# Investigation of Toxic Heavy Metal Removal Activity of Activated Coconut Husk as Solid Support Medium for Isolated Bacterial Strains

Sa Aung Ko Ko[1], Saw Sandar Maw[2], and Zaw Khaing Oo[3]

***Abstract*—Excessive released of industrial wastewater is the major problem for many people's life. These toxic metals can cause some chronic diseases to human life and this is the biggest problem for human life. Many natural by-products have toxic metals adsorption activities and lower costs and higher removal activity than other synthetic products. In our country, natural by-products are very plenty and by using these gives benefits in cash in the industrial wastewater treatment. In this research, activated coconut husk was used as solid support medium for isolated bacterial strains. Ten bacterial strains isolated from streams around the Mandalay Industrial Zones, Myanmar and identified their toxic heavy metal removal activities by using semi-synthetic waste water such as $Pb^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ about 100ppm in lab scale. According to the Atomic Absorption Spectrometer results, the amount of these three metals reduced and the quality of water such as BOD, COD, TDS, TSS, pH and DO can discharge into the river.**

**Key words---activated coconut husk, Atomic Absorption Spectrometer, BOD and discharge, toxic heavy metal**

## I. Introduction

Most industrial wastewater is usually hazardous to human and much aquatic life. Industrial wastewater contain many organic pollutants and toxic heavy metals such as $Ni^{2+}$, $Cr^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Cd^{2+}$ and $Cu^{2+}$. Excessive release of heavy metals into the environment due to industrialization and urbanization has posed a great problem [1].

Heavy metal contamination exists in aqueous wastes of many industries, such as metal plating, mining operations, tanneries, chloralkali, radiator manufacturing, smelting, alloy industries and storage batteries industries, etc. Treatment processes for heavy metal removal from wastewater include precipitation, membrane filtration, ion exchange, adsorption, and coprecipitation/adsorption [2].

Today, many researchers try to remove toxic metals by using biotechnological methods. Removal of toxic metals by using biotechnological methods is lower costs and more safety than other treatment methods. In biotechnological methods, many natural by-products such as sugarcane, rice husk, sawdust, coconut husk and oil palm shell have been investigated for treatment of industrial wastewater [3].

For the treatment of industrial wastewater, these natural by-products can be used as biofilms. Many microorganisms possess toxic metal removal activities just like as natural by-products.

Therefore, natural by-products can be used biofilms or appropriate solid support medium for microorganisms. Biofilms have three mechanistic pathways such as: (1) microbial accumulation, (2) microbially mediated precipitation and (3) microbial transformation [4].

In microbial accumulation, microorganisms can serve passive or active accumulation of toxic metals. Microorganisms possess an abundance of functional groups on their cell surface and extracellular polymers that can passively sequester metal ions. In this process, Cyanobacteria (*Spirulina*), yeast, algae, plants (*Lemna* sp., *Sphagnum* sp.), *Bacillus subtilis, Chlorella vulgaris, Sargassum natans, Ascophyllum nodosum, Halimeda opuntia, Palmyra pamata, Chondrus crispus, Chlorella vulgaris, Rhizopus arrhizus, P. chrysogenum* [5].

In microbially mediated precipitation, metals and radionuclides precipitate as minerals of sulfide, hydroxide, phosphate and carbonate by microbial activities. Many Sulfate reducing bacteria are *Desulfobacter, Desulfococcus, Desulfosarcina, Desulfobacterium, Desulfonema, Desulfovibrio, Desulfotomaculum, Desulfomonas, Thermodesulfo bacterium* and *Desulfobilbus* [6].

In microbial transformation, microorganisms can transform metals or metalloids via reduction /oxidation or alkylation reactions. For example, many aerobic and anaerobic bacteria reduce Cr (VI) to the less toxic and less soluble Cr (III). Moreover, some microorganisms can be used in removal of heavy metals. In bacteria species, *Pseudomonas sp, Bacillus sp, Rhizopus arrhizus, ChloreIla vulgaris and Spirulina platensis* can remove toxic heavy metal [7].

Algae and fungus have these toxic metal removal activities such as *Sargassum, Ulva reticulata, Padina sp,Aspergillus niger, Fusarium, Candida albicans* and so on [8]. In this research, activated coconut husk was used as solid support medium for isolated bacterial strains. These bacterial strains isolated from industrial wastewater, identified their toxic metal removal activities in lab scale.

Manuscript received Nov. 1, 2016.

Sa Aung Ko Ko, Department of Biotechnology, Mandalay Technological University, Myanmar.

Saw Sandar Maw, Department of Biotechnology, Mandalay Technological University, Myanmar.

Zaw Khaing Oo, Department of Biotechnology, Mandalay Technological University, Myanmar.

## II. Materials and Methods

### A. Sampling of Industrial Wastewater

Industrial wastewater was sampled from Nyaung Nhint Pin village and near Truck Bus Terminal on 67th street, Pyigyitagon Township, Mandalay Region, Myanmar.

### B. Characterization of wastewater

The physicochemical characteristics of wastewater such as pH, biological oxygen demand, total solid, total dissolved solid and total suspended solid was measured.

### C. Isolation of Bacterial Strains

For removal of toxic heavy metal, ten bacterial strains were isolated from streams near the Mandalay Industrial Zone, Mandalay Region in Myanmar.

### D. Screening on growth of isolated bacterial strains on culture media with different metals concentration

The growth condition of isolated bacterial strains on different metals concentration were tested by culturing in nutrient media with different heavy metal ($Pb^{2+}$, $Zn^{2+}$ and $Cd^{2+}$) concentration ( 50 mg/L, 100 mg/L, 200 mg/L, 400 mg/L, 800 mg/L and 1000 mg/L).

### E. Treatment of Semi-synthetic wastewater

Isolated bacterial strains cultured (2.3cm high x 2.3cm length x 2.3cm width) in nutrient broth media for 24 hours with activated coconut husk (2.3cm high x 2.3cm length x 2.3cm width). After 24 hours incubation, activated coconut husk was used as an inoculums for the treatment of semi-synthetic wastewater with 100ppm concentration of different heavy metals. The treated metal solution was determined by atomic absorption spectrometer (AAS).

## III. Results and Discussion

There are two sampling sites for the treatment of industrial wastewater. These two sampling sites are shown in Fig .1. The sampling time, date, pH and dissolved oxygen are shown in Table 1. The pH and Dissolved oxygen of wastewater from Nyaung Nhint Pin village are 5.8 and 0.10 respectively. The pH and dissolved oxygen of the other site, near Truck Bus Terminal on 67th street, are 6.8 and 0.04.

The physicochemical characteristics of these two sites are shown in Table 2. The BOD value of wastewater from Nyaung Nhint Pin village is 1350 mg/L. This value is more than the EPA's typical wastewater standard. The TS, TDS and TSS of this site are 3530 mg/L, 1686 mg/L and 1815 mg/L respectively.

The wastewater near Truck Bus Terminal on 67th street has 44 mg/L of BOD value. This value is lower than the EPA's typical wastewater standard. The value of TS, TDS and TSS of wastewater near Truck Bus Terminal on 67th street are 1290 mg/L, 1294 mg/L and 1260 mg/L respectively. In comparison of these two sites, the first site, wastewater from Nyaung Nhint Pin village needs to treat for discharge into the water.

Ten bacterial strains isolated from streams near the Mandalay Industrial Zone, Mandalay Region in Myanmar for the treatment industrial wastewater and removal of toxic heavy metals. There are seven rod shape bacteria and three bacterial strains are cocci shape.

In Gram's staining reaction, the seven bacterial strains are gram positive bacteria and the left three are gram negative bacteria. These facts are shown in Table 3. Some biochemical test results are expressed in Table 4. In sugar fermentation test, eight isolated strains are positive in glucose fermentation expect B1 and B2.

The isolated strains (B1, B7, B8 and B9) are positive in sucrose fermentation and the left are negative. In urease test, B1, B2, B3 and B8 are negative and the others are positive. Isolated strains, B1, B5, B6 and B7 are positive and the other six strains are negative in lipolytic activity. In starch hydrolysis activity, only B2 has negative activity and the left nine isolated strains has positive activity. There are no strains in citrate utilization activity.

For the growth condition of isolated bacterial strains on different metal concentration, all isolated strains cultured in nutrient agar media with $(PbNO_3)_2$, $ZnSO_4$ and $CdSO_4$ solution (50, 100, 200, 400, 800, 1000mg/L). In $(PbNO_3)_2$ solutions, isolated strains (B3, B7 and B10) was grew grow in 50mg/L and 100um/l. The left strains can grow well in 50 mg/L to 400 mg/L of $(PbNO_3)_2$ concentration. In $ZnSO_4$, isolated strain, B3, can grow only 50 and 100 mg/L, isolated strain, B2, cannot grow 800 and 1000 mg/L and isolated strain, B7, can grow 50, 100 and 200mg/L.

Isolated strains, B5 and B9 cannot grow well in all concentration of (50, 100, 200, 400, 800, 1000mg/L) of synthetic metal solutions. The other strains can grow all concentration. In $CdSO_4$, isolated strains, B3 and B7 can grow only 50 and 100mg/L concentration and isolated strains, B10, cannot grow well in all concentration. The left strains can grow well in all concentration.

For the treatment of semi-synthetic industrial wastewater, activated coconut husk was used as support medium for isolated bacterial strains. All isolated bacterial strains were cultured in nutrient broth media with activated coconut husk. After 48 hours incubation, only activated coconut husk was used as inoculums for the treatment of semi-synthetic wastewater in 100 mg/L of metal solution ($Pb(NO_3)_2$, $ZnSO_4$ and $CdSO_4$) with nutrient media in water batch shaker. After 48 hours treated in water batch shaker, the amount of metal concentration decrease 7.6 mg/L in coconut husk and 5.6 mg/L in coconut husk combine isolated bacterial strains for $Pb^{2+}$. For $Zn^{2+}$, the amount of metal concentration were decreased 0.05 mg/L in coconut husk and 0.02 mg/L in coconut husk combine isolated bacterial strains. For $Cd^{2+}$, the amount of metal concentration were decreased 15.23 mg/L in coconut husk and 14.95 mg/L in coconut husk combine isolated bacterial strains. These results are shown in Table 5. Therefore, the toxic heavy metal removal activities of activated coconut husk and isolated bacterial strains are good and their activities can be used in the treatment of industrial wastewater.

According to the above data, these isolated bacterial strains have toxic heavy metals removal activities and the activated coconut husk can remove toxic heavy metal.


Fig. 1. Sampling Sites of Industrial Wastewater at Nhint Pin village and near Truck Bus Terminal on 67th street, Mandalay Industrial Zone, Mandalay Region, Myanmar

TABLE I. SAMPLING SITES, TIME, PH AND DISSOLVED OXYGEN

| No. | Sample | Location | Date | Time | Temperature | pH | Dissolved Oxygen |
|---|---|---|---|---|---|---|---|
| 1 | S1 | Nyaung Nhint Pin village | 26.1. 2016 | 10:15 AM | 34ºC | 5.8 | 0.10 |
| 2 | S2 | Near Truck Bus Terminal on 67th street | 26.1. 2016 | 10:44 AM | 20ºC | 6.8 | 0.04 |

TABLE II. PHYSICOCHEMICAL CHARACTERISTICS OF TWO SAMPLING SITES FROM MANDALAY INDUSTRIAL ZONE, MANDALAY REGION, MYANMAR

| No. | Sample | Total solid (mg/L) | Total dissolve solid (mg/L) | Total Suspended Solid (mg/L) | Biological Oxygen Demand (BOD) |
|---|---|---|---|---|---|
| 1 | S1 | 3530 | 1686 | 1815 | 1350 |
| 2 | S2 | 1290 | 1294 | 1280 | 44 |

TABLE III. GRAM'S STAINING REACTION AND SHAPE OF ISOLATED BACTERIAL STRAINS

| No. | Strains No. | Gram's Positive/ Negative | Shape |
|---|---|---|---|
| 1 | B1 | + | rod chain |
| 2 | B2 | + | small rod chain |
| 3 | B3 | - | rod |
| 4 | B4 | - | cocci |
| 5 | B5 | + | small rod |
| 6 | B6 | + | cocci chain |
| 7 | B7 | - | small rod |
| 8 | B8 | + | cocci |
| 9 | B9 | + | small rod |
| 10 | B10 | + | small rod |

TABLE IV. BIOCHEMICAL TESTS OF ISOLATED BACTERIAL STRAINS

| No. | Strain No. | Glucose fermentation | Sucrose fermentation | Urease test | Citrate utilization | Starch hydrolysis | Lipolytic activity |
|---|---|---|---|---|---|---|---|
| 1 | B1 | - | + | - | - | + | + |
| 2 | B2 | - | - | - | - | - | - |
| 3 | B3 | + | - | - | - | + | - |
| 4 | B4 | + | - | + | - | + | - |
| 5 | B5 | + | - | + | - | + | + |
| 6 | B6 | + | - | + | - | + | + |
| 7 | B7 | + | + | + | - | + | + |
| 8 | B8 | + | + | - | - | + | - |
| 9 | B9 | + | + | + | - | + | -- |
| 10 | B10 | + | - | + | - | + | + |

TABLE V. COMPARISON OF SEMI-SYNTHETIC WASTEWATER TREATMENT AND SEMI-SYNTHETIC CONTROL

| No. | Treatment Type | Remain Metal concentration (mg/L) in semi-wastewater | | |
|---|---|---|---|---|
| | | $Pb^{2+}$ | $Zn^{2+}$ | $Cd^{2+}$ |
| 1 | Activated Coconut Husk | 7.6 | 0.05 | 15.23 |
| 2 | Activated Coconut Husk with isolated bacterial strains | 5.6 | 0.02 | 14.95 |

## IV. CONCLUSION

In this study, ten bacterial strains isolated and they were used together as community for the treatment of semi-synthetic wastewater ($Pb^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ solution) with activated coconut husk. In my views, activated coconut husk have toxic heavy metals adsorption activities and isolated bacterial strains posses toxic metals removal activities. According to the results of AAS, their heavy metal removal activities were the highest percentage in $Zn^{2+}$ solution and the other two metals can also be removed by activated coconut husk as solid support medium for isolated bacterial strains. Therefore, these isolated strains can be used in the treatment of industrial wastewater.

## ACKNOWLEDGEMENT

The author would like to express his thanks to Dr. Myint Thein, Rector, Mandalay Technological University, for his kindness, help, permission and suggestion for completion of this paper.

The author would like to express his gratitude to Dr. Myo Myint, Associated Professor and Head, Department of Biotechnology and to his teachers from Department of Biotechnology at Mandalay Technological University. The author also appreciates and thanks to corresponding people who helped directly and indirectly for this research.

## REFERENCES


[1] V.K Gupta and S. Sharma., "Process development for the removal of lead and chromium from aqueous solution using red mud – an aluminum industry waste", *Water Res.* 35(5): 1125 – 1134 pp, 2001.

[2] K.. Thamaraiselvi, and C Namasivayam,, "Removal of Heavy Metal from Industrial Wastewaters by Adsorption onto Activated Carbon Prepared from an Agricultural Solid Waste", *Bioresource Techn.*, 76: 63-65 pp, 2001.

[3] N. A. Khan*, S. Ibrahim and P. Subramaniam, *"Malaysian Journal of Science",* 23: 43 – 51, 2004.

[4] M. Tsezos, A. Hatzikioseyian, E. Remoudaki, *National Technical University of Athens, School of Mining and Metallurgical Engineering, Laboratory of Environmental Science and Engineering, Heroon Polytechniou 9, Zografou 15780, Athens, Greece.*

[5] B. Volesky, "Biosorption of Heavy Metals", CRC press,[6] F. Widdel and Bak,, "Gram-negative mesophilic sulphate-reducing bacteria", The Prokaryotes, Balows A., Truper H.G., Dworkin M., Harder W., and Schleifer K.- H., Eds, 2nd edition, Springer – Verlag, New York, 1991, 3352-3378, 1990.

[6] B.E. Rittmann and P.L. McCarty, "Environmental Biotechnology:Principles and applications", Mc Graw Hill Series in Water Resources and Environmental Engineering, 2001.

[7] D. H. NIES, "Resistance to cadmium, cobalt, zinc and nickel in microbes. *Plasmid"*, 27: 17-28, 1992. https://doi.org/10.1016/0147-619X(92)90003-S


Sa Aung Ko Ko is now a Ph.D student of Mandalay Technological University, Mandalay, Myanmar. He was borned in Hinthada Township, Ayeyarwady Region, Myanmar. His date of the birth is 22 apiral, 1985. He got his B.S degree with Biotechnology from Technological University ( Pathein), Myanmar in 2006. He obtained his M.S degree with Food Biotechnological from Mandalay Technological University, Mandalay, Myanmar in 2012. Currently, he is also a demonstrator at Mandalay Technological University. He has over three years working experience in Food Biotechnology research for Master degree program and nearly six years working experience in Environmental Biotechnology research. Now, he is also doing his Ph.D research in Environmental Biotechnology at Mandalay Technological University. He will include in International Conference on Science and Engineering in December 10 to 11, 2016 at Yangon Technological University, Yangon, Myanmar by oral presentation about "Removal of Heavy Metal by Using Isolated Bacteria from Industrial Wastewater in Laboratory Scale".