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Isolation, Identification and Heavy Metal Biosorption Assessment of Yeast Isolates Indigenous to Abandoned Mine Sites of Itogon Benguet, Philippines

ABSTRACT

Water samples collected from abandoned mining sites in Itogon, Benguet, Philippines were screened for metal resistant microorganisms, in particular yeasts that will be used to remove toxic metals such as Zn, Cu, Pb, Cr and Ni from aqueous media. Among the five yeast strains selected and five heavy metals tested, Nodulisporium sp. exhibited the highest removal efficiency of 80% and biosorption capacity of 56.7 mg g^{-1} for Pb. This was based on the model equation for each metal that was generated to derive optimum response for removal efficiency. The metal accumulation potential for all selected yeast isolates was generally higher at the lower initial metal concentration of 25 mg L^{-1}, indicating rapid metal absorbing ability of the isolate and that adsorption sites in the biomass are taking up available metal ions more quickly. An increased removal capability was observed when the best isolate was applied in a semi-continuous treatment system thru an Aerobic Cascading Filter Bed Baffled Reactor (ACFBBR). The reactor design including the packing material remarkably enhanced the contact between the yeast biomass and Pb contaminated wastewater resulting in a much greater biosorption capacity of 170.14 mg g^{-1} as compared to the biosorption of 56.7 mg g^{-1} achieved during the batch adsorption experiment.

Key words: *metal resistant, yeast isolate, abandoned mine site, Itogon Benguet, ACFBBR*

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INTRODUCTION

Contamination brought about by toxic heavy metals as a consequence of human activities is a key concern due to its adverse impact on environment and public health. Unlike other contaminants, all heavy metals, in spite of some are essential micronutrients, have toxic effects on living organisms. The bioaccumulation of toxic metals can occur in the body and food chain. These can enter into water via drainage, atmosphere, soil erosion and all human activities by different ways. As the heavy metals concentrated more in the environment, these enter biogeochemical cycles, leading to toxicity (*Govind and Madhuri 2014*).

Nonetheless, the negative effect can be minimized through proper remedial actions and management. A number of methods have been developed for the removal of heavy metals from liquid such as precipitation, evaporation, ion exchange, membrane process and others. These approaches, however, are found to be ineffective or rather expensive and usually generate toxic slurries (*Das et al. 2008*). In contrast, biological treatment has gained an increasing attention for heavy metal removal and recovery due to upright performances and low cost (*Siddiquee et al. 2015*). Also, it is being considered a favorable technique for the bioremediation of heavy metal-bearing wastewater since it can degrade pollutants in the wastewater and simultaneously transforms heavy metals (*Wang et al. 2010*).

Bioremediation using heavy metal-tolerant microorganism is an alternative method to remove or recover heavy metals efficiently from polluted environment, and isolation of heavy metal-tolerant microbes as bioremediation agent is fundamentally important (*Zhu et al. 2015*). Microorganisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating heavy-metal ions (*Joshi and Sahu 2014*). Yeasts, in particular, have developed a complex defense system to neutralize heavy metal toxicity (*Ayangbenro and Babalola 2017*).

Most studies have shown that sites polluted with toxic metals can lead to the establishment of a tolerant or resistant microbial population that are likely capable of absorbing metals. As a result, *Bautista- Hernandez et al.* (*2012*) made an assessment on the capacity for

biosorption of resistant bacterial strain isolated from a polluted site. Similarly, the study of *Colin* (*2012*) placed particular emphasis on the potential use of metal-resistant microorganisms that are indigenous to contaminated areas for the development of toxic metal bioremediation strategies. Also, *Xie et al.* (*2010*) isolated indigenous bacterial strains with high heavy metal resistance from a mine tailing area . Further, *Velmuragan et al.* (*2010*) found heavy metal-adsorbing biosorbents from contaminated soil sample collected from a mine in South Korea. On this context, one of the most promising location to get heavy metal resistant microorganism would be from abandoned mine sites where extreme environmental conditions would allow only metal-tolerant organisms to thrive. In the Philippines, the province of Benguet has a long history of mining. The town of Itogon, Benguet is particularly of interest since it hosted the operations of Benguet Corporation (BC), the oldest mining company in the country. After a complete stop in its mining operations in 1998, BC left several inactive mine structures (*ESSC 2003*).

There are limited researches done on the isolation of metal resistant yeast from abandoned mine sites of Benguet Province including their potential use for the biosorption of heavy metals. Moreover, fewer adsorption investigations have been conducted with toxic metal resistant yeast than metal-tolerant bacteria, and dead biomasses are more often examined and reported than live biomasses (*Velmuragan et al. 2010*). Consequently, only selected studies have been published regarding the possible usage of live yeast as potential biosorbent. Along this line, this study aims to isolate and identify metal resistant yeasts that are indigenous from the abandoned mine sites of Benguet, and evaluate their potential to remove heavy metals from aqueous media. Further, this research initiative intends to design a cost-effective biological treatment system that will capitalize on the use of the best metal-tolerant yeast to remediate heavy metal-bearing wastewater.

MATERIALS AND METHODS

Sampling and Sample Collection

The project team together with the technical staff of BC and the Department of Science and Technology, Cordillera Administrative Region (DOST-CAR) performed an ocular inspection and sampling activities on May 17, 2016 at the project area (Itogon, Benguet) in which six sites were identified. On each of the identified location, a total of 5 L of water sample was collected and preserved according to the Standard Methods for the Examination of Water and Wastewater, 18th edition. The pH of the samples was determined on-site using Lovibond Sensodirect 150 set, (pH/conductivity/oxygen) water tester while other water quality parameters such as turbidity and heavy metals were analyzed at the laboratory of the Industrial Technology Development Institute of the Department of Science and Technology (ITDI-DOST), Bicutan, Taguig City.

Isolation of Organism

Around 2 mL of the collected water sample was weighed into 100 mL flask, mixed with 20 mL peptone buffer (0.1%) and then agitated and incubated at 30° C for 30 min. From this solution, about 0.1 mL of the liquid microbial supernatant was plated on each disk. Plates were incubated at 30°C ± 2°C for 3 to 7 days. The initial isolation was performed on 5 and 10 mg L^{-1} concentrations of mixed metal. Potato Dextrose Agar plates (PDA) with 0.1 % tartaric acid, and supplemented with 5 mg L^{-1} and 10 mg L^{-1} concentrations of Zn(II), Cu(II), Pb(II), Cr(VI) and Ni(II) from metal salts, in particular $ZnSO_4$, $CuSO_4.5H_2O$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, and $NiSO_4$ were prepared to isolate metal-tolerant yeast strains. The colonies formed from the initial concentrations were subsequently grown on 15 and 20 mg L^{-1} concentrations of heavy metals. Afterwards, the colonies that developed were transferred to plates spiked with much higher metal concentrations of 60 and 100 mg L^{-1}, respectively to screen for highly metal resistant isolates.

Purification of best isolates

For purification purposes and for the rapid growth of yeasts, the isolates were enriched in Yeast Peptone Dextrose liquid media containing 60 mg L^{-1} to 100 mg L^{-1} of mixed heavy metals using an inoculation of 10% (v:v), to obtain and maintain highly resistant heavy metal cultures. Selected yeast isolates were submitted to the Philippine Genome Center in University of the Philippines, Diliman for DNA extraction, sequencing and identification.

Biosorption tests

The capability assessment of the best microbes to remove heavy metals in simulated wastewater was primarily done through batch biosorption experiment. Selected yeast isolates were assessed for their performance for the removal of zinc, copper, lead, chromium and nickel in aqueous solution. Samples containing 25 mg L^{-1} and 50 mg L^{-1} mg L^{-1} of individual metal were prepared and inoculum of 10% (v/v) was added to make

50 mL solution and constantly shaken using a rotary shaker at 150 rpm for a contact time of 5 d at room temperature. After the biosorption test, the samples were filtered and the filtrate was analyzed for heavy metals using PinAAcle 500 Perkin Elmer solid state detector with double beam optics, flame atomic absorption spectrometer (Flame AAS).

Design of experiment

For the assessment of the biosorption performance of the five isolates in increasing concentrations of heavy metals, design of experiment was generated using the Minitab® 17 Statistical software. Two factors, yeast isolate and initial concentration were considered in the design along with five levels for isolate (yeast species) and two levels for initial concentration (25 mg L^{-1} and 50 mg L^{-1}), and the removal efficiency as the response variable. General factorial design was used in the experiment since it can be used in situations where the factors considered have different number of levels. Also, this design can provide an estimate with the interactions between factors, their significance to the response (i.e., the percentage removal) and in providing basic response optimization based from the model produced (*Wheatley 2014*).

Biosorption experiments were carried out in replicates and average values were used in the analysis. The biosorption capacity (Q), i.e., amount of metal ion (mg) biosorbed per g (dry mass) of biomass, was calculated using the following equation (*Rafida 2008*):

$$Q = \left(\frac{C_i - C_f}{m}\right)V \quad (1)$$

where:
Q = mg of metal ion biosorbed per g of biomass;
Ci = initial metal ion concentration, mg L^{-1};
Cf = final metal ion concentration, mg L^{-1};
m = dry mass of biomass in the reaction mixture, g;
V = volume of the reaction mixture, L.

Continuous treatment System using Lab-Scale Aerobic Cascading Filter Bed Baffled Reactor (ACFBBR)

The laboratory-scale experiment using ACFBBR was carried out in ITDI laboratory at ambient temperature of 27° C to 30° C. The ACFBBR was fabricated using acrylic sheet with a total volume of 18.9 L (30 cm x 14 cm x 45 cm) and an effective volume of 7.5 L. It consisted of four sets of vertical and standing baffles, installed in series and in descending level along the bioreactor to particularly remove heavy metals from the wastewater (**Figure 1**). The four chambers of these reactors were packed with perforated spent softdrink plastic caps that served as the niche for the growth and development of the selected isolates. Each plastic cap was perforated with at least eight equidistant holes of 1/8 inch in diameter through the use of a Di Acro Ouidail sheet metal punch press. Three sampling ports were mounted along the side of the reactor to facilitate effluent sampling and additional ports at the bottom of each chamber for sludge handling. Air stones connected to small air pumps were also placed inside the reactor in order to facilitate aeration of 2 L min^{-1} for each compartment. Wastewater loading was done in downflow mode using a variable metering pump (Iwaki Company, Japan), and maintained a flowrate of 20 L min^{-1}.

Start-up Procedure

Prior to wastewater loading, the reactor was inoculated with the isolate with the highest removal efficiency. The inoculum, 10% (v/v), was introduced into the reactor's chamber which was then filled to its capacity with culture media spiked with 30 mg L^{-1} of Pb. The ACFBBR was then allowed to undergo acclimatization for one week in order to facilitate growth of the selected yeast around the packing material and for the isolate to adapt to its new environment. Acclimatization was determined during the period where a steady-state condition was reached. Steady state condition can be described as a stable condition that does not change over time. It took approximately a week before the bioreactor set-up exhibited steady state pH values that ranged from 5.0-6.0 as well as temperature readings which were noted in the range of 28-31°C. After this period, a daily loading of simulated 25 mg L^{-1} Pb

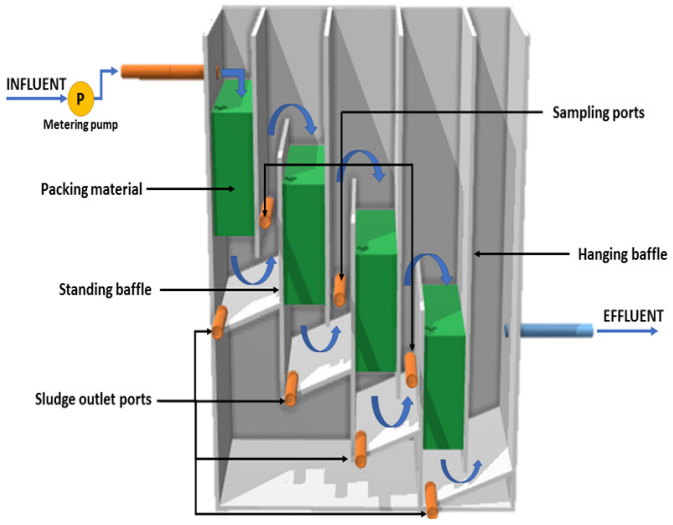

Figure 1. Design configuration of the Aerobic Cascading Filter Bed Baffled Reactor (ACFBBR).

bearing wastewater commenced. Effluent sampling was collected twice a day and analyzed for residual metal.

RESULTS AND DISCUSSION

Water samples were collected from five sampling sites in Itogon, Benguet. This mining town is located at the southern end of the Central Cordillera Mountain Range, north of Luzon and is generally mountainous in terrain with deep valleys and steep slopes (www.itogon.com.ph). The identified sampling points (**Table 1**) are comprised mainly of water bodies (rivers and creek) and tailings storage facility (TSF) from four Barangays of Virac, Ucab, Poblacion and Loacan (**Figures 2** and **3**).

Except for sampling site 1 (Balatoc), the pH values obtained from the other areas were almost neutral and below the DENR standard. The color of the water samples appeared as dark brown, light brown and clear. Turbidity was highest at Balatoc site, a heavily silted and turbulent stream which registered a reading of 621 FAU. All sampling sites exhibited elevated levels of chromium, copper and lead that were way above the regulatory limits (*DENR 2016*). For other metals however, only site 1 (Balatoc), yielded Zn concentration that is higher than the DENR standard for water quality. (**Table 2**).

Table 1. Sampling Sites in Itogon Benguet.

Site	Label	Location (Barangay) in Itogon Benguet
1	Balatoc	Virac
2	Ambalanga River, Phase 3, Pink Tunnel, Narba Tunnel	Ucab
3	Tailing Storage Facility 1 (SF1, Gold Creek)	Ucab
4	Convergence Ambalanga, Acupan River	Poblacion
5	Tailing Storage Facility 2 (TSF 2 , Active)	Poblacion
6	Antamok 440	Loacan

Screening and Identification of Isolates

Isolation of heavy metal-tolerant yeasts were conducted by selecting colonies that exhibited good growth on solid agar media supplemented with increasing heavy metal concentrations. Yeast growth numbering to about 50 colonies formed in the initial concentration of 5 mg L^{-1} mixed metal of Zn, Cu, Pb, Cr and Ni. Thereafter, the number of colonies decreased to 10 when the metal concentration was doubled to 10 mg L^{-1}. A similar quantity of yeast growth was observed when metal concentration was amplified to 15 mg L^{-1}. Again, the same number of colonies performed well in the screening tests and exhibited the greatest resistance up to a concentration of 20 mg L^{-1} of mixed metals. The isolated yeast strains were enriched and finally tested to a much higher dose of heavy metal of 60 mg L^{-1} and 100 mg L^{-1}, respectively. At this point, no significant growth was seen on the 100 mg L^{-1} media but there were 5 yeast isolates that survived the 60mg L^{-1} set-up. The number of colonies formed with increasing mixed metal concentrations, became the basis for the succeeding tests to consider metal concentrations lower than 60 mg L^{-1} (**Figure 4**).

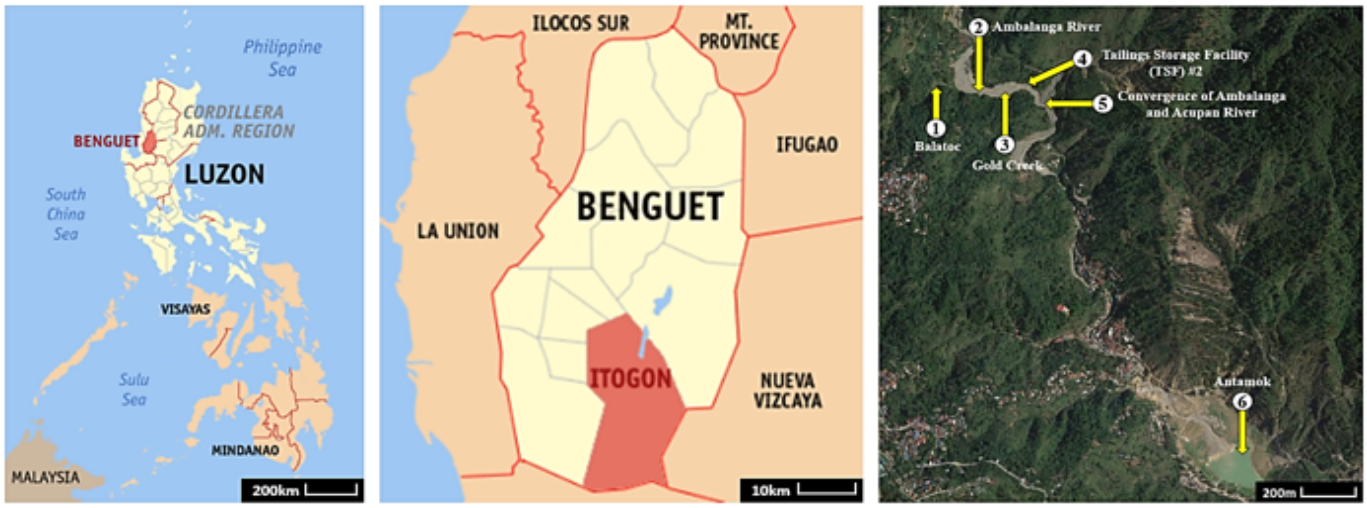

Figure 2. Location map of the six sampling sites in Itogon Benguet. (a) Philippine map with the province of Benguet highlighted (https://goo.gl/wqvodB), (b) Map of Benguet with Itogon highlighted (https://goo.gl/cJL8ey), (c) Sampling sites in Itogon, Benguet (https://goo.gl/RjzfUi).

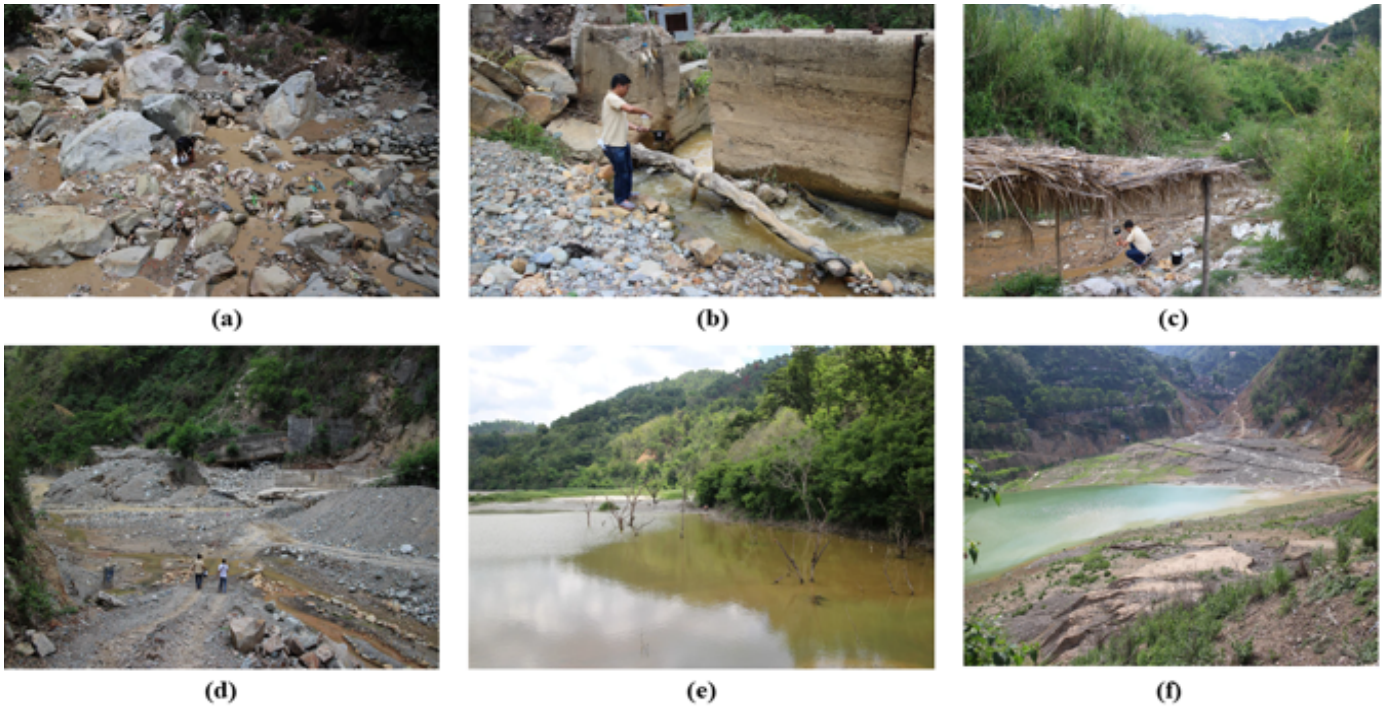

Figure 3. Sampling sites in Itogon Benguet (a) Balatoc, (b) Ambalaya river, (c) TSF-19, Gold creek (d) Convergence Ambalanga -Akopan river, (e) TSF-2, (f) Antamok.

Table 2. Characteristics and heavy metal content of water samples from Itogon Benguet.

Parameters	1	2	3	4	5	6	Water Quality**	Effluent Quality***
pH	6.26	6.33	6.65	6.78	6.96	6.84	6.5-9.0	6.0-9.5
Color (descriptive)	Light brown	Light brown	Dark brown	Light brown	Clear	Clear	-	-
Turbidity, FAU (formazin attenuation unit)	621	50	145	176	10	29	-	-
Chromium, mg L^{-1}	0.53	0.39	0.63	0.34	0.45	0.45	0.01a	0.02a
Copper, mg L^{-1}	1.37	0.24	0.62	0.56	2.70	0.38	0.02	0.04
Lead, mg L^{-1}	4.90	0.21	0.69	0.77	0.23	0.27	0.05	0.10
Zinc, mg L^{-1}	4.79	0.17	0.89	0.84	0.27	0.17	2.0	4.0
Nickel, mg L^{-1}	0.15	0.06	0.13	0.10	0.11	0.10	0.2	1.0
Silver, mg L^{-1}	0.14	0.04	0.04	0.03	0.07	0.04	-	-

Note: * Based from DENR Administrative Order 2016-08 for Class C waters (Inland waters), DAO 2016
** Applicable to sites 1,2,3,4 and 6 , *** Applicable to site 5, [a] Hexavalent chromium , Cr(VI)

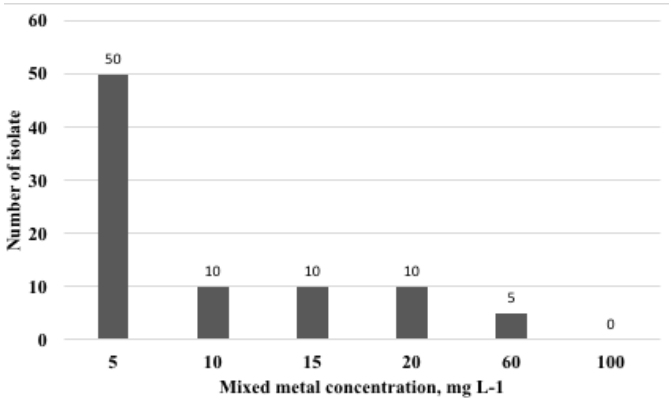

Figure 4. Number of yeast isolate that survived at different mixed metal (Zn, Cu, Pb, Cr and Ni) concentrations.

Molecular Identification of Yeast Isolates

The molecular identification of the best five yeast isolates was done at the Philippine Genome Center, University of the Philippines, Diliman. The DNA extraction process includes the mechanical lysis of yeast cells, specifically through bead beating. After DNA isolation, capillary sequencing was employed. Universal primers were used, i.e ITS1 (5'-TCTGTAGGTGAACCTGCGG-3") and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') that amplify a region of the ribosomal DNA. Comparison of sequences in GenBank via nucleotide BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) showed five DNA sequenced yeasts

with their percentage sequence similarity (**Table 3**). Most of the selected isolates originated mostly from the riverbanks of Ambalanga and Acupan, although one isolate came from an active tailings storage facility (TSF2) in Bgy. Poblacion.

Biosorption Tests

After performing the biosorption experiment and the analysis of residual heavy metal from the samples, the data gathered was then analyzed using the Minitab® 17 Statistical Software. Significance of the factors, their interactions and the model generated was assessed using analysis of variance (ANOVA). Also, interaction plots were produced in order to compare the removal efficiency of each isolate at different heavy metal concentrations. Further, a basic response optimization was performed in selecting the best microorganism for the removal of each heavy metal at a certain concentration.

As a result of the initial assessment on the tolerable limits applicable with the isolates, only two levels were considered, the high (50 mg L^{-1}) and low (25 mg L^{-1}) concentrations in relation to the observed growth rates of the yeasts at different metal concentrations.

Zinc Biosorption

Among the five isolates, only four showed capability to remove zinc contaminant from aqueous solution. However, this is apparent only at lower concentration of 25 mg L^{-1} and at a lesser percentage removal. At higher concentration of 50 mg L^{-1}, the absence of metal uptake was noted for all isolates. Removal efficiency was maximum at 41%, attributed to isolate A (*Rhodotorula toruloides*) followed by isolate E, *Nodulisporium* sp. with the attainment of 30.5% performance efficiency (**Figure 5**).

A factorial ANOVA revealed the existence of significant main effect for the type of yeast isolate ($F(4,10) = 216.59$, $p < 0.05$) and initial concentration ($F(1,10) = 1025$, $p < 0,05$) as factors on the zinc removal efficiency. Further, it can be deduced that an interaction exists between these factors ($F(4,10) = 216$, $p < 0.05$) and that such interaction is also significant with respect to the percent removal of zinc as the response variable. The interaction between the type of isolate and the initial concentration of heavy metal suggests that the isolates tend to have better removal rates at lower concentration (25 mg L^{-1}) than in higher concentration (50 mg L^{-1}) (**Figure 6**).

Table 3. Molecular identification of isolates and their sampling origin.

Yeast Isolate Code	Species Name	Sequence Similarity, %	Sampling Site
A	*Rhodotorula toruloides*	99	S4
B	*Candida tropicalis st.*	76	S2
C	*Papiliotrema laurentii*	99	S4
D	*Candida maltosa*	98	S4
E	*Nodulisporium* sp.	99	S5

Copper Biosorption

All the five isolates indicated copper metal adsorption potential at both concentrations of 50 mg L^{-1} and 25 mg L^{-1}, respectively (**Figure 7**). Higher removal efficiencies were favored at the low level than at the elevated concentration. Out of the five isolates, two yeasts, namely *Candida maltosa* and *Papiliotrema laurentii* were found to sequester more than 40% copper from aqueous solution.

The main effect and interaction for the Cu metal were assessed. As suggested by the ANOVA, the main effect

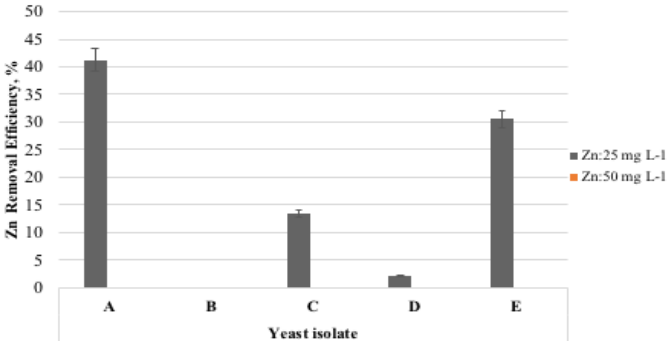

Figure 5. Removal efficiency of five yeast isolates at different initial Zn concentration.

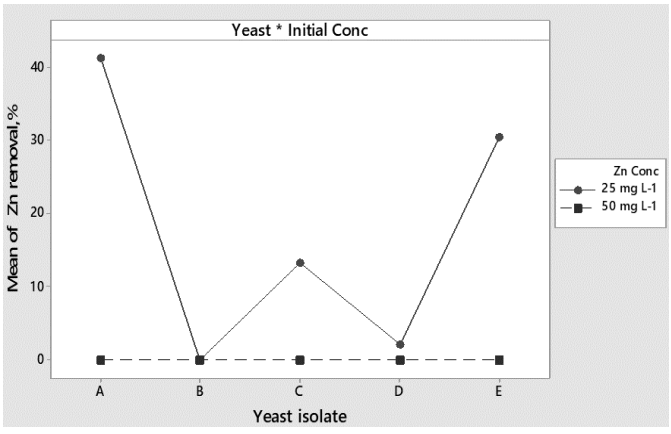

Figure 6. Interaction plot between yeast type and initial Zn concentration.

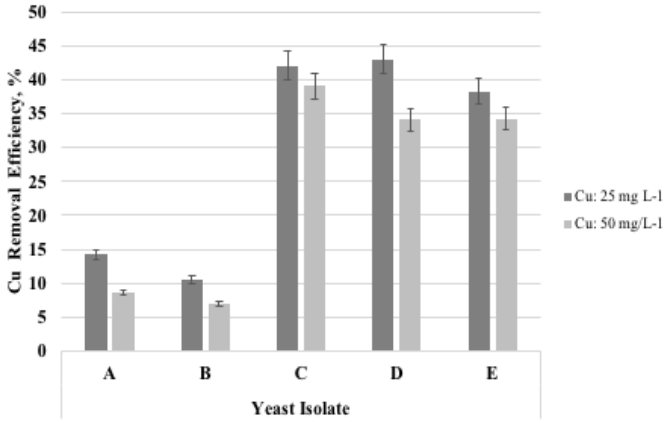

Figure 7. Removal efficiency of five yeast isolates at different initial Cu concentration.

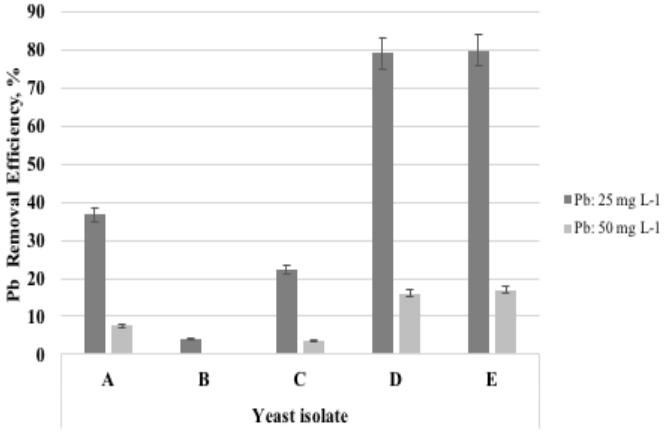

Figure 9. Removal efficiency of five yeast isolates at different initial Pb concentration.

for the yeast isolate used ($F(4,10) = 728.6$, $p < 0.05$) and initial concentration ($F(1,10) = 96.1$, $p < 0.05$) as factors on the copper removal efficiency was found to be significant. Similarly, there is the presence of an interaction between these factors ($F(4,10) = 4.11$, $p < 0.05$) with respect to the response variable (removal efficiency) (**Figure 8**).

Lead Biosorption

Lead biosorption experiments yielded significant results (**Figure 9**) as to the capability of the isolates to remove Pb in simulated wastewater. Except for isolate B, all the yeasts showed potential for treating wastewater at 25 mg L^{-1} and 50 mg L^{-1}. Again, removal rate was more pronounced at the minimum concentration of 25 mg L^{-1} where isolates E and D gave remarkable removal efficiency of more than 78 %.

The main effect for the type of yeast isolate ($F(4,10) = 492.9$, $p < 0.05$) and initial concentration ($F(1,10) = 1815.2$, $p < 0.05$) as factors on the lead removal efficiency was significant as indicated by the ANOVA. Moreover, interaction (**Figure 10**) was found to exist between these factors ($F(4,10) = 201.27$, $p < 0.05$) along with the response variable (Pb removal efficiency).

Low percentages of chromium removal (**Figure 11**) was observed for the five isolates which occurred at 25 mg L^{-1} initial concentration. However, higher concentration of metal did not show any chromium removal for all yeast strains.

The highest removal efficiency of less than 20% was ascribed to isolate E (*Nodulisporium* sp.) The main effect on the type of yeast isolate ($F(4,10) = 4.2$, $p < 0.05$) and initial concentration ($F(1,10) = 154.4$, $p < 0.05$) as factors on the lead removal efficiency was significant as revealed in the factorial ANOVA results. On the other hand, the effect of interaction (**Figure 12**) between the type of isolate and the initial concentration of the contaminant ($F(4,10) = 2.8$, $p > 0.05$) was statistically nonsignificant.

Nickel Biosorption

The biosorption tests performed for nickel indicated

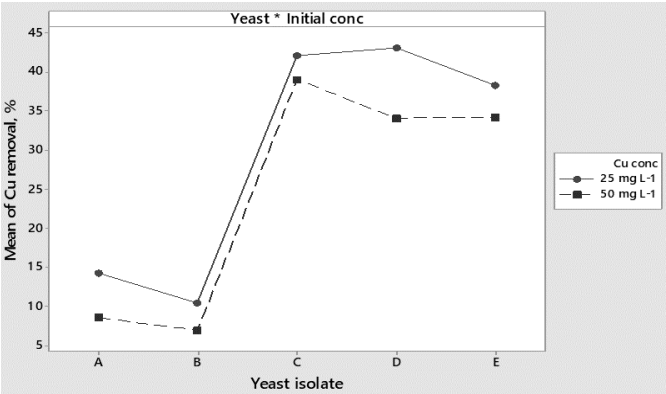

Figure 8. Interaction plot between yeast type and initial Cu concentration.

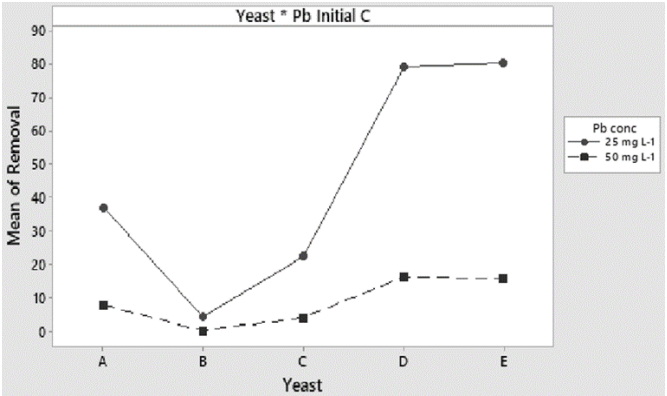

Figure 10. Interaction plot between yeast type and initial Pb concentration Chromium Biosorption.

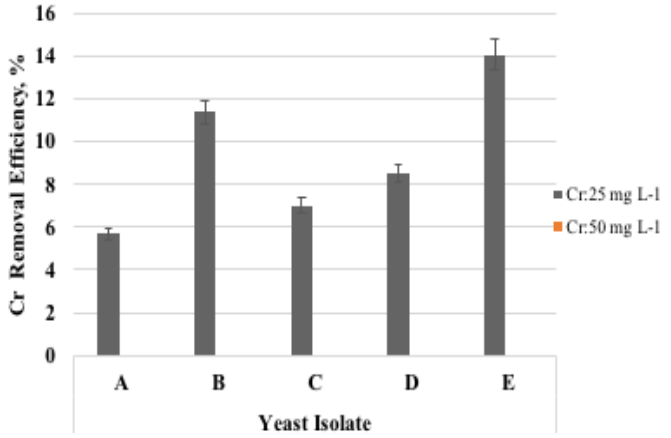

Figure 11. Removal efficiency of five yeast isolates at different initial Cr concentration.

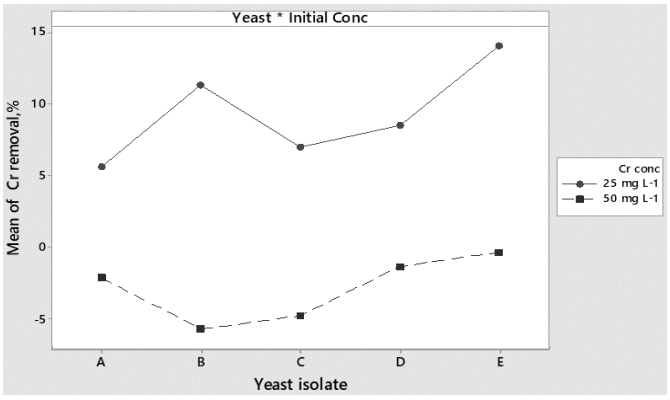

Figure 12. Interaction plot between yeast type and initial Cr concentration.

metal removal capacity of the five isolates at both concentrations. Greater removal efficiencies were noted to occur at 25 mg L^{-1} as compared to 50 mg L^{-1}. Among the isolates, the highest capability of treating more than 40% of Ni from simulated wastewater was achieved thru isolate E (*Nodulisporium* sp.). A comparison of removal efficiencies of the yeast isolates was made at initial concentrations of 25 mg L^{-1} and 50 mg L^{-1} (**Figure 13**).

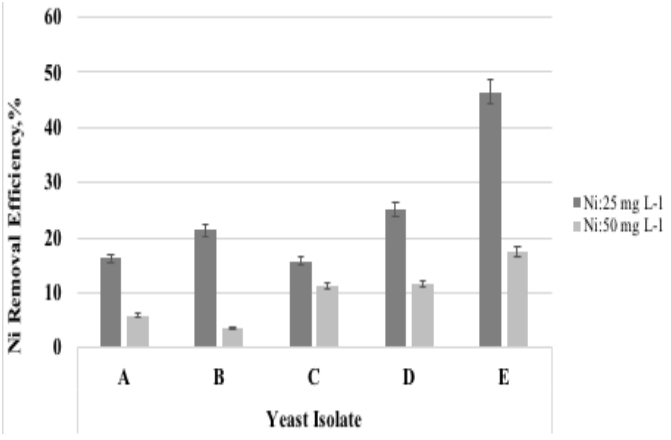

Figure 13. Removal efficiency of five yeast isolates at different initial Ni concentration.

The presence of significant main effect for the type of yeast isolate ($F(4,10) = 47.6$, $p < 0.05$) and initial concentration ($F(1,10) = 187.6$, $p < 0.05$) as factors on the lead removal efficiency was revealed thru ANOVA. In the same manner, interaction was also statistically significant between the type of isolate and initial concentration ($F(4,10,) = 14.1$, $p < 0.05$) (**Figure 14**).

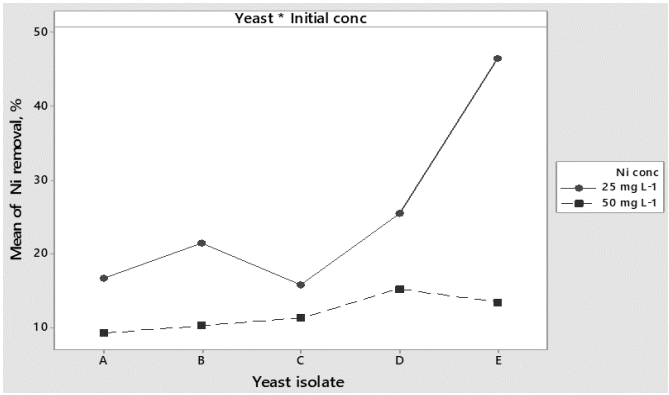

Figure 14. Interaction plot between yeast type and initial Ni concentration.

Means Comparison for the Best Yeast Isolate

A one-way ANOVA, in particular the Fisher Pairwise Comparison test using Minitab statistical tool was applied to the Pb biosorption results that obtained the highest removal efficiency. The means comparison test will assess if the difference between the means of the Pb removal efficiencies of the isolates were significant. Using the LSD method at 95% confidence interval, the grouping information (**Table 4**) presents the comparison between the performances of the isolates in the removal of lead from aqueous solution.

Based on the mean comparison test, both yeast isolates E and D have significantly different removal efficiency as compared to the rest of the isolates. However, the difference between the respective removal efficiency of these two isolates was not statistically significant, implying that either isolate E or isolate D can be utilized to attain maximum removal efficiency.

Table 4. Comparison of means using the Fisher LSD method.

Isolate	Mean (Removal Efficiency), %	Grouping*		
E	80.0	a		
D	79.1	a		
A	41.2		b	
C	22.4		b	c
B	4.1			c

*Means that do not share the same letter are significantly different

Model and Response Optimization Equation

Model equation for each metal was also generated to derive optimum response (removal efficiency) using the Minitab software. In general, the higher the R^2 (coefficient of determination), the better the model fits the data. The value of R^2 is always between 0 and 100%. On the other hand, low values for s (standard deviation =) suggest a better fit while higher values indicate a worse fit (*Frost 2014*). Based on the values of s and R^2, the models generated for all biosorption tests were adequate and significant and thus can be used to obtain optimum removal efficiency for each metal of concern.

Response optimization shows that a zinc removal efficiency of 41.2% at a concentration of 25 mg L^{-1} is attainable by using yeast isolate A (*Rhodotorula toruloides*). This corresponds to a biosorption capacity of 29.31 mg of zinc /g *R. toruloides*. Similarly, the performance efficiency of 43.4% was obtained for copper at a concentration of 25 mg L^{-1}. The use of isolate D (*Candida maltosa*) resulted in a biosorption capacity of 28.54 mg g^{-1} (**Table 5**).

On the other hand, greater removal efficiency of 80% was obtained by yeast isolate E (*Nodulisporium* sp.) at an initial lead concentration of 25 mg L^{-1}. The performance efficiency gave an equivalent biosorption capacity of 55.65 mg g^{-1} dry yeast which is comparable, if not better than the biosorption capacity of several studies. In particular, *Quayyum et al.* (*2016*) reported biosorption capacity of 39.55 mg g^{-1} for fungal isolates, *Rhizomucos pusillus* and *Aspergillus flavus* while a review carried-out by *Mustapha and Halimoon* (*2015*) cited that *Aspergillus niger* has Pb biosorption capacity of 34.4 mg g^{-1}. Additionally, *Li et al.* (*2015*) examined the biosorption capacity of *Ceriporia lacerata*, a strain of white-rot fungus which were observed to be 6.79 mg g^{-1} and 7.76 mg g^{-1} for initial copper (II) concentration of 100 and 200 mg L^{-1}, respectively (**Table 5**).

However, the response optimization for chromium

Table 5. Model equations for the optimum removal efficiency of different heavy metals and biosorption capacity of the best isolate.

Heavy Metal	Model Equation (Removal Efficiency)	Maximum Removal Efficiency, %	Biosorption capacity, mg g^{-1}	Optimum initial concentration, mg L^{-1}	Best Isolate
Zn	$8.736 + 11.8Y_A - 8.7Y_B - 2.05Y_c - 7.6Y_D + 6.5Y_E + 8.7Ci_{25} - 8.7Ci_{50} + 11.8Y_A*Ci_{25} - 11.8Y_A*Ci_{50} - 8.7Y_B*Ci_{25} + 8.7Y_B*Ci_{50} - 2Yc*Ci_{25} + 2Yc*Ci_{50} - 7.6Y_D*Ci_{25} + 7.6Y_D*Ci_{50} + 6.5Y_E*Ci_{25} - 6.5Y_E*Ci_{50}$ (S = 1.2, R2 = 99%)	41.2	29.31	25	*Rhodotorula toruloides* (Y_A)
Cu	$4.9 - 1.6Y_A - 2.0Y_B + 1.4Y_C + 1.2Y_D + 1.0Y_E + 0.3Ci_{25} - 0.3Ci_{50} + 0.1Y_A*Ci_{25} - 0.1Y_A*Ci_{50} + 0.02Y_B*Ci_{25} - 0.02Y_B*Ci_{50} - 0.1Y_C*Ci_{25} + 0.1Y_C*Ci5_0 + 0.08Y_D*Ci_{25} - 0.08Y_D*Ci_{50} - 0.1Y_E*Ci_{25} + 0.1Y_E*Ci_{50}$ (S = 0.1 , R2 = 99%)	43.4	28.54	25	*Candida maltosa* (Y_D)
Pb	$26.6 - 4.4Y_A - 24.5Y_B - 13.6Y_C + 20.9Y_D + 21.7Y_E + 17.8Ci_{25} - 17.8Ci_{50} - 3.2YA*Ci_{25} + 3.2Y_A*Ci_{50} - 15.7Y_B*Ci_{25} + 15.7Y_B*Ci_{50} - 8.3Y_C*Ci_{25} + 8.3Y_C*Ci_{50} + 13.7Y_D*Ci_{25} - 13.7Y_D*Ci_{25} + 13.6Y_E*Ci_{25} - 13.6Y_E*Ci_{50}$ (S = 1.8, R2 = 99%)	80	55.6	25	*Nodulisporium* sp.(Y_E)
Cr	$3.2 - 1.4Y_A - 0.4Y_B - 2.1Y_C + 0.36Y_D + 0.36Y_E + 6.0Ci_{25} - 6.0Ci_{50} - 2.2Y_A*Ci_{25} + 2.2Y_A*Ci_{50} + 2.4Y_B*Ci_{25} - 2.4Y_B*Ci_{50} - 0.2Y_C*Ci_{25} + 0.2Y_C*Ci_{50} - 1.1Y_D*Ci_{25} + 1.1Y_D*Ci_{50} + 1.1Y_E*Ci_{25} - 1.1Y_E*Ci_{50}$ (S = 2.2, R2 = 94%)	14.1	9.56	25	*Nodulisporium* sp.(Y_E)
Ni	$17.4 - 6.4Y_A - 4.9Y_B - 3.9Y_C + 0.9Y_D + 14.4Y_E + 7.5Ci_{25} - 7.5Ci_{50} - 2.4Y_A*Ci_{25} + 2.4Y_A*Ci_{50} + 1.4Y_B*Ci_{25} - 1.4Y_B*Ci_{50} - 5.3Y_C*Ci_{25} + 5.3Y_C*Ci_{50} - 0.6Y_D*Ci_{25} + 0.6Y_DCi_{50} + 7.0Y_E*Ci_{25} - 7.0Y_E*Ci_{50}$ (S = 2.4, R2 = 98%)	46.5	31.67	25	*Nodulisporium* sp.(Y_E)

Legend: Ci – initial concentration , Y - yeast

gave the lowest removal efficiency among the metals studied. Yeast E (*Nodulisporium* sp.) showed biosorption capacity of 9.56 mg g^{-1} at the initial concentration of 25 mg L^{-1}. In contrast, the response optimization for nickel gave a better removal efficiency of 46.5 % which was achieved using the same isolate (*Nodulisporium* sp.). At the initial concentration of 25 mg L^{-1}, a biosorption capacity of 31.76 mg g^{-1} *Nodulisporium* sp. was obtained for nickel.

Based from the results of the study, the five isolates revealed potential to remove heavy metals particularly at lower concentration of 25 mg L^{-1}, suggesting that greater performance can be achieved at much lower or possibly in residual concentration. The selected isolates tend to be more selective in removing heavy metals. In particular, *Nodulisporium* sp. has the greatest potential in removing Pb in aqueous solution. Without any modification, this yeast species can remove at least 80% of Pb and was likewise found to be better than the rest of the isolates in removing various metals of concern such as Ni and Cr.

Continuous treatment System using Aerobic Cascading Baffled Reactor

The semi-continuous treatment of Pb contaminated wastewater using the ACFBBR equipped with perforated plastic packing material containing the Nodulisporium sp. resulted in much improved metal removal capacity. Similar with the batch adsorption test, an average removal efficiency of 80% was also achieved after passing a total volume of 23.3 L of wastewater (**Figure 15**). Although the same efficiency was noted between batch and semi-continuous treatment modes, the operation of the ACFBBR registered a tripled value on the biosorption capacity of 170.14 mg g^{-1}, a value that is remarkably higher than the batch adsorption test. The result signified promising potential of *Nodulisporium* sp. when applied in a continuous treatment system. The design of the ACFBBR provided good contact between the biosorbent and the wastewater since the reactor configuration allowed a gentle cascading flow.

In addition, the plastic packing material (**Figure 16**) that was used has a uniform size and corrugated outer and inner surfaces which could enhanced yeast growth. Moreover, Scanning Electron Microscope (SEM) image revealed rough surface of a plastic cap which basically improved slime growth (**Figure 17**). *Gacho et. al* (2010) observed that uniform size and perforations on packing materials allowed random packing without compromising any restrictions on flow. The high void fraction or space not occupied by the packings itself permitted free flow

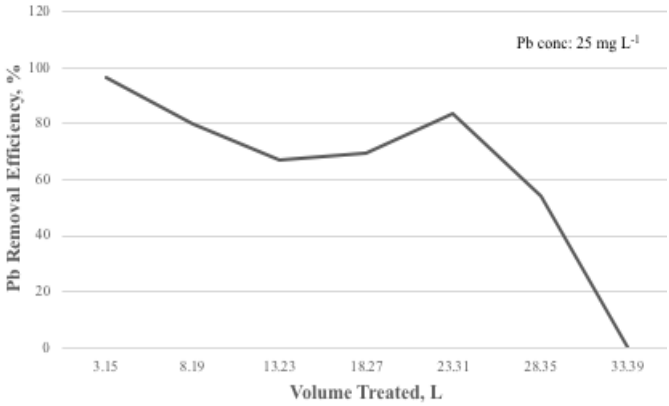

Figure 15. Removal efficiency attained by the ACFBBR during the semi-continuous treatment run.

Figure 16. The plastic cap employed in the experiment was of screw type with corrugated grip.

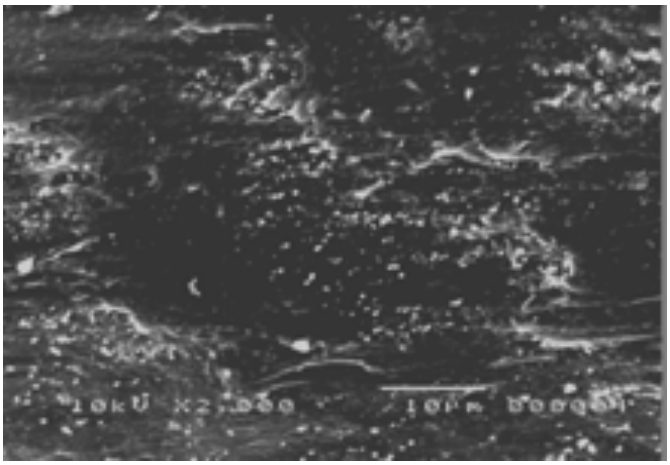

Figure 17. Corrugated grip feature of the plastic captured using Scanning Electron Microscopy (SEM). Magnified picture of the packing material taken using SEM showed rough surface.

and significant contact with the wastewater. Saturation of adsorption sites, however, occurred when the performance of the ACFBBR abruptly declined and until removal was no longer observed at 33.4 L.

CONCLUSIONS AND RECOMMENDATIONS

The screening and isolation of microbes from water samples collected from abandoned mining sites in Itogon Benguet yielded several metal-tolerant isolates which were found to be capable of removing various heavy metals from aqueous media. The selected microbes were molecularly identified as species of yeast.

Except for Cr, the calculated p-values for most of the heavy metals of concern (Zn, Cu, Pb, Cr and Ni) revealed that factors such as the type of yeast isolate and initial concentration are significant. It was also deduced that an interaction exists between these factors and that the interaction is also significant in terms of the response (% removal of metal). Means comparison revealed that either *Nodulisporium* sp. or *Candida maltose* have strong potential for Pb sequestration in aqueous media. Similarly, *Candida maltosa* and *Rhodotorula toruloides* can be applied for the removal of Zn and Cu, respectively.

Among the yeast isolates, *Nodulisporium* sp. registered highest removal efficiencies for at least three metals, namely Pb, Cr and Ni. The tolerance property of *Nodulisporium* sp. to several metals can be attributed to the fact that the isolate has already acclimatized to an extreme environment, having been collected from an active mine tailings storage facility in Benguet. Compared with other microbes in the literature, some of which were either treated or dead biomass, the biosorption capacity of *Nodulisporium* sp. of 56.65 mg g^{-1} was relatively higher than those reported for *Rhizomucos pusillus*, *Aspergillus flavus*, *Aspergillus niger* and *Ceriporia lacerate*.

The capability of *Nodulisporium* sp. was further enhanced when it was applied in a semi-continuous treatment system using the Aerobic Cascading Filter Bed Baffled Reactor (ACFBBR). The use of a non-degradable packing material with corrugated inner and outer surfaces was noticed to favorably enhanced slime growth and retention times. The spent plastic caps employed in the experiment was of screw type with corrugated grip design which provided good adherence of the filamentous yeast to the material. The packing material and the alternating cascading baffled design of the reactor greatly improved the contact between Nodulisporium sp. and the Pb contaminated wastewater and resulted in a much increased biosorption capacity of 170.14 mg g^{-1}.

The isolates gave better removal efficiencies at lower concentration of 25 mg L^{-1}, suggesting better application for sites with residual amount of toxic metals. The metal accumulation potential for the selected yeast isolates was generally higher at the lower initial concentration of the metal ions. This characteristic correlates well with the results of the statistical analysis wherein surface saturation of adsorbent is highly dependent on the initial concentration of metal ions. A study carried out by *Oves et al.* (*2013*) indicated that better metal uptake of biosorbent materials at the lowest concentration could probably be due to a rapid metal absorbing ability of the yeast biomass. At low concentrations, available adsorption sites took up the available metal ions more quickly. Whereas, *Bhattia et al.* (*2011*) suggested that at higher metal concentration, metal ions diffuse onto the biomass surface by intraparticle diffusion and therefore, the hydrolysed ions are likely to diffuse very slowly. Similarly, *Wang and Chen* (*2009*) observed that with a decrease in metal ion concentration, the biosorption rate increases rapidly while with higher metal ion concentrations a substantial decline in metal removal rate is reported which could probably be due to the saturation of a number of adsorption sites.

Though many studies on biosorption employed chemical and physical treatment to improve adsorption capacity of biomass, this study, on the other hand, showed that even inactivated yeast relatively possess high capacity for the treatment of specific metals such as Pb from aqueous media. This study is quite similar with several research initiatives such as those of *Ceribasi and Yetis* (*2001*) and *Vasudevan et al.* (*2001*) where species of live and inactive biomass have been applied in biosorption for metal removal. In addition, the advantage of yeasts over other microbes is that they are easy to cultivate and can produce a high biomass yield. As a result, the yeast has potential to sequester toxic metals from polluted wastewaters or any aqueous media and would be very useful for application in continuous treatments system.

Overall, the selected yeast isolated from abandoned mining site in Itogon Benguet presents desirable opportunity as low-cost means to protect the environment from heavy metal contamination.

Further studies, particularly batch adsorption testing should be carried-out to examine the effect of other factors such as pH, contact time and temperature. In addition, kinetic study must be carried-out to optimize the contact time needed to practically remove the contaminants. Moreover, there is a need to investigate other ways for the yeast biomass to adhere to the plastic carrier material which could better improve the performance of the bioreactor. Also, biosorbent characterization on biological, physiological and molecular level, and the determination of surface morphology, functional groups and particle size should be performed as these

are important properties for elucidating the mechanism involved in the removal of heavy metals in aqueous media. Most importantly, microbial biomass with the potential for metal uptake needs to be explored fully in terms of practical large scale application of continuous treatment system, in particular ACFBBR in water and wastewater management, as well as how to recover metal for reuse or handle the metal as hazardous waste.

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