al. 2012) and has been used for identification (Nilsson et al. 2009; Smith et al. 2013), for species level phylogenies (Harrower et al. 2011) and considered the gold standard for phylogenetic species level recognition (Porter et al. 2011). The GenBank accession number of Vanrija sp. HMAT2 for ITS1, ITS2, and 5.8S ribosomal rRNA genes was assigned GenBank KX348772.

The genus Vanrija was recently proposed by Weiss et al. (2014) and has been followed by several reports including Takashima et al. (2015). The strain HMAT2 had a different structure in the ITS1, ITS2, and 5.8S rRNA genes exhibiting a difference of 48 nucleotides and 15 nucleotides gap compared to a Type Strain GenBank AB035588 of the same genus of Vanrija. Furthermore the 5.8S gene of HMAT2 was unique and had no sequence that matched in searching the ITS2 database (http://its2.bioapps.biozentrum.uni-wuerzburg.de; Ankenbrand et al. 2015). The 5.8S rRNA provided a critical function in protein translocation as well as in ribosome movement (Rampersad 2014) and exhibit an extreme conservation among the eukaryotes (Freire et al. 2012). Based on these data we strongly we strongly argue that Vanrija sp. HMAT2 is a novel strain. Figure 2 showed the acid-neutralization capacity of HMAT2 while Figure 3 pictured the morphological structure at 400x magnification using light microscopy.

**Removal of Heavy Metals from Wastewater Effluents**

Microbial-assisted removal of heavy metals from industrial wastewater effluents was subsequently studied using either synthetic or simulated mine wastewater, and effluents obtained from electroplating and leather tanning plants. The three bioreactor systems used in the study were shown in Figure 4.

Shown in Figure 4 were column bioreactors and the treatment of industrial effluent using corncob-immobilized HMAT2. Control experiments also proved that no significant heavy metals were bound to the immobilizing agent consisting of corncobs (data not shown). The 3L one-tank bioreactor system was used to study biosorption of heavy metals from synthetic effluents and acid neutralization using either free or immobilized cells. On the other hand, the 50L and the 150L two-tank bioreactor systems (data not presented in this paper) were utilized primarily for scale-up validation studies using actual tannery wastewater effluents. The inoculants were propagated separately in two L-Erlenmeyer flasks. Prolonged biosorption of tannery wastewater for three weeks resulted in a clearer wastewater solution (Figure 5).
Treated tannery wastewaters were pre-treated on-site to reduce the pollution load in compliance with the DENR. Heavy metals recovery efficiencies were summarized in Table 1. Heavy metals removal efficiency ranged from 68.65% to 100% indicating that biosorption of heavy metals vary. The increase of pH during the biosorption process could indicate that the HMAT2 was resistant to heavy metals and had capacity for neutralizing acidic compounds. It was suggested that during biosorption of heavy metals, the strain was not adversely affected; the heavy metals were bound to the cell wall, thus preventing adverse metabolic effects inside the cell.

Copper removal in simulated wastewater reacted with free cells of \textit{Vanrija} sp. HMAT2 was characterized by a rapid phase of 50% reduction in the initial 20 h (Figure 6) whereas the rapid biosorption phase of Mn was observed at 24 h, which could result due the difference in affinity or selectivity for metal ions (Herrera-Estrella and Guevara-Garcia 2009). After 48 h, maximal removal of 97.29% Cu (Figure 6) and 94.22% Mn (Figure 6) were observed in amended simulated acid mine tailing with an increase of pH to 8.9.

Corncob-immobilized HMAT2 was highly active in acid milieu of pH 3.0 (Figure 7, 8, 9) and even at a high concentration of Cr (107.3 mg/L) in actual untreated tannery wastewater (Figure 9A). Treatments of actual electroplating effluents resulted in the removal of 68.65% Cr and 82.69% Ni (Figure 7). Actual electroplating wastewater treatments accounted for the removal of 92.31% Cu and 98.68% Zn (Figure 8). In contrast, actual tannery wastewater treatments resulted in the removal of 99.15% Cr (Figure 9A, untreated) and 100% Cr (Figure 9B, pre-treated). As shown in Figures 7, 8 and 9 heavy metal removal rates for nickel, copper, zinc, and chromium exhibited a rapid phase within the initial 4 h. Cu, Ni, Zn, and Cr were reduced at an initial rate of at least 50% within the first 4 h. In the treated tannery wastewater (Figure 9B), Cr was totally reduced to 100% within the first 4 h using corncob-immobilized HMAT2.

### Table 1. Efficiency of Heavy metals removal from test industrial wastewater effluents.

<table>
<thead>
<tr>
<th>Type of Wastewater Effluent, Heavy Metal</th>
<th>Metal Content Final Concentration (ppm)</th>
<th>Removal Efficiency (%)</th>
<th>Time (hrs)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic Wastewater/ Simulated Mine Tailing Treated with free-cell \textit{Vanrija} sp. HMAT2</td>
<td>Cu</td>
<td>0.226</td>
<td>97.29</td>
<td>48</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>0.51</td>
<td>94.22</td>
<td>48</td>
<td>3.94</td>
</tr>
<tr>
<td>Electroplating Wastewater Treated with immobilized \textit{Vanrija} sp. HMAT2</td>
<td>Zn</td>
<td>0.125</td>
<td>98.68</td>
<td>120</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Cr</td>
<td>2.543</td>
<td>68.65</td>
<td>120</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>3.579</td>
<td>92.31</td>
<td>120</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Ni</td>
<td>41.92</td>
<td>82.69</td>
<td>120</td>
<td>3.0</td>
</tr>
<tr>
<td>Actual Tannery Wastewater Treated with immobilized \textit{Vanrija} sp. HMAT2</td>
<td>Untreated Tannery Wastes, Cr</td>
<td>0.91</td>
<td>99.15</td>
<td>120</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Treated Tannery Wastes, Cr</td>
<td>(undetected)</td>
<td>100</td>
<td>20</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Figure 5. Tannery wastewater samples after prolonged treatment.

Figure 6. Effect of HMAT2 free-cell suspension treatment on Acid Synthetic Mine Wastewater (Simulated Mine Tailing). Copper concentration (blue bar with corresponding pH in red); and Manganese concentration (green bar with corresponding pH in purple) in wastewater effluent. Zero hour corresponded to the four-bar column next to the control.

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Compared to other literature (Cited in, El-Morsey et al. 2013) biosorption of immobilized fungal cells was 60.94% for Cu (*Aspergillus* sp.), 97.21% for Cd (*Penicillium* sp.) and 73.27% for Pb (*Cephalosporium* sp.). A similar adsorption capacity for Cu, Cd and Pb in immobilized cells of *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. was reported (Johncy Rani et al. 2010). Immobilized cells have many advantages such as high biomass loading, minimum clogging in continuous flow systems and reusability. However, it was reported (Gurel et al. 2010) that immobilized *Rhizopus arrhizus* in rice bran exhibited a greatly reduced biosorption for Ni resulting in ten times reduction.

The Zygomycetes sp. such as *Mucor* sp. and *Rhizopus* sp. are reported to be efficient biosorbent agents while immobilized biomass of *Cunninghamella echinulata* was able to remove ions from a natural environment of up to 95%. Maximum copper removal of 98% was obtained for *Rhizopus arrhizus* under optimal conditions (El-Morsy et al. 2013). A psychrophile yeast NIOCC#PY13, *Cryptococcus vishniacii* (Singh et al. 2013) has been described to be tolerant to 200 mg/l of Cu, Pb, and Zn and exhibited metal uptake capacity of 98% for Pb, 96% for Zn and 76% Cu in 10 mg/l of metal containing solution for 3 days. Ngô 2013 reported two acid tolerant yeasts which were found to be resistant to heavy metals in cultures containing 0.1 to 250 mM. *Cryptococcus* sp. AH-13 was more resistant to Cd, Cu, Zn, Co, Hg, Ag, Fe, Mn, Ni (except Pb) on the YG (Yeast extract-Glucose) solid medium than *Candida palmioleophila* KB-6. Strain AH-13 was inhibited in cultures with 0.5 mM Cd, 1.5 mM Cu and 1.5 mM Zn.

At pH 4, reported removal rates for Pb, Cu and Zn were 96.15%, 81.11% and 80.55% while higher rates of 95.88%, 95.04% and 83.24% were removed at pH 5 (El Morsy et al. 2013). Higher removal rates for heavy metals (Cu, Co, and Fe) were observed with immobilized *Rhizopus delemar* column on polyurethane foam, which achieved 92% removal during 5 cycles (El Morsy et al. 2013). Data in Table 1 showed that Cu removal rates were consistently higher at 97.29% for free-cells and 92.31% for immobilized HMAT2 compared with immobilized *Rhizopus delemar* and with the psychrophile yeast NIOCC#PY13, *Cryptococcus vishniacii* (Singh et al. 2013).
Data from Basak et al. 2014, revealed that Zn removal efficiency of yeast biofilm increased with the increase in pH of 2 to 6. At an optimal pH 6.0 Candida rugosa biofilm showed maximum Zn removal of 44% in biofilm biomass dry wt. 0.93 g/m2, whereas Cryptococcus laurentii biofilm showed 37% Zn removal in biofilm biomass dry wt. was 0.84 g/m2 at 12 h. In a continuous column mode (height of 12 cm and biomass dry wt. of 2.98 g/m2), C. rugosa biofilm formed on gravels depleted the Zn maximally at 95.29% with a flow rate of 1 ml/min in a residence time of 180 min. However the pH of the column was not reported. In comparison, the removal efficiency for Zn in this study was quite high at 98.68% (Table 1) compared with biofilm and gravel-immobilized yeasts in column and with the psychrophile C. vishniacii.

Based on this study, biosorption of simulated acid mine tailings occurred with the greatest change in pH from pH 3.7 to 8.9. This result was similar to the biosorption of Zn (El-Morsy et al. 2013) by A. niger, Penicillium chrysogenum and Clavicipis paspali, which was accompanied by a corresponding increase in pH up to 9, however another paper reported that the optimal uptake of Zn and Pb by Rhizopus arrhizus was achieved at a neutral pH and pH 5, respectively. Treatment of electroplating wastewater exhibited a change in pH from 3.0 to 6.6 and untreated tannery effluent exhibited both an intermediate change in pH from 3.0 to 6.8. The treated tannery effluent exhibited the least in the change of pH from 7.2 to 7.3; however this experiment resulted in 100% reduction in Cr within a relatively short time of 20 h.

Recent experiments with the yeast Mucor racemosus (El-Morsy et al. 2013) results in the optimal time for biosorption of 15 minutes after contact. This result is similar for Saccharomyces cerevisiae (Volesky & Philips 1995) and that most metal biosorption was achieved in 5-15 min with residual and slower additional metal deposition (El-Morsy et al. 2013). In Candida utilis, biosorption occurred within 10 minutes (Pesti et al. 2000).

Using laboratory scale 3L column reactor for biosorption for corncob-immobilized Vanrijia sp. HMAT2 experiments, we showed in this paper that heavy metals Zn, Cu, Ni, and Cr exhibited a rapid initial phase within the first 4 h. This was also observed even in the zero time samples due to the quick biosorption reaction, which occurred within minutes. The presence of other metal ions could affect the biosorption efficiency. Based on the report of Mutter et al. 2002, Cd(II) and Zn(II) inhibited biosorption of Cr(VI), whereas a Pb(II) or Cu(II) exerted a positive effect on Cr(VI) biosorption. In our experiments using 3L column, a combination of Zn, Cu and Ni, which occurred in electroplating wastewaters greatly reduced the biosorption of Cr to 68.65% level.

While this paper was in writing Opulencia et al. (2015) reported a Cu-resistant T21 strain of Bacillus megaterium, which could reduce Cu content of semiconductor wastewater by 65.95%. Based on our data (Table 1) for the biosorption of several heavy metals using the HMAT2 strain, we could achieve a greater reduction of 92.31% in electroplating wastewater and 97.29% in acid mine tailing for Cu biosorption.

A yeast strain of Cryptococcus humicola MCN2 (Kwon et al. 2002) was isolated from coke-plant wastewater which was capable of degrading free and metallocyanides and thus can be used for bioremediation of highly toxic cyanides. MCN2 was also reported to have better cyanide removal efficiency for aerobic treatment (about 100%) compared to anaerobic treatment rate of 90%. In comparison, the novel yeast strain Vanrija sp. HMAT2 immobilized in corncobs resulted in 100% removal of Cr from the treated tannery waste effluent using aerobic column bioreactor.

CONCLUSION

A team of researchers from the Industrial Technology Development Institute (ITDI) took soil samples within the PHILEX Mine site in Benguet and successfully isolated two microbes capable of reducing acidic mine tailings. One of the new isolates, Vanrija sp. HMAT2 was also able to remove heavy metals. This research represented a pioneering report on the ability of microbial-assisted wastewater treatment using the novel HMAT2 strain, which exhibited a multi-metal biosorption for Cu, Cr, Mn, Ni, and Zn and was highly active in acidic mine tailings. As shown in this paper the aerobic process using immobilized HMAT2 for heavy metal biosorption of contaminated electroplating and tannery wastewaters was characterized by a rapid phase of biosorption occurring within a few hours of reaction. In contrast, free cell biosorption utilizing HMAT2 exhibited a longer reaction phase of about 20 to 24 hours. Many researchers have succeeded in discovering microbes that can remove heavy metals by biosorption but nobody has reported locally, a microbe that could neutralize acid and remove several heavy metals such as Cr, Cu, Mn, Ni, and Zn in highly acidic mine tailings. This reduction in heavy metal content was significant and could shed light on the bioprocess of utilizing microbes for removing heavy metals from wastewaters.
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