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Biosorption of Heavy Metals in Automobile Panel Workshop Effluent using *Bacillus safensis* LAU13

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Abstract

Heavy metals are natural constituents of the environment, but improper application for human activities has altered their geochemical compositions and biochemical balance. There is a need to remediate heavy metals released into natural resources through anthropogenic activities. This study centres on remediation (by biosorption) of Mn^{2+}, Co^{3+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Hg^{2+}, Se^{4+}, Cr^{3+}, Pb^{4+} and Cd^{2+} from wastewater obtained from automobile panel workshop, Ogbomoso, Nigeria using *Bacillus safensis* LAU13 inoculum. Initial concentrations of heavy metals in the effluent samples after digestion were determined through Atomic Absorption Spectrometery (AAS). The glucose enriched wastewater samples were autoclaved, the inoculums cultured and subjected to bio-treatment using 250, 500, 750 and 1000 µL dosages of *Bacillus safensis* LAU13 inoculum, a Gram-positive, mesophilic, spore-forming, aerobic and chemo- heterotrophic bacterium. Optical density of inoculated samples was determined through spectrophotometer and the growth monitored for 48 h. Residual pellets were subjected to Scanning Electron Microscopy (SEM). Results showed that 100% removal was achieved for Mn^{2+} and Co^{3+}. Cr^{3+} was reduced to a very minimal concentration, Zn^{2+}, Cu^{2+}, $Pb^{4+}$and Se^{4+} were above average in the remediation. $Fe^{2+}$and Cd^{2+} were remediated below average but very low remediation of Hg^{2+} with less than one percent removal. Optical density increased significantly showing rapid growth of microorganism irrespective of glucose supplement, and 250 µL inoculum dosage has the optimum performance. SEM micrographs indicate certain accumulated aggregates taken as remediated heavy metals. The study confirmed efficiency of biosorption technique as an alternative to the conventional treatment methods of huge cost, and high precipitates and slurry. *Bacillus safensis* LAU13 is therefore recommended as a bio-remediating agent in treating automobile workshop effluents with high amount of manganese, cobalt, chromium, zinc, copper, lead and selenium.

1. Introduction

Heavy metals are often the group name used for metals and semi-metals that have been connected with contamination and possible toxicity. Heavy metals bioaccumulate and tend to be dangerous since they have long biological half-lives [1, 2, 3, 4]. Some point sources of heavy metal pollutants include industrial effluent from mining, metal processing, tanneries, pharmaceuticals, pesticides, organic chemicals, rubber and plastics, lumber and wood products. Heavy metals even at low

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concentration can cause toxicity to other forms of life. Heavy metal pollution has become a very serious threat today and of great environmental concern as they are non-biodegradable and thus persistent. Heavy metals have the ability to enter the human body through inhalation, ingestion and dermal contact absorption [4, 5, 6]. They also accumulate in soils, plants and in aquatic biota [3, 7, 8]. It is necessary to remove these toxic heavy metals from waste water before its disposal to avoid health hazards.

Bioremediation is the use of biological organisms, most times, using micro-organisms to remediate contaminants especially from polluted water. The main goal of bioremediation is to stimulate microorganisms with nutrients and other metabolites that enable them to destroy the contaminants. The use of microorganisms have many advantages over the conventional remediation methods, such include cost- effectiveness, little or no by-products, reusability and more [9]. Microorganisms are responsible for the maintenance and sustainability of any ecosystem as they adjust rapidly towards changes and deterioration of the environment. Certain metabolic enzymes that can be employed for the safe contaminants removals are produced through them, they achieve this either by direct destruction of chemical or through transformation of the contaminants to a less toxic substance.

Bacillus safensis is a gram positive, meso-philic, spore forming, aerobic and chemo-heterophic bacterium. It is a rod-shaped motile bacterium that is tolerant to heavy metals, salinity, ultra-violet and gamma radiations [10, 11, 12]. The habitat of *Bacillus safensis* is a wide range colony, many of which make the survival of some micro-organisms possible. Its unique physiological and genotypic characteristics dictate its survival in extreme environments [13]. The bacterium belongs to the *Bacillus pumilus* groups and is to *Bacillus pumilus*, *Bacillus altitudinis*, *Bacillus xiamenesis* and *Bacillus invictae* so closely. *Bacillus safensis* possesses some plant growth promoting traits and because of its ability to produce various industrial enzymes and individually applicable secondary metabolites it has promising biotechnological applications. It may be regarded as a safe industrial microorganism because its pathogenicity has never been evidenced [14]. Strains of *Bacillus safensis* is capable of producing industrial enzymes like amylase [12], keratinase [14], lipase [15], and is also being applied as a plant growth promoting bacterium [12]. *Bacillus safensis* displays growth capacity at a pH range of 4.0 – 9.0 and within in a temperature range of 10 – 50 ºC, its optimum growth is at pH 7 and 37 ºC [12, 16]. There has been a recent report of keratinase production by a new isolate of *Bacillus safensis* LAU13 [14, 17]. The isolate was found to be capable of degrading whole feather, and its keratinase used for dehairing of goat skin, removal of blood stain and bio-synthesis of silver, gold and silver-gold alloy nanoparticles [13, 14, 18, 19, 20, 21]. Further reports about the use of *Bacillus safensis* to produce some secondary metabolites; including arachidonic acid, carotenoids and biosurfactants have been made [22, 23, 24]. These are for diverse applications which were documented in an earlier review on the biology and biotechnological applications of *Bacillus safensis* [13]. These unique abilities of *Bacillus safensis* make it an ideal candidate for various biotechnogical applications, remediation inclusive.

The most toxic heavy metals are cadmium, copper, lead, mercury and chromium, all of which are included in the Environmental Protection Agency's list of priority pollutants [25]. Earlier research works established the application of bacteria for biosorption of heavy metals [26, 27, 28, 29, 30, 31] reported the use of some strains of *Bacillus safensis* for

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the remediation of nickel. There were also publications regarding the isolation of metal and salt-tolerant strains of *Bacillus safensis* [11, 12] as potential organisms for remediation of heavy metals.

One of the industrial activities which generate heavy metals as waste is the automobile panel works. During various sanding automobile processes, heavy metals such as Nickel, Arsenic, Copper, and Zinc are generated which later find their ways into our water sources either through percolation into the ground water or by runoff into surface water. Thus, effluents from human activities especially industrial activities should be properly treated before being discharged into the nearby water bodies. Bioremediation process is a very important, proven and improving process that is used in the remedial of heavy metals present in industrial wastewater such as one from automobile panel workshop.

Therefore this research focuses on how to improve the bioremediation process by using a bacteria called *Bacillus safensis* LAU 13 as an absorbent for the heavy metals present in automobile panel work wastewater which can assist reduction of heavy metals effluents into water bodies and impact positively in water treatment and management system as a whole. This study centres on remediating heavy metals from the automobile panel works wastewater using *Bacillus safensis* LAU13. The specific objectives of the project are to identify and determine the concentration of heavy metals in the automobile panel works wastewater, determine the effectiveness of *Bacillus safensis* LAU13 in the bioremediation of automobile panel work wastewater.

2. Materials and Methods

2.1 Preparation of the wastewater

Preliminary experimental study was conducted on the wastewater samples collected from the Gbade automobile panel workshop, Ogbomoso, Nigeria located on latitude and longitude 8.15839 ^{0}N and 4.26086 ^{0}E respectively. The wastewater samples were collected into clean 2-litre plastic containers, air-tightened and taken directly to the laboratory for analyses. To breakdown the complexity of samples, their digestion was carried out before the AAS analysis. About 10 mL of the wastewater was taken into a beaker, then 10 mL of Concentrated (70 wt%, 15.7 N) HNO_3 was added to it to generate a rapid reaction [28]. The wastewater – nitric acid mix was placed in the fume cupboard and heated in a mantle at 100 ˚C for 30 minutes. The sample was then removed from the cupboard and distilled water added making it up to 100 mL before being filtered through Whatman filter paper No.1. The filtrate was later poured into a container for the Atomic Absorption Spectrometry (AAS) analysis to determine the initial and final concentrations of heavy metals present in both the wastewater and in the control samples [29, 30].

2.2 The Bacterium

Bacillus safensis LAU13 inoculum used in this study is a bacterium previously isolated in the Industrial Microbiology and Nano biotechnology laboratory, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso from feather waste dumpsite [14]. Before the application, it has been regularly sub-cultured and maintained on yeast extract agar and stored at 4 ^{o}C.

2.3 Preparation of sample for inoculation

A 500 mL portion of wastewater sample was measured into a beaker and a pH meter was used to take the initial pH value which was 7.2**.** Sodium hydroxide (NaOH) was added to the wastewater to increase the pH of the water to 7.5, which is the pH value that can aid best growth of our inoculum (*Bacillus safensis* LAU13)**.** The adjusted pH value

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wastewater (240 mL) was measured into a beaker and 3.12 g of the nutrient broth was dissolve into it and mix thoroughly to form a solution with a spatula. The sample (10 mL) was measured and transferred into twenty - four (24) different test tubes. Glucose was added at different proportion into the test tubes ranging from (0.0 – 1.0 g) at intervals of 0.2 g making six (6) test tubes for each proportion of glucose. The test tubes were cotton - plugged at their open ends to prevent board, the inoculums (*Bacillus safensis* LAU13) was transferred into them with use of wire loop from the nutrient medium. The air-tight cultures were then incubated at 37 °C for 24 h. The 18-h broth culture of *Bacillus safensis* LAU13 containing approximately 1 × 106 cfu / mL was then added aseptically at 250, 500, 750 and 1000 µL with micropipette to the samples with various quantities of supplement (glucose) ranging from 0.2 to 1.0 grams. After inoculation, the samples were

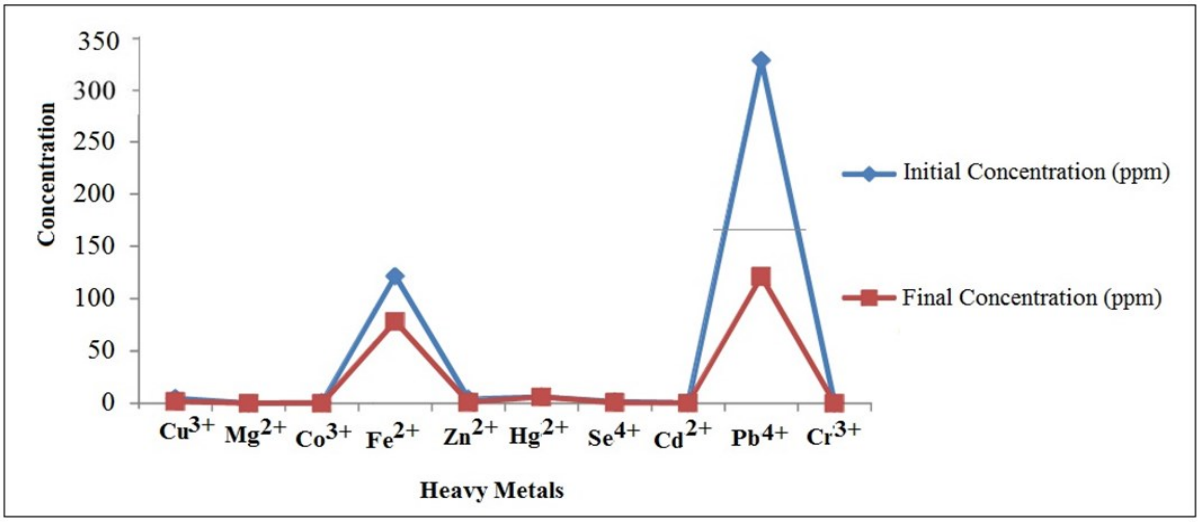

Fig . 1: The trends of initial and final concentrations of heavy metals in Automobile panel wastewater

further contaminations [30]. The water level inside the autoclave was checked, samples were loaded into the autoclave, the lid of the autoclave was closed, ensured that it was air-tight when closing by tightening the two knobs in opposite direction at once. The autoclave was switched on until it commenced oozing at 121°C, it was stopped after oozing the third time, and the contents were then allowed to cool down before being removed.

2.4 Culturing of the inoculum (*Bacillus safensis* LAU13)

To aid the growth of the bacteria, 10 mL of distilled water, into which nutrient broth had been added, was injected into six (6) different cultures in test tubes. The cotton-plugged tubes were autoclaved after which in an a septic condition inside the fume cup-transferred onto an incubator shaker at 100 rpm agitation speed.

2.5 Optical density measurement

The optical density test for monitoring the growth in the inoculums was carried out at various time intervals of 24 and 48 h. The samples were taken out of the incubator shaker, after 24 h and 20 ml of it was poured into the spectrophotometer curvette under aseptic condition for reading the optical densities corresponding to the supplements. This procedure was repeated at the 48th h to determine their respective values.

2.6 Scanning electron microscopy (SEM) analysis

Specimens for Scanning Electron Microscopy (SEM), taken from the residual pellets

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after the remediation process were prepared by carbon taping. They were stuck on the carbon tape plate and subjected to detail - obscuring conducive gold coating [30] and subjected to SEM analysis at the Metallurgy Department, Kwara State University, Malete, Ilorin, Nigeria.

2.7 Biosorption studies

The pH of samples was determined using digital Systronics pH meter, System 361 model and then modified to 7.5 with 1 N NaOH. The wastewater samples were supplemented with glucose to enhance the growth of the bacterium [29]. The samples were autoclaved at 121 °C for 15 min. The sterilized supplemented wastewater samples were then inoculated with the culture and were incubated at 37 °C on a rotary shaker. At 24 h interval, samples were withdrawn for the determination of optical density at 600 nm as a measure of growth. Thereafter, samples were centrifuged at 4000 rpm for 15 min, and the supernatants were subjected to AAS analysis to determine the residual concentration of heavy metals.

Table 1: Optical densities of inoculum at varying grams of supplement at 24 h

Inoculum (µL)	Supplement (g)					
	0.0	0.2	0.4	0.6	0.8	1.0
250	0.3	0.1	0.2	0.1	0.2	0.2
500	0.2	0.2	0.2	0.2	0.2	0.1
750	0.2	0.2	0.1	0.1	0.1	0.1
1000	0.3	0.3	0.2	0.1	0.1	0.2

3. Results and Discussion

3.1 Atomic absorption spectrophotometry (AAS) analysis

Figure 1 represents the initial and final concentrations of the heavy metals present in the samples. From Figure 1, it was observed that *Bacillus safensis* LAU13 inoculum completely removed manganese (Mg^{2+}) and cobalt (Co^{3+}) present in the wastewater sample. Also chromium was greatly reduced to a very minimal concentration. Zinc (Zn^{2+}), Copper (Cu^{3+}), Lead (Pb^{4+}) and Selenium (Se^{4+}) were above average bioremediated with Iron (Fe^{2+}) and Cadmium (Cd^{2+}) was below average remediated. It was however noted that there was very low removal of mercury (Hg^{2+}), having less than one percent removal after the treatment. Bacteria have been known to possess a wide variety of bioremediation potentials, which are beneficial from both in environmental and economic standpoint. The remediation potential of *Bacillus safensis* is in line with earlier works on the use of bacteria for wastewater treatment [9,26,29].

Bioremediation has advanced potential of cleaning pollutants, and toxic metals though persistent in nature are removable using microorganisms [25, 31]. Measured growth and optical density in the treatment process indicates the activity levels of microorganisms. From Tables 1 and 2, it was observed that after taking initial optical densities at 24 h and final optical densities at 48 h, the optical densities increases significantly showing that there was rapid growth of microorganism in the wastewater overtime. At 0 g of supplement the optical densities increase rapidly at the various dosages of inoculums showing that without the supplements the bacteria still grows. The highest optical density was observed at the least dosage of 750 µL confirming that the inoculums does not require supplement to perform optimally. With the 0.2 g of the glucose supplement, the rate of increase of the optical densities of the 250 to 750 µL

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dosages were found as lower compared to that of no supplements.

And also at 1000 µL dosage of inoculums at 48 h the optical density decreased showing that there were no more growth of the bacteria. Using 0.4 g supplement, good growth of the organisms was observed due to increment in the optical densities taken at both the 24th and 48th h respectively. Highest optical density was observed at 750 µL dosage of the inoculum. The optical densities for 0.6 g supplement showed appreciable increase in value indicating gradual growth of the inoculum, it had the highest observable growth at 250 µL of dosage of the inoculum. For 0.8 g supplement there was also increase on the optical densities of the samples at all the dosages just as in the case of no supplement. At the 1.0 g of supplement, there was moderate growth of the organism noted between 250 to 750 µL dosages, but this dropped at the 1000 µL dosage perhaps signifying death of the organisms. Generally it was found that the growth of the bacteria is largely independent of the glucose supplement applied. Again the optimum dosage for the bacteria growth was noted as 750 µL. The findings established earlier reports of *Bacillus safensis* as a bio-control agent [37] and a bio-remediating organism [27].

Table 2: Optical densities of inoculum at varying grams of supplement at 48 h

Inoculum	Supplement (g)					
(µL)	0.0	0.2	0.4	0.6	0.8	1.0
250	0.5	0.2	0.3	0.3	0.3	0.4
500	0.5	0.2	0.3	0.3	0.3	0.3
750	0.6	0.2	0.4	0.2	0.2	0.1
1000	0.6	0.3	0.3	0.3	0.3	0.2

3.2 Scanning electron microscopy (SEM) micrographs results

The SEM micrographs of residual pellets after centrifuging and oven dry in gin. Plate 1clearly show accumulation of certain irregularly-shaped whitish fine particles and porosity surface texture with small aggregates onto which the sorption by the bacteria might have occurred. There were inter - sparse deposits

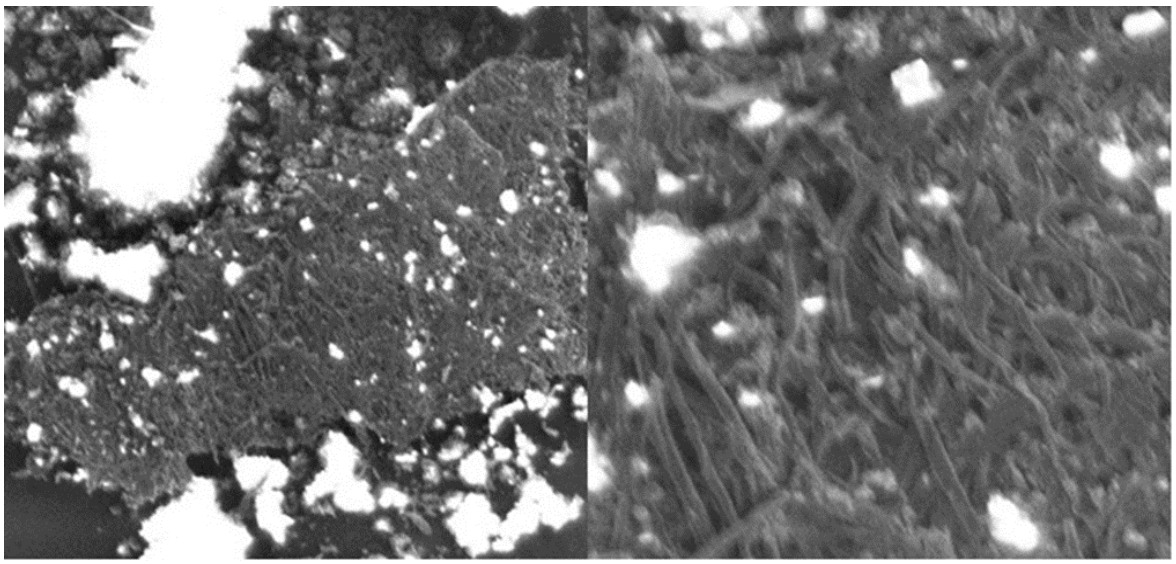

Plate . 1: SEM micrograph of residual pellets after the bio-sorption process at X75 (left) and X500 (right).

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observed to be the remediated heavy metals from the wastewater samples. This corroborates findings of some earlier researchers [16, 30, 33, 34] on biosorption of heavy metals by *Bacillus safensis* and other organisms. *Bacillus safensis* LAU13 could therefore be a potential adsorbent for bioremediation of automobile panel work wastewater.

4. Conclusion and Recommendations

In the biosorption study of *Bacillus safensis* LAU13 on automobile panel work wastewater, the organism completely removed manganese and cobalt. Chromium concentration was reduced to barest minimum while zinc, copper, lead, and selenium were considerably remediated above their average initial concentrations. The sorption of iron and cadmium by the bacterium was below average in the study but lack potential of remediating mercury from the wastewater. Glucose supplement has minimal effect on the growth of the organism and the optimum inoculum dosage was achieved as 250 µL. SEM micrographs of the residual pellets after the biosorption indicate bioaccumulation of certain aggregates supposed to be heavy metals. *Bacillus safensis* LAU13 is found as a potential sorbent for bioremediation of heavy metals, especially manganese and cobalt in the automobile panel effluent and is therefore recommended for such application prior to the discharge of effluents into larger water body.

Conflict of interest

The authors declare that they have no conflict of interest.

Author's contributions

OSO: Conceptualization, Methodology, Software, Resources, Supervision, Writing - Reviewing and Editing.
LA: Methodology, Resources, Supervision, Writing - Reviewing and Editing.
AWM: Data collation, Methodology, Software, Resources, Field work, Laboratory analysis, Formal analysis, Writing- Original draft preparation, Visualization, Investigation, Validation.
OOO: Data collation, Methodology, Software, Resources, Field work, Laboratory analysis, Formal analysis, Writing - Original draft preparation, Visualization, Investigation, Validation.
AAE: Methodology, Resources, Supervision, Writing - Reviewing and Editing.

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