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Effect of Fungi Bioaugmentation on Solidification and Stabilization (S/S) Method of Industrial Wastewater Sludge-Removal of Heavy Metal

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Abstract: The rapid growth industrial development and urbanization increases the production of industrial wastewater sludge that contains heavy metals, pathogens and organic compounds can impact on human health and the environment. This study aims to treat the industrial wastewater sludge by using the combination of solidification and stabilization (S/S) method and fungi bioaugmentation method, which specifies in measuring the effect of fungi bioaugmentation on S/S method based on the compressive strength of the S/S matrices and leaching behaviour of heavy metals concentrations. The materials used in sludge remediation were Portland cement to act as a binder and *Aspergillus brasiliensis* ATCC 16404 as the degrading agent. The experimental work involved in this study includes compressive strength test, leaching test (TCLP) and measure the concentration of heavy metals by using ICP-MS equipment followed British standards (BS) and United States Environmental Protection Agency (US EPA). After 7 days, the effect of fungi bioaugmentation and S/S method on Sample D recorded the lowest compression strength and only Arsenic samples passed the leaching test. Thus, increase the curing period up to 72 or 90 days can be implemented to improve the results.

Keywords: Bioremediation, Hazardous Waste, Solidification and Stabilization, Heavy Metals

1. Introduction

Sludge or biosolids is the residue that float or sink in wastewater treatment plants. It describes as a solid or semi-solid slurry residue that contains lots of nutrients and pollutants [1]. The main contributors of wastewater sludge are from various processes. Domestic wastewater increase in sludge production

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from laundry, bathing and food waste in sewage plant. Meanwhile, industrial wastewater produced sludge from textile mills, paper mills from pulp and paper industry and food industry.

There are several approach and technology that can limit the release of harmful contaminants from hazardous wastes such as solidification and stabilization (S/S) method and bioaugmentation, which focus in this study. S/S method refers to a group of cleanup methods that prevent or slow the release of harmful chemicals from wastes as well as kept the contaminants from leaching above safe levels into surrounding environment [2]. Bioaugmentation is the addition of archaea or bacterial cultures required to speed up the rate of degradation of a contaminant and is favored because microorganisms that were originally in the environment do not accomplish their task when it came to breaking down chemicals in the sludge [3].

Following that, remediation of sludge by using both S/S method and fungi bioaugmentation can minimize the adverse impact to human and the environment. Fungi functions as degrading agent while Portland cement will be used as a binder in the S/S method. Fungi also act as absorbent to enhance the immobilization of heavy metals in the S/S method. Treated sludge will be less hazardous and can be dispose safely into the landfills and eventually helps in limiting the amount of heavy metals on land for long term use.

The overall goal of this study is to evaluate the effectiveness of fungi bioaugmentation on the solidification and stabilization (S/S) method of industrial wastewater sludge. The specific objectives of this study are to measure the effect of fungi bioaugmentation on S/S method based on the compressive strength of the S/S matrices as well as to evaluate the effect of fungi bioaugmentation based on leaching behaviour of heavy metals concentrations.

S/S is one of the favoured techniques that aims to immobilize pollutants by transforming them into a less soluble shape and encapsulating them with the formation of a durable matrix [4]. The S/S approach has begun with a solidification procedure involving the combining of waste with a binding agent, which is a fluid that helps loose materials hold together while the stabilization process is a chemical reaction with pollutants that takes place in the solidification process [2].

From previous study, the novel binder SPC which composes of single superphosphate and calcium oxide use to stabilize and solidify lead (Pb), zinc (Zn), and cadmium (Cd) contaminated industrial site soil. Results from Toxicity Characteristic Leaching Procedure (TCLP) test revealed that SPC addition of 4%, 4%-10% and 4%-8% to the contaminated soil gave notable reduction in leachability of Pb below 5 mg/L, Zn below 100 mg/L and Cd below 1 mg/L of standard [5]. Another research used a new limestone calcined clay cement binder in S/S method with aimed to encapsulate the contaminated soil in a monolith mass of high structural integrity and decrease the metal toxicity for high content of Zn contaminated soil. Analysis from TCLP test shown that curing time of 28 days improve the 88-99% efficiency of solidification with values of 11.89 and 1.22 leaching factor of Zn and Pb [6].

The term bioaugmentation literally refers to a biological increase defined as the inclusion of microorganisms capable of biodegrading recalcitrant molecules in the contaminated setting [7]. In recent study, *Aeromonas hydrophila* LZ-MG14 was used in the bioremediation of highly toxic dyes like malachite green (MG) and hexavalent chromium (Cr(VI)) that commonly found in textile wastewater. The study results showed that strain LZ-MG14 successfully removed 96.8% of MG (200 mg L^{-1}) and 93.71% of Cr (VI) (0.5 mmol L^{-1}) within 12 h with a significant increase in biomass [8]. In addition, another study uses *Achromobacter sp.* JL9 and carbon-to-nitrogen ratio to investigate whether the method can improve sulfamethoxazole (SMX) degradation and nitrate-N and ammonia-N removal in the Moving Bed Biofilm Reactor system. From results, the efficiency of SMX degradation was adversely affected when hydraulic retention time decreased but, optimal SMX degradation efficiency was 33.06% when HRT at appropriate level which is above 8 h with the augmentation of strain JL9 [9].

2. Methodology

2.1 Collection of Raw Materials

Sludge and Portland cement were the materials that need to be collected before starting the experimental work. Specific location for collection of sludge samples was from industrial wastewater treatment in Evergreen Fibreboard Berhad, which the largest factory in the industrial area in Parit Raja, Batu Pahat, Johor. The factory has been established more than 20 years and its operation is still ongoing. During the fibreboard production, cleaning process is required to remove any impurities that is required to be treated. During this process, the sludge produced require RM 500 every month to process half tons of sludge and transport by licensed contractor. This factory produced fibreboard from tropical and rubber tree. The sludge then transported to Kualiti Alam Sdn Bhd before undergone through specific processes and disposed into landfill. For Portland cement, it was purchased from a hardware store in Parit Raja.

Following the standard procedure from US EPA of SESDPROC-306-R3, 10 kg of the raw sample were collected from industrial wastewater treatment in Evergreen Fibreboard Berhad and placed in high density polyethylene (HDPE) plastic bags, stored at room temperature and kept until being analysed.

2.2 Preparation of Fungi Strain

Specially type of fungi that used for this study was *Aspergillus brasiliensis* ATCC® 16404TM (previously known as *Aspergillus niger*) were purchased from Bio Focus Scientific Sdn Bhd, Malaysia that focuses to be used as bioabsorbent in measuring the leachability of heavy metals. This type of fungi was chosen because the high capability in absorbing the heavy metals in wastewater and contaminated soil.

To obtain the living culture from the freeze-dry ampoules, the recommended procedure from the supplier was followed. Firstly, 6 ml of sterile distilled water withdrew from test tube and applied directly to the pellet to be stirred until suspension formed. Next, the suspension was aseptically transfer into a single test tube of distilled water and let to sit at room temperature for at least 2 hours or longer undisturbed because rehydration might increase viability of some fungi. Then, a few of suspension was spread on the surface of potato dextrose agar (PDA) and incubated at 20°C. After 2 or 3 days of incubation time, the viability of spores was noticeable. 10 ml aliquot of saline solution was poured on the plate before an L-shaped spreader used to lift-off the spores.

In order to re-growth the mould, 3.9 g of the Difco Potato Dextrose Agar (PDA; OXOID) powder were mixed with 10 ml of distilled water in a bottle before 1 ml of the fungal spore suspension was spread evenly on the plate while conducting in a biohazard safety cabinet and incubated at 28 °C. The incubation time is needed for 3 days for mycelium growth and more than 3 days for sporulation. Meanwhile, for experimental purposes, 1 ml of the fungal spore suspension was dropped into the 1 ml of saline solution. The suspension was adjusted to an absorbance of between 0.9 and 1.0 at 600 nm, to give between 5×10 –1×10−1 spore.ml.

2.3 Design and Preparation of S/S Matrices

This study focuses on the combination of S/S method and fungi bioaugmentation, thus the sludge will be treated by using two different methods, that is (i) S/S method only and (ii) combination of S/S method and fungi bioaugmentation. S/S matrices was design based on the ratio of mixture as indicated in Table 1. There were five S/S matrices that were prepared for this study which were Sample A, B and C contain 100% cement, 50% cement + 50% sludge and 100% raw sludge respectively. Meanwhile, Sample C contain 50% cement with 50% sludge + fungi and last Sample D contain 100% sludge + fungi.

There were three steps included for preparation of S/S matrices which are mixing, casting and curing. First step, the mixing process involves the industrial sludge samples inoculated with 1 x 10^{10} spores/ml fungi and followed with adding Portland cement to be mix approximately for 5 minutes until no lumps left. After mixing, the finished mixture is casted in 3 layers, compacted manually and shake about 50 hits to yield good packing mixture in each of the mould that have 100 mm^3 size. The process continued with leaving the samples at controlled temperature and humidity to cure for 7 days for compressive strength test.

Table 1: Ratio of mixture in S/S matrices

Sample	Portland Cement (%)	Sludge	Sludge + Fungi (%)	Water Content	Total Weight of Sample (g)
A	100	-	-	0.45	250
B	50	50	-	0.45	250
C	0	100	-	0.45	250
D	50	-	50	0.45	250
E	0	-	100	0.45	250

2.4 Compressive Strength Test

The compressive strength test used to establish the compressive force or crush resistance of a sample. After curing process completed, the cube placed on bearing plate and aligned properly with the center of thrust in the compression test machine to obtain the value of maximum load carried by sample. The results then will be compared with the British Standard (PBS EN 1015–11) compliance to determine the minimum requirement for further applications of treated sludge as a secondary raw material in construction. If the findings shown the strength is higher than 0.35 MPa, it shows that it can dispose to the landfill.

2.5 Leaching Test

This test requires the leaching fluids to be prepared in screw-capped polyethylene bottles that will be filled with crushed cube sample and leachant. This step prepared according to the ratio 1:10 from European Leaching Protocol for Hazardous Waste aims to stimulate the earlier leaching period. Following the US EPA Method 1311, half-filled of distilled water in 1000 ml conical flask shaken with 80 ml acetic acid. Following this, an additional distilled water poured into the same conical flask until reached the highest limit line then shake again. Next, 400 ml of reagent water and 5.7 ml of acetic acid were filled in a screw-capped polyethylene bottle. 20 g of crushed cube sample with size less than 9.5 mm, were mixed with leachant in the bottles. Then, the bottles left agitated an end-over-end manner at 30 rpm for 18 hours. Lastly, the leachate were filtered using 7 μm fiber filters and stored at a pH of less than 2 with nitric acid.

2.6 ICP-MS Analysis

Analysis test was conducted using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) that used the filtered sample to ionize with inductively coupled plasma to obtain the concentration of heavy metals. The samples were analysed to obtain concentration of Chromium (Cr), Zinc (Zn) and Arsenic (As) and then compared with US EPA standard limits for TCLP test.

3. Results and Discussion

The results and discussion section presents data and discussed on the outcomes of completed two tests: compressive strength and leaching. The goal of this study was to compare the remediation of industrial sludge by using S/S method and bioaugmentation.

3.1 Compressive Strength Test

Table 2 shows the variations of compressive strength as a function of different ratios of materials in each sample. The findings of compressive strength for the S/S matrices revealed that Sample A shown the maximum value of compressive strength which was 25.8 MPa. Then, the strength was reduced when 50% sludge was replaced in Sample B which was 2.8 MPa. Addition of fungi in sample reduced the compressive strength to 0.62 MPa in Sample D. Meanwhile, sample with no cement, Sample C and E both recorded the lowest strength at 0.0 MPa from this test.

In qualitative point of view, Sample C and E shown the lowest compressive strength values than Sample B and D because the compressive strength values of the solid matrices are greatly dependent on the water/cement ratio and the degree of hydration of the cement. Cement need appropriate hydration to bond with water and causing the hardening of samples [10]. In comparison of S/S method, Sample D recorded the lowest compressive strength than Sample B and C due to cement binding properties that did not solidify and stabilized the pollutants with fungi. Following that, Sample D had the lower strength value than Sample B due to the tough cement environment which includes varied temperatures and a very high pH, which reduces the fungal microbial metabolic activities in immobilizing heavy metals. Hence, Sample A, B and D surpassed the requirement for safe disposal at municipal landfill, which were higher than 0.35 MPa except for Sample C and E.

Table 2: Compressive strength results after 7 days of curing

Sample	Compressive Strength (MPa)			
	Reading 1	Reading 2	Reading 3	AVG
A	28.300	25.100	23.900	25.8
B	3.300	3.400	1.500	2.8
C	0.000	0.000	0.000	0.0
D	0.554	0.330	0.363	0.62
E	0.000	0.000	0.000	0.0

3.2 Leaching Test

The leachate from the TCLP test kept and undergone analysis test using ICP-MS equipment to determine the leaching concentration of six heavy metals which are Cr, Zn and As then were compared with US EPA regulatory limits for TCLP test.

3.2.1 Arsenic (As)

Figure 1 revealed the concentration values of Arsenic (As) in leaching test for 7 days of curing. The findings shown that sample containing cement 0% + 100% sludge + fungi, Sample E had the highest concentration of As at 4.64 mg/L, whereas sample containing 100% cement, Sample A, had the lowest concentration of As at 0.42 mg/L. Sample C, which included 100% sludge yielded 2.84 mg/L of As, whereas the other two Sample D and B had the lowest concentrations, at 1.48 mg/L and 0.92 mg/L respectively.

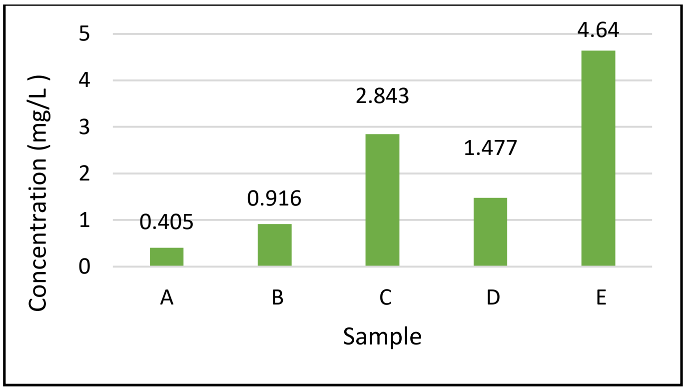

Figure 1: Concentration of Arsenic in each sample (mg/L)

In the case of Sample D and E, fungi bioaugmentation approach in S/S matrices was less effective in removing As from wastewater sludge than other sample with no fungi included. Sample C recorded higher concentration than Sample B on account of no cement as a binding agent in the S/S method to reduce As toxicity. Fungi in Sample D unable to absorb initial high As content in the S/S matrices even with the presence of cement. Nonetheless, all samples passed the leaching test since their concentrations were less than the permitted limit of 5 mg/L in compliance with US EPA standard.

3.2.2 Chromium (Cr)

According to the Figure 2 shown the concentration of Chromium (Cr) after curing for 7 days, the highest Cr concentration is Sample E, which includes cement 0% + 100% sludge + fungi, with 67.9 mg/L, followed by Sample C with 64.63 mg/L that contains 100% sludge. Following that, the Cr concentration in Sample D with cement 50% + 50% sludge + fungi was 61.57 mg/L, while both Sample A and B had the lowest concentrations of 40.68 mg/L and 24.50 mg/L respectively.

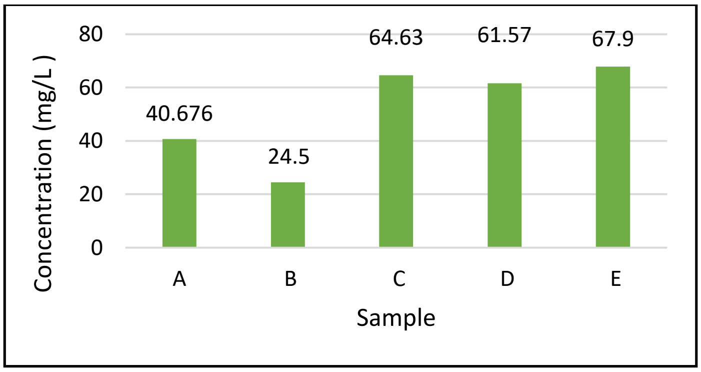

Figure 2: Concentration of Chromium in each sample (mg/L)

In the case of Sample D and E, fungi bioaugmentation approach in S/S matrices was less effective in removing Cr from wastewater sludge than other sample. The analysis revealed that Sample D which included fungi and cement, had a greater Cr content than Sample B, indicating that the added fungi did not aid in immobilization of heavy metals might due to poor solidification. These results were contradicted to a previous study that obtained low solidified/stabilized chromium amounts in concrete specimens [11]. Similar to Sample D results, fungi exhibited a greater Cr content in Sample E than in Sample C, despite the fact that neither sample included Portland cement. Overall, for Cr concentration, all samples above the permitted limit of 5 mg/L for leaching test per US EPA standard, indicating that they are hazardous and unfit for disposal.

3.2.3 Zinc (Zn)

According to the findings of Zinc (Zn) concentration in leaching test after 7 days of curing in Figure 3, the highest Zn concentration is Sample E comprises cement 0% + 100% sludge + fungi with 401.33 mg/L. Sample C on the other hand, recorded Zn concentration at 378.33 mg/L, which contains cement 0% + 100% sludge. Following that, Sample A included 100% Cement and measured 117.5 mg/L, whereas Sample D included cement 50% + 50% sludge + fungi and measured 88.03 mg/L. Sample B had the lowest leached Zn content of 57.23 mg/L and includes 50% cement + 50% sludge.

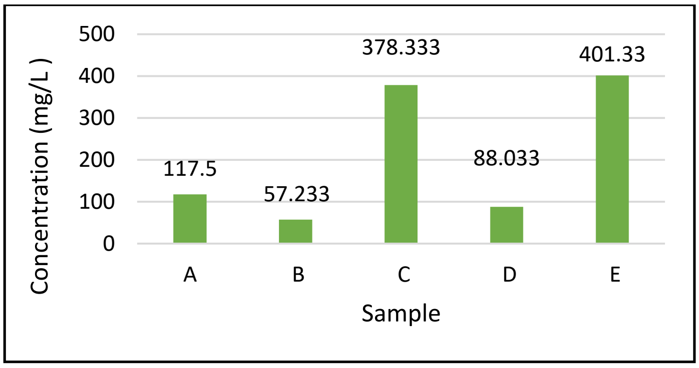

Figure 3: Concentration of Zinc in each sample (mg/L)

In qualitative point of view, Sample E marked the highest concentration because of the high initial toxicity of metals. Similar to Sample E, high concentration of Zn in Sample C is because the absence of binder unable to encapsulate very high toxicity of Zn in stronger matrix, rather than Sample B. Fungi that contains in Sample D did not success to absorb Zn and not enough hydration provided by Portland cement compared to Sample B. It also contradicts with the concentrations of heavy metals decreased with the presence of magnesium oxide (MgO) binder in the samples from earlier study [12]. Except for Sample C and Sample E, all samples of the leached Zn content did meet the corresponding landfill requirements under 300 mg/L.

Overall, the sludge remediation applying both methods were deemed a failure since data revealed that the concentration of most samples with fungi were higher than the concentration of samples without fungi. Table 3 showed the difference of percentage removal by comparing S/S method only and combination of S/S method and bioaugmentation.

Table 3: Comparison of heavy metals concentration with or without fungi

Types of heavy metals	Removal of S/S method only (%) (Sample C – Sample B)	Removal of S/S method & bioaugmentation (%) (Sample C – Sample D)
Arsenic (As)	68	48
Chromium (Cr)	62	5
Zinc (Zn)	85	77

This study intended to do compressive strength and leaching tests over 7 and 28 days, respectively. However, owing to Movement Control Order 3.0, only 7 days' worth of samples were successfully analysed, while the remaining 28 days' worth of samples could not be examined since the laboratory was closed. If 28-day samples can be collected, this investigation may yield a favourable outcome since, according to earlier studies, compressive strength was improved due to longer curing times for fungi to digest heavy metals. As a result, it was predicted that the concentration of heavy metals in sample D would be lower than in sample B after 28 days of curing.

Furthermore, due to a lack of data on compressive strength and leaching test for 28 days of curing, a comparison on different curing periods cannot be inferred. The leaching of heavy metals concentrations based on three variables (Chromium and Zinc) for all samples were declared hazardous by reason of exceeding the permissible limit according to US EPA regulations. Only Arsenic was below the US EPA permissible limit and safe for disposal to the municipal landfill.

4. Conclusion

Based on the results, it was indicated that the fungi bioaugmentation on the solidification and stabilization (S/S) method was not effective in the remediation of industrial wastewater sludge for short period, i.e., 7 days of curing. The findings from compressive strength showed that only Sample A, B and D exceeded 0.35 MPa in accordance to US EPA and can dispose safely to the municipal landfill. Thus, it can be concluded that the first objective, the effect of fungi bioaugmentation on S/S method based on the compressive strength of S/S matrices was not achieved. From the leaching test, the findings revealed that Barium (Ba) in Sample E was the highest concentration which was 1550 mg/L whereas the lowest concentration was Arsenic (As) in Sample A which was 0.41 mg/L after 7 days curing. In accordance to US EPA regulations, only Arsenic samples passed the permissible limits of more than 5 mg/L and declared as non-hazardous.

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