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Potential of the Bioaugmentation Method to Enhance the Remediation Process in the Solidification/Stabilization Method

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Abstract: In the published literature, Stabilization/Solidification (S/S) method was excellent in remediating heavy metals, whereas bioremediation was excellent in remediating organic contaminants. However, tremendous papers only focus on specific contaminants, while the polluted environment is not due to specific contaminants. In this paper, both methods were combined and targeted to remediate both group contaminants, namely organic contaminants and heavy metals. The study focused on the effect of the bioaugmentation method on S/S method with the use of Portland cement as a binder in the S/S method. *Sphingobacterium spiritivorum* (bacteria) and *Aspergillus brasiliensis* (fungi) as degrading agents in the bioaugmentation method. The S/S matrices were tested for leaching behaviour which is the concentration of Chemical Oxygen Demand (COD) and heavy metals after 14 and 28 days of hydration. Bacteria augmentation showed a better COD removal compared to without augmentation (S/S method only). Bacteria augmentation also shows better removal compared to fungi augmentation for both hydration days. In addition, by using the S/S method only, COD concentration was increased from 14 to 28 days. For heavy metals, chromium shows a positive result; bacteria augmentation shows the lowest concentration after 14 and 28 days of hydration. While other metals, S/S method only shows the lower concentration compared to samples that augment with bacteria and fungi. Nonetheless, the concentrations of heavy metals in all samples did not exceed the USEPA limit. Therefore, this study contributed to the possibility of combining the S/S and bioaugmentation methods by adding degrading agents to remediate contaminants in the sludge, especially for sludge that contains high organic contaminants.

Keywords: Remediation, combination method, bioaugmentation, organic contaminant, heavy metals

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1. Introduction

Fibreboard sludge is waste that is produced during industrial wastewater treatment. The rapid development of industrial activities has led to increased sludge production. The rising population and expanding economy in Malaysia have been linked to the ongoing rise in sludge production [1]. Normally, the composition of sludge varies depending on the industrial activity specifications. Excessive production of sludge will affect human health and environmental conditions due to hazardous substances in the sludge composition. Sludge comprises a variety of harmful and hazardous elements including heavy metals, organic substances, pathogenic microorganisms and dioxins [2][3]. The sludge origin during treatment (primary sludge, secondary sludge, and digested sludge) and wastewater treatment plant (WWTP) type (industrial vs. municipal) both affect the composition of sludge [4]. The sludge becomes more harmful to both human health and the environment when it is disposed of inappropriately [5]. Therefore, a proper treatment method must be used to remediate the sludge to ensure it becomes less harmful and can be disposed of properly.

Currently, the treated sludge is disposed of at a sanitary landfill in Malaysia. The sludge is treated physically, chemically or biologically [6]. There are three treatment methods for sludge remediation (i) physical treatment of the sludge where in this treatment, the large volumes of dissolved and colloidal particles are still present which must be eliminated before discharge such as floating and grading process, (ii) biological treatment where this treatment is used the microorganisms including bacteria and fungi as degrading agents to degrade the organic contaminants in the sludge such as bioremediation and (iii) chemical treatment such as chemical oxidation and chemical precipitation process where chemical materials are used to remove the unnecessary substances in the sludge such as heavy metals. However, the most popular methods that are usually used to degrade the contaminants in the sludge is S/S method due to these methods are environmentally friendly and low in cost [7]. According to Kualiti Alam Malaysia, in the S/S treatment method, cement, lime and sand were mixed into the sludge to immobilize the contaminants. The cement was added to increase the physical properties of the sludge and make it more solid. After that, the solidified waste was transferred to the secured landfill.

The S/S method is defined as a treatment method that can immobilize the contaminants in the sludge by converting them into less harmful products. This method may increase the strength and durability of the products while also providing low permeability for those that can be reused. Besides, the products from the S/S method that are used in construction such as building materials have their own benefits such as the fact that they may reduce greenhouse gas emissions and the amounts of residue that are usually disposed of at landfills [8]. Moreover, the S/S technique is frequently utilized for sludge containing significant amounts of heavy metals including chromium (Cr), copper (Cu), zinc (Zn), arsenic (As) and lead (Pb). The S/S method has the ability to solidify the sludge and stabilize the heavy metals in the sludge [9]. Heavy metals can be treated using the S/S technology which has been shown to be effective, fast and affordable and during this process, the heavy metals are either enclosed in a properly structured matrix or changed into a stabilized phase that is difficult to dissolve [10]. The addition of binders such as cement and clay in the S/S technique is able to contribute to the immobilization process of transforming the heavy metals in the sludge into less harmful [11]. In addition, during this S/S treatment, the sludge will interact with the binders in many ways including precipitation, chemisorption, surface complication and ion exchange [12]. Cement is commonly used as a binder since it contains a large proportion of calcium oxide (CaO) that can prevent the contaminants from reacting chemically with the surface of stabilized waste [13]. The S/S method has been investigated using a range of binders including Portland cement, fly ash, volcanic ash, lime and pozzolan [14]. In fact, heavy metals can be effectively immobilized using cement-based S/S technology even without the use of extra additives [7]. The cement-based S/S technique also proved that this technique not only efficiently reduced carbon emissions and energy use but also increased the effectiveness of heavy metals stabilization [10]. The facts showed that the heavy metal concentration in the sludge may be immobilized by using the S/S method.

However, the S/S method is not able to degrade the organic contaminants in the sludge due to the organic contaminants may inhibit the hydration of the binder. This condition may affect the safety of solidified samples where it will become harmful when the binder is not able to absorb or react with the toxicity of organic contaminants to form a stable component. Organic contaminants frequently contribute to the negative impact on the hydration of Portland cement [15]. Therefore, to ensure both heavy metals and organic contaminants can be immobilized, it is important to combine the S/S treatment with the biological treatment such as the bioaugmentation method.

Bioaugmentation is one of the bioremediation methods that is usually used by researchers to degrade the organic contaminants in the sludge. In this method, microorganisms such as bacteria and fungi will be used as degrading agents to degrade organic contaminants. The biological process is the most popular and cost-effective method due to its ability to treat different types of sludge without any chemical usage [16]. Besides, according to recent research findings, biologically improved treatment is one of the key methods for removing amine from the environment [17,18]. Several studies have been conducted over the last decade to investigate strategies for bioaugmentation techniques for sludge treatment. Table 1 shows examples of contaminants that have been removed by using bioaugmentation treatment.

Table 1 - Examples of contaminants removed by using bioaugmentation method

Types of Contaminants	Medium of Bioaugmentation	Types of Bacteria used	Ref.
Lignin (highly complex polymer of phenol)	Industrial wastewater sludge	*Comamonas* and *Pandoraea* (bacteria) and *Aspergillus* (fungus)	[19]
2,4,6-Trichlorophenol	Industrial wastewater sludge	*Desulfitobacterium* sp.	[20]
Pyridine	Industrial wastewater sludge	*Rhizobium* sp.	[21]
Benzene, toluene and styrene (BTS)	Sewage sludge	*Pseudomonas putida* and *Rhodococcus ruber*	[22]
Nonylphenol (NP)	Sewage sludge	*Bacillus safensis*	[23]
Nitrogen	Aerobic granular sludge (AGS)	*Pseudomonas mendocina, Brucella* sp., *Pseudomonas putida* and *Paracoccus* sp.	[24]
Triisobutyl phosphate (TiBP)	Activated sludge (AS)	*Pseudomonas*	[25]

Furthermore, the researchers stated that by adding specific microbial strains with specialized functions to wastewater treatment systems, bioaugmentation can increase the removal efficiencies of the targeted contaminants and improve the stability of the operating system [26]. In addition, many studies conducted over the past few decades have demonstrated that bioaugmentation can greatly increase the removal efficiency of various contaminants including inorganic nitrogen and phosphorus contaminants in wastewater treatment reactors [27]. Nevertheless, in the bioaugmentation treatment, the selection of strains is certainly a key point [28] to ensure the microbes have high adaptability and mechanism in the actual wastewater treatment system [29]. Thus, it was demonstrated that the effectiveness of biological treatment depends on the types of degrading agents that are used to remediate organic contaminants. Previous studies showed that the use of *Pseudomonas putida* and *Rhodococcus ruber* in the bioaugmentation strategy contributed to the increase in the degradation rate of BTS in the sewage sludge which is 97.9% [22]. Then, the research also demonstrated the viability of employing bioaugmentation to remove additional types of contaminants from sewage sludge, such as PAHs, PBDEs, pharmaceuticals, and personal care products using bacteria or fungi [30].

Nonetheless, high concentrations of heavy metals in the sludge could also reduce the efficiency of biological treatment. Hence, a combination of the S/S and the bioaugmentation methods was implemented to immobilize both heavy metals and degradation of organic contaminants. This combination method was tested in petroleum drill cutting to degrade total petroleum hydrocarbons (TPH) and it was discovered that the TPH was reduced by 15% more than the control sample of the S/S treatment without the bioaugmentation method [30]. Based on the finding, the sludge can also use this combination method approach. The combination based on the studies [30], the S/S, and bioremediation treatment for the treatment of drill cutting is not widely established. Since the modified bacteria eventually break down the organic contaminants, combining the S/S with additional technologies like biological treatment may provide a new research avenue [31].

Besides, numerous abiotic factors including irreversible sorption of contaminants to the cementitious matrix, volatilization, and reductive dichlorination of chlorinated organic in the presence of Fe (II) [32] were implicated in the contaminant losses in previous studies treating artificially contaminated soils with two organic contaminants such as 2-chlorobenzoic acid and phenol [31]. Nonetheless, the previous study [30] only used bacteria as the degrading agents in the S/S matrices. In the research by Kogbara et. al., (2016) [30], Portland cement and a bacterium consortium were used to test the viability of TPH breakdown by bacteria mixed with the embedded in granulated S/S drill cutting. Prior to disposal, the hydrocarbon content of petroleum drill cuttings was reduced by adding compost made up of the bacteria and nutrition sources to S/S matrices [31]. The usage of fungi as degrading agents in the bioaugmentation process was not well documented.

This study aimed to measure the leaching concentration of the contaminants in the sludge by using the S/S method and the combination of S/S and the bioaugmentation method. The leaching results will also be compared between these single and combine methods. For the S/S method, Portland cement will be used as a binder. Bacteria (*Sphingobacterium spiritivorum*) and fungi (*Aspergillus brasiliensis*) will be used as degrading agents in the combination of S/S and the bioaugmentation method to enhance the immobilization of organic contaminants in the sludge.

2. Materials and Method

2.1 Raw Materials Preparation

In this research, two raw materials were prepared which were fibreboard sludge and Portland cement. 40 kg of fibreboard sludge was collected from the wastewater treatment factory. The sludge that was used in this study was produced from the cleaning process during fibreboard production in wastewater treatment. A sealed plastic bag was used to store the sludge at room temperature until the analysis was required. Portland cement was obtained from a local hardware store.

2.2 Bacteria Preparation

The bacteria (*Sphingobacterium spiritivorum*) that was used in this study was isolated from sewage sludge and preserved at -80ºC in microbeads (microbankTM). 5.6 g of Nutrient Agar (C1225) powder was mixed with 200 ml of distilled water in a bottle (USEPA, Method FNES6). After that, the bottle was shaken 3 to 4 times before being sterilized in an autoclave machine for about 45 minutes. The mixture of agar in the bottle was poured into several Petri dishes and it was left for 10 to 12 minutes to ensure the agar became hardened. Then, a single colony of *S. spiritivorum* was picked up by using an inoculation loop. To separate single bacterial cells, the inoculation loop was streaked on a petri dish using the quadrant method to reduce the bacterial load at each streak. These processes were conducted in a biohazard safety cabinet. The Petri dishes were sealed with parafilm and upside-down incubated at 37ºC for 3 to 4 days due to the isolating of bacteria that were active on day 3 or 4. The bacteria were optimized at a temperature 30 – 37ºC.

2.2.1 Bacteria Broth Preparation

The bacteria were revived by transferring a few of the frozen beads into universal bottles containing nutrient broth and the broth was incubated at 30°C for 3 days. To prepare bacteria broth, 26 g of Nutrient Broth powder was mixed with 2 L of distilled water in a bottle for a cube mould (USEPA, Method FNES6). The broth was sterilized in an autoclave machine for about 45 minutes. The sterilized broth was mixed with 4 Petri dishes of growth bacteria and conducted in a biohazard safety cabinet. The bottle was incubated at 30°C for 3 days before the broth could be used as a degrading agent in the bioaugmentation.

2.3 Fungi Preparation

Aspergillus brasiliensis ATCC® 16404TM was a fungal strain that was used in this research. First of all, 7.8 g of Potato Dextrose Agar (PDA) powder was mixed with 200 ml of distilled water in a bottle according to USEPA, Method FNES6. After that, the bottle was shaken 3 to 4 times before being sterilized in an autoclave machine for about 45 minutes. The mixture of PDA agar in the bottle was poured into several Petri dishes and left for 10 to 12 minutes to ensure the agar become hardened. Then, 1 ml of the fungal spore suspension was dropped onto the PDA agar. These processes were conducted in a biohazard safety cabinet. The Petri dishes were sealed with parafilm and incubated at 28ºC for 7 days due to sporulation occurring completely after 7 days. The fungi were optimized at a temperature of 30ºC.

2.3.1 Fungi Broth Preparation

In order to prepare the fungi broth, the standard procedure USEPA, Method FNES6 was used. Firstly, 48 g of Potato Dextrose Broth powder was mixed with 2 L of distilled water in a bottle for a cube mould. The broth was sterilized in an autoclave machine for about 45 minutes. The sterilized broth was mixed with 4 Petri dishes of growth fungi and conducted in a biohazard safety cabinet. The bottle was shaken 4 to 5 times and the solution was used as a degrading agent in the mixture of cement and sludge. The mixture was added to the cement to form and cure the S/S matrices for 14 and 28 days. There were three steps that were involved in the preparation of the S/S matrices including mixing, casting and curing. For approximately 5 minutes, the samples were mixed to confirm there was no lump left. Three layers were used to cast the samples and the samples were compacted by hand and shaken for 50 hits to yield a mixture with good packaging. After the process of casting, the samples were triplicated for two hydration durations which were 14 and 28 days to ensure adequate air drying occurs at a controlled temperature and humidity condition.

2.4 S/S Method and Combination Method (S/S Method and Bioaugmentation Method)

Two methods which were (i) the S/S method only and (ii) a combination of S/S method and bioaugmentation were used to treat the sludge. Portland cement as a binder was used to mix with the sludge for the first method Tables 2 and 3 show the ratio mixtures of the S/S matrices for the S/S method only and the combination of S/S method and the bioaugmentation method respectively. 70% of Portland cement and 30% of sludge were used as the ratio in the S/S method only and the combination of S/S and the bioaugmentation method due to this ratio was gave the optimum result based on the study conducted by [33]. Then, 10×10^{10} cfu/ml strains of bacteria (*Sphingobacterium spiritivorum*) and fungi (*Aspergillus brasiliensis*) were inoculated into the sludge and in the combination of S/S and bioaugmentation methods.

The mixture was added with the cement to form and cure the S/S matrices for 14 and 28 days. There were three steps that were involved in the preparation of the S/S matrices including mixing, casting and curing. For approximately 5 minutes, the samples were mixed to confirm there was no lump left. Three layers were used to cast the samples which the samples were compacted by hand and shaken for 50 hits to yield a mixture with good packaging. After the process of casting, the samples were triplicated for two hydration durations which were 14 and 28 days to ensure adequate air drying occurs at a controlled temperature and humidity condition.

Table 2 - Ratio mixture in (i) S/S method only for 14 and 28 days

Sample	Duration (Day)	Portland Cement (%)	Sludge (%)	Water Content	Total Weight of Sample (kg)
SS1	14	70	30	0.45	2.50
SS2	14	70	30	0.45	2.50
SS3	14	70	30	0.45	2.50
SS4	28	70	30	0.45	2.50
SS5	28	70	30	0.45	2.50
SS6	28	70	30	0.45	2.50

*SS – Stabilization/Solidification method
(Total number of samples = 24)

Table 3 - Ratio mixture of bacteria and fungi inoculation for the mixture on S/S method for 14 and 28 days

Sample	Duration (Day)	Portland Cement (%)	Sludge (%)	Sludge + Bacteria* (%)	Sludge + Fungi* (%)	Water Content	Total Weight of Sample (kg)
SSB1	14	70	30	0	0	0.45	2.50
SSB2	14	70	0	30	0	0.45	2.50
SSB3	14	70	0	0	30	0.45	2.50
SSB4	28	70	30	0	0	0.45	2.50
SSB5	28	70	0	30	0	0.45	2.50
SSB6	28	70	0	0	30	0.45	2.50

*SSB – Combination of Stabilization/Solidification and Bioaugmentation method
*Use 10×10^{10} cfu/ml
(Total number of samples = 24)

2.5 Leachability Test

In the Toxicity Characteristic Leachability Procedure (TCLP) test, the cube samples were crushed of less than 9.5 mm in size due to the particle sizes needed in the TCLP test to follow the provided standard method (USEPA, Method 1311). A 1000 ml conical flask was half-filled with distilled water and 5.7 ml of concentrated acetic acid, CH_3COOH and shaken to mix. Then, the distilled water was filled again until the highest limit line of the 1000 ml conical flask and shaken again. Next, 400 ml of reagent water (a mixture of distilled water and 5.7 ml of concentrated (CH_3COOH) was poured into a beaker and checked for pH. After that, the reagent water was added with 20 g of crush from cube samples and put this mixture in the screw-capped polyethylene bottles. A speed of 30 rpm was used to agitate the bottles for 18 hours. 7 µm of fiber filters were used to extract the collected leachate. The leachate was stored with concentrated nitric acid, HNO_3 at a pH of less than 2. After that, an ICP-MS analyzer was used to analyze the concentration of heavy metals in the extracted and measured leachate.

2.6 Chemical Oxygen Demand Test

The total amount of organic contaminants in the collected leachate was indirectly measured by using a Chemical Oxygen Demand (COD) test. The mass of oxygen that was consumed per litre of solution was also shown in this COD test. The quantity of oxygen that was consumed in water by contaminants during the chemical oxidation process was also measured by the COD test. First, the leachate was stored with concentrated nitric acid HNO_3 at a pH of less that 2 and High Range COD vials was filled with a 2 ml diluted filtered sample. It was heated for two hours in the DRB200 reactor. The quantity of organic contaminants present was then determined by analysing the leachate using DR6000 equipment. The standard method of EPA-600/4-79-020, USEPA Method 410.3 was used to conduct the test.

3. Results and Discussion

This section was divided into two subsections. The first subsection discussed the comparison results for Chemical Oxygen Demand (COD) concentration between the S/S method only and the combination of the S/S method and the bioaugmentation method. In the second subsection, the concentration of heavy metals was discussed in detail between the S/S method only and the combination of the S/S method and the bioaugmentation method.

3.1 Concentration of COD for S/S Method Only and Combination of the S/S and Bioaugmentation Method After 14 and 28 Days

Figure 1 shows the results for the concentration of Chemical Oxygen Demand (COD) for the S/S method only and the combination of the S/S and bioaugmentation methods after 14 and 28 days of hydration. Based on the figure, by comparing the types of degrading agents, bacteria augmentation (*Sphingobacterium spiritivorum*) showed a better COD removal compared to without augmentation (S/S method only). In addition, bacteria augmentation also shows better removal compared to fungi augmentation (*Aspergillus brasiliensis*) for both after 14 and 28 days. The samples of bacteria augmentation in the combination method had the lowest COD concentration when compared to the samples without augmentation (S/S method only) and fungi augmentation. It was observed that the bacteria augmentation was more efficient at degrading the contaminants and this finding can be supported by [34] that state the bacteria is more potential to encourage the elimination of COD concentration. This showed that the *Sphingobacterium spiritivorum* had more ability to absorb and degrade the organic contaminant in the sludge which resulted in a decrease in COD. Oxidizable organic materials can be absorbed by bacteria which serves as a source of nutrition for their growth and lowers COD concentration [35].

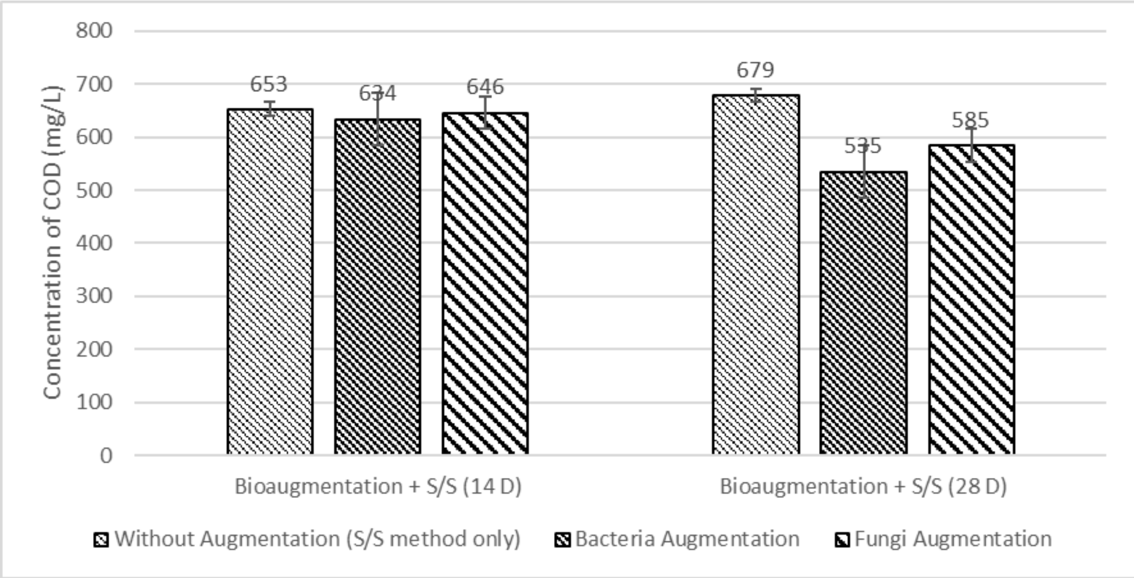


Fig. 1 - Concentration of COD for S/S method only and combination of bioaugmentation and S/S method after 14 and 28 days

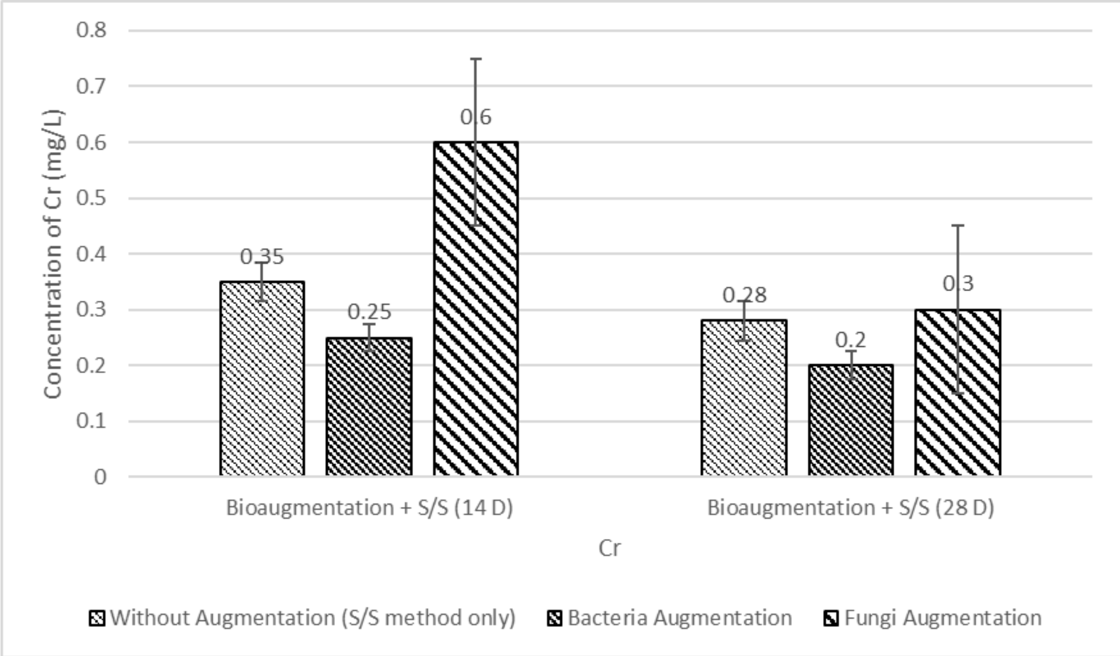
The COD removal concentration values ranged from 535 mg/L to 679 mg/L. The S/S method showed a 3.98% increase in COD removal from 14 days to 28 days. When compared to the sample without augmentation (S/S method only) with the bacteria augmentation and fungi augmentation in the combination method of bioaugmentation and the S/S method, it demonstrated the reduction of COD removal to 2.90% and 1.07% respectively after 14 days. For the COD removal in the combination method after 28 days, it showed a reduction of 21.21% and 13.84% with the bacteria augmentation and fungi augmentation respectively. The results demonstrated that the combination method treatment degraded more organic contaminants with a longer curing period, resulting in a lower COD concentration. The concentration of COD also decreased after 28 days for both bacteria augmentation and fungi augmentation due to these microbes having a high capacity to remove the contaminants.

From the results, it was clear that the combination of bioaugmentation and the S/S method was more effective in reducing the COD concentration in the fibreboard sludge. The removal of COD for all samples after 28 days was lower than for all samples after 14 days. Then, the COD concentration for the S/S method (without augmentation) showed the highest concentration of COD removal for both after 14 and 28 days. It was explained that the S/S method only was unable to immobilize the contaminants in the fibreboard sludge in this study. The results of the S/S method only showed that the concentration of COD increased proportionally with the curing period. Since the S/S method approach frequently results in organic contaminants having a negative impact on the hydration properties of Portland cement, this method may become unsuitable to breakdown the treated contaminants [30].

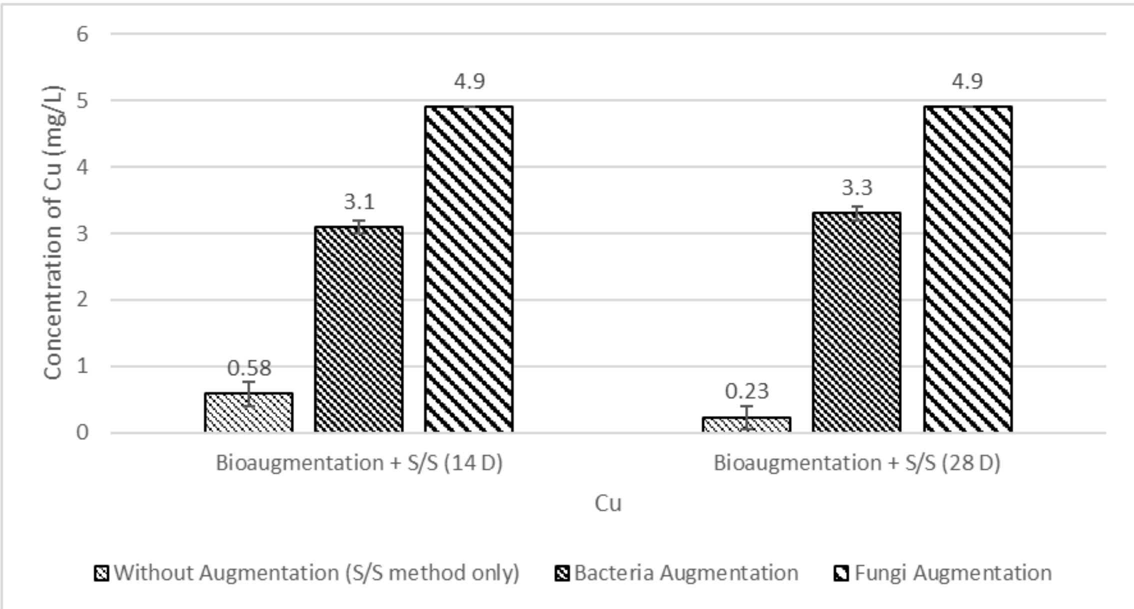
Based on the graph, it was concluded that the most effective agent in degrading contaminants was bacteria (*Sphingobacterium spiritivorum*) followed by fungi (*Aspergillus brasiliensis*) in the combination of bioaugmentation and the S/S method. However, in order to degrade the contaminants, the type of bacteria and fungi are crucial. In this case, it was discovered that bacteria (*Sphingobacterium spiritivorum*) had a higher bioavailability rate [36] than fungi (*Aspergillus brasiliensis*), resulting in a higher COD removal rate with the bacteria augmentation (*Sphingobacterium spiritivorum*) that with the fungi augmentation (*Aspergillus brasiliensis*). In fact, in degrading the contaminants, fungi may act as efficiently as bacteria, but in this study [36], it was demonstrated that the contaminants in the fibreboard sludge are more tolerable to the bacteria (*Sphingobacterium spiritivorum*). This study also indicated the potential of bioaugmentation of the fibreboard sludge that has been treated to effectively degrade the contaminants.

3.2 Concentration of Heavy Metals for S/S Method Only and Combination of the S/S and Bioaugmentation Method after 14 and 28 Days

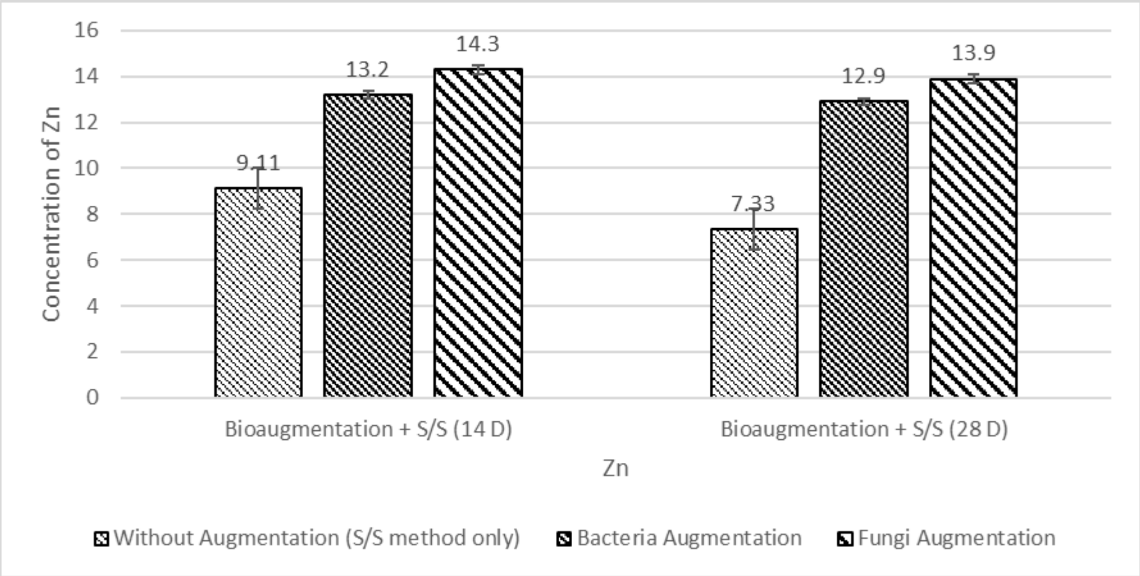
In this study, heavy metals such as chromium (Cr), copper (Cu), zinc (Zn), arsenic (As) and lead (Pb) were measured. Figure 2 shows the results for the concentration of heavy metals for the S/S method only and the combination of S/S and the bioaugmentation method after 14 and 28 days. In fact, the concentration of heavy metals should follow the trend where the concentration of heavy metals after 28 days was lower compared to the concentration of heavy metals after 14 days.


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Concentration of Cr (mg/L)
0.35
0.25
0.6
0.28
0.2
0.3
Bioaugmentation + S/S (14 D)
Bioaugmentation + S/S (28 D)
Cr
Without Augmentation (S/S method only)
Bacteria Augmentation
Fungi Augmentation

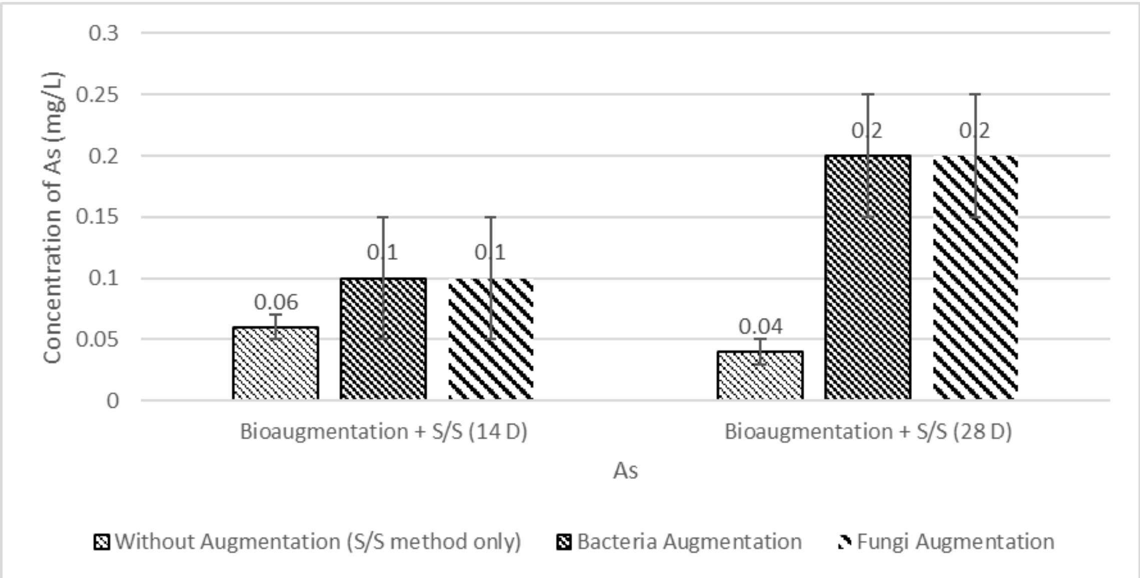
(a)


6
5
4
3
2
1
0
Concentration of Cu (mg/L)
0.58
3.1
4.9
0.23
3.3
4.9
Bioaugmentation + S/S (14 D)
Bioaugmentation + S/S (28 D)
Cu
Without Augmentation (S/S method only)
Bacteria Augmentation
Fungi Augmentation

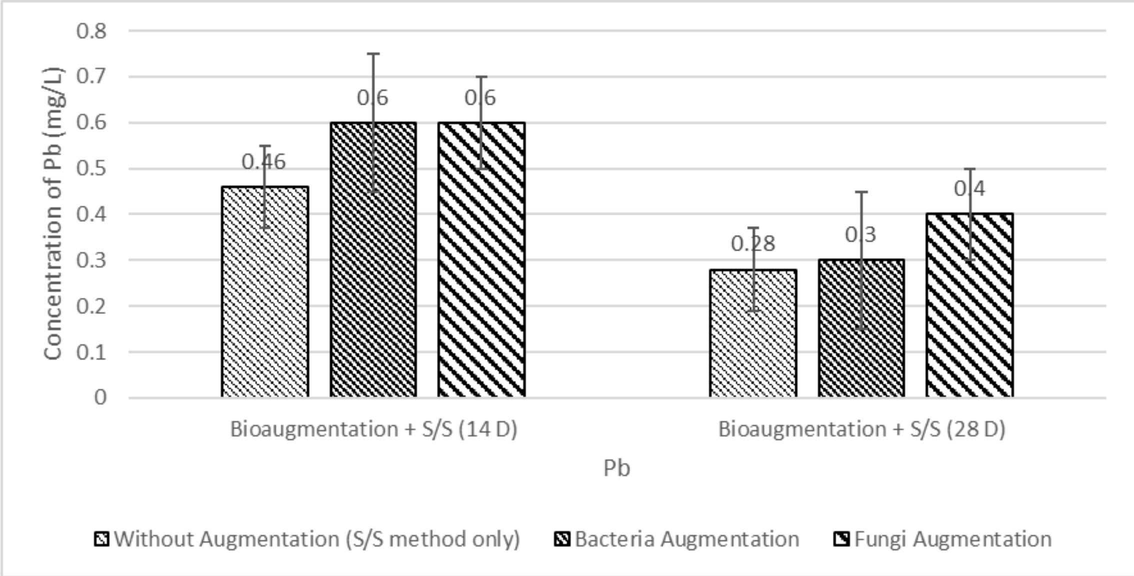
(b)


16
14
12
10
8
6
4
2
0
Concentration of Zn
9.11
13.2
14.3
7.33
12.9
13.9
Bioaugmentation + S/S (14 D)
Bioaugmentation + S/S (28 D)
Zn
Without Augmentation (S/S method only)
Bacteria Augmentation
Fungi Augmentation

(c)


0.3
0.25
0.2
0.15
0.1
0.05
0
Concentration of As (mg/L)
0.06
0.1
0.1
0.04
0.2
0.2
Bioaugmentation + S/S (14 D)
Bioaugmentation + S/S (28 D)
As
Without Augmentation (S/S method only)
Bacteria Augmentation
Fungi Augmentation

(d)



(e)

Fig. 2 - Concentration of Heavy Metals for S/S method only and Combination of the S/S and Bioaugmentation method after 14 and 28 days for (a) Cr; (b) Cu; (c) Zn; (d) As; and (e) Pb

From the results, the range values of Cr (0.2 mg/L – 0.6 mg/L), Cu (0.23 mg/L – 4.9 mg/L), Zn (7.33 mg/L – 14.3 mg/L), As (0.04 mg/L – 0.2 mg/L) and Pb (0.28 mg/L – 0.6 mg/L). It showed that Zn had the highest range values of the heavy metal concentration while As had the lowest range values of the heavy metal concentration. Nonetheless, the concentrations of heavy metals in all samples did not exceed the USEPA limit which were Cr<5 mg/L, Cu<100 mg/L, Zn<500 mg/L, As<5 mg/L and Pb<5 mg/L.

Besides, by comparing the types of degrading agents, the samples without augmentation (S/S method only) showed a better capability in immobilize the heavy metals compared to the the bacteria augmentation and the fungi augmentation except for Cr. According to the figure, it was observed that the samples without augmentation (S/S method only) showed a constant decrease in all the heavy metals concentrations (Cr, Cu, Zn, As and Pb) after 28 days. It was proved that the concentration of heavy metals decreased in inverse proportion to the curing period. As the curing period lengthened, the S/S matrices were more developed and were capable of encasing additional contaminants [37]. In this condition, it also showed that the decreasing concentration of heavy metals for the samples without augmentation (S/S method only) was due to the increase of the S/S matrices that were able to encapsulate the contaminants when the curing period was increased.

However, the samples with the bacteria augmentation and the fungi augmentation showed irregular trend results after 28 days where the concentration of Cu and As were increased after 28 days for the bacteria augmentation while the concentration of Cu with the fungi augmentation remained constant after 28 days. The increase in Cu and As concentrations in the bacteria augmentation samples after 28 days was assumed due to the bacteria could not absorb the heavy metals to the fungi. It was also assumed that the absorption of Cu and As only occurred after 14 days. This situation led to the increase of the Cu and As concentration where the concentration will be increased as long as the curing period increases. A constant result of Cu after 28 days for the fungi augmentation sample was assumed due to the same absorption level after 14 days and 28 days. In fact, since the heavy metals ions did not destroy the concentration of heavy metals remained constant [38].

Based on the results, when comparing the concentration of heavy metals for the bacteria augmentation and the fungi augmentation, the fungi augmentation showed a better result in absorbing the heavy metals compared to the bacteria augmentation due to the fungi got an ability in absorbing the heavy metals efficiently. In the fungi augmentation sample, there was a 50%, 2.80%, 33.33% decrement in the Cr, Zn and Pb concentrations respectively while in the bacteria augmentation sample, there was a 20%, 2.27% and 50% decrement in the Cr, Zn and Pb concentrations respectively. The concentration of Cu and As was excluded due to the irregular trend result that was presented.

Therefore, it was concluded that in destroying the heavy metals for the fibreboard sludge, the S/S method only was the most efficient method that was able to destroy the concentration of heavy metals (Cr, Cu, Zn, As and Pb) followed by the fungi augmentation and the bacteria augmentation.

4. Conclusion

In remediating the contaminants, the sample with the bacteria augmentation (*Sphingobacterium spiritivorum*) showed better COD removal compared to the fungi augmentation (*Aspergillus brasiliensis*) and without augmentation (S/S method only) for both after 14 and 28 days. The result proved that the combination of the S/S and bioaugmentation method was effective in reducing the COD concentration in the fibreboard sludge since the removal of COD for all samples after 28 days was lower than for all samples after 14 days.

The concentrations of heavy metals; Cr, Cu, Zn, As and Pb did not exceed the USEPA limit and Cr only shows a positive result. For other metals, the combination method was not effective compared to the S/S method. Thus, this combination method is highly recommended for sludge that contains high organic contaminants such as fibreboard sludge. It also recommended determining the potential of mixed degrading agents in the bioaugmentation process as bacteria and fungi show different potential in degrading the contaminants.

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