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The dynamic duo of bioremediation: A case study on the efficacy of *Penicellium spp* and *Bacillus spp*

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Abstract

Anaerobic digestion (AD) is a biological process in which microorganisms break down organic matter with biogas. It is a series of chemical reactions during which organic material is decomposed through the metabolic pathways of naturally occurring microorganisms in an oxygen-depleted environment. Recently, anaerobic digestion (AD) has been recognized as one of the best options for treating food waste/cattle rumen mixtures since it results in two valuable final products, biogas and digestate that may be utilized for electricity production and as soil fertilizer, respectively. However, putting the digestate directly into the soil may bring environmental concerns because they contain certain pathogens and heavy metals which could be detrimental to plant and human health when consumed as food. The experimental study carried out in this project has shown that heavy metals contained in food waste/cattle rumen mixtures can be removed using indigenous microbes (*Bacillus spp* and *Penicellium spp*). From the results of the experiment carried out, it was deduced that bioremediation is effective in removing heavy metals from AD sludge. This finding is of special importance because this means that digestate can be freely used as manure without worrying about plants absorbing dangerous heavy metals which are more than their allowable limit from the soil. The use of indigenous microbes is cheap since they are readily available in the digestate.

Keywords: Anaerobic digestion (AD); Municipal solid waste (MSW); Heavy metals (Pb, Mn, Zn and Cu); Biogas; Fertilizers; Nutrients

1. Introduction

Anaerobic digestion (AD) is a biological process in which microorganisms break down organic matter with biogas as the end product. It is a series of chemical reactions during which organic material is decomposed through the metabolic pathways of naturally occurring microorganisms in an oxygen-depleted environment. The full process occurs in four stages namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Amani et al., 2010). Anaerobic digestion produces two main products: digestate and biogas. The solid or semi-solid material leftover after AD is called digestate, while liquid exiting from the digester is called effluent. Anaerobic digestion (AD) digestate, also called Sludge (slurry and dregs), is a nutrient-rich biofertilizer, being the organic product of microbial fermentation after producing biogas in methane-generating. All kinds of organic residues and waste, such as human fecal, chicken dung, vegetable waste, food waste, kitchen waste, corn silage, pig liquid manure, cattle liquid manure, slaughterhouse waste, could be the input materials of biogas plant, therefore, biogas residue is rich in organic matters, NPK elements and vitamin which are essential for optimal crop yield, making them high-grade fertilizers. It can well be used in agriculture to conserve and recycle nutrients and to reduce waste discharge and use of chemical fertilizers.

Biogas sludge is composed of organic compounds, macronutrients, and a wide range of micronutrients, non-essential trace metals, organic micro pollutants, and microorganisms (Singh RP, Aarawal M. 1993.). Some of the major threats

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that arise from the use of these wastes as fertilizers include the emission of pathogens (Venglovsky et al., 2009), also, the treatment and disposal of sludge from the biogas plants is a very sensitive problem because sludge may be a good fertilizer but contain heavy metals which could cause environmental risks, a large volume of soil is required to cover the waste in order to prevent the leaching of potentially toxic compounds including metals (Ahlberg et al 2006).

Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Mueller, 1996). For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products.

1.1. Statement of problem

In the last decade, the use of sludge residues has been proven as a potential source of organic pollutants into the environment (Richardson and Ternes, 2011). Metal ions including sodium, potassium, calcium and magnesium are commonly present in the digestate of anaerobic digesters. Unlike other toxic substances, heavy metals are not biodegradable and can accumulate to potentially toxic concentrations; this makes the disposal of bio-digested slurry after biogas production a major concern for the environment (Gaur 1990, Gaind 1991). These bio-digested slurry or sludge need to be managed in a sustainable way to avoid depletion of natural resources, minimize risk to human health, reduce environmental burdens and maintain an overall balance in the ecosystem.

Objectives of the study

The aim of this study is to carry out bioremediation of anaerobic digested sludge using *Penicellium spp* and *Bacillus spp* to make it safe for use as a bio-fertilizer.

- To establish that bioremediation is an efficient means of removing heavy metals from AD sludge.
- To investigate the action of *Penicellium spp* and *Bacillus spp* two in bioremediation of AD sludge.
- To evaluate of the efficiency of *Penicellium spp* and *Bacillus spp* in purification of AD sludge.
- To compare the performance of *Penicellium spp* and *Bacillus spp* in purification of AD sludge.

1.2. Methods of Heavy Metal Removal

Removal of heavy metals can be carried out by a number of conventional treatments, such as reverse osmosis, electrodialysis, ultrafiltration, chemical precipitation, and ionic exchange. These methods, however, have the disadvantage of requiring high operation costs. The ionic exchange resins, for example, have been commercially known for their effectiveness as pollutant adsorbents in wastewater treatments, but their high cost hinders their application at industrial levels. Chemical processes, although simple to perform, end up being even more expensive because of the active agent that cannot be recovered for future uses. Besides, the final product is a highly concentrated.

Reverse osmosis: a process where heavy metals are separated through a semipermeable membrane by using a pressure higher than the osmotic pressure, which is caused by the the dissolved solids in waste waters. The high pressures required for this process are the main reason for the high operating costs of reverse osmosis

2. Material and methods

The study was carried out in different stages namely

- Co-digested cattle rumen and food waste collection.
- Characterisation of Digestate
- Determination of heavy metals
- Inoculation of indigeneous micro-organisms into digestate
- Analysis of result using existing models and statistical analysis

2.1. Equipments

Equipments used for these experiments include;

- Electronic weighing balance
- Incubator
- Conical flasks

- Measuring cylinder
- Atomic Absorption Spectrophotometer
- Visible spectrometer
- Stainless steel bowls for sample preparation and mixing
- A stirrer
- Water source
- Burette
- Pipette
- Test tubes
- Automatic Stirrer
- Syringes
- Autoclaving Machine
- Agar Plates
- Agar
- Measuring cylinders
- PH Meter
- Thermometer
- Conductivity meter
- Heater
- 100 ml (about 3.38 oz) volumetric flask
- Whatman filter paper
- Funnels

2.2. Collection of Samples, Preparation, Homogenization And Storage

Digestate was collected from cannisters at ecotoxicology Laboratory in the Faculty of Biochemistry, Delta State University, Abraka, Delta State. After sample collection, the sample bottles were packed with an identifying label and the bottle lids were shut close immediately after sample collection to prevent contamination. The samples on getting to the lab were prepared for immediate analysis according to prescribed methods. To maintain the sample integrity, materials were analyzed within 1-2 days of sampling.

2.3. Characterisation of Digestate

For the purpose of characterizing the digestate, samples were neatly labelled and transported in sterile containers to quality lab, located at Mail in oil Laboratory, 12, Ogborikoko, Warri Delta State. Digestate was characterized for physio chemical parameters using prescribed methods:

- Hydrogen Ion Concentration (pH)
- Determination of TS (Total Solids)
- Standard Procedure for Determination of Electrical Conductivity in Water
- Determination Of Heavy Metals in Samples Using Nitric- Dichloric Acid Digestion (Qauam/23)

2.4. Sample Processing

- Samples were placed in a glass petri dish and dried in the oven at 350 °C
- After 24hrs of drying, any lump present was broken up with a clean glass rod to expose inside for drying
- After drying, samples were ground to fine powder using mortar and pestle.

2.5. Digestion Procedure

- 0.25 - 1 g of ground sample was weighed and put into a conical flask
- 5 ml of the Nitric- Dichloric acid was added to form a mixture and let soak overnight
- A small glass funnel was inserted to act as a reflux condenser, it was heated for 1hr at 150 degrees Centigrade.
- Gradually, the temperature was increased to 235 °C. When dense white fumes occur, the sample was heated for another 2hrs.
- Sample was removed from the block, cooled to about 100 °C and 1 ml of 1:1 HCI was added, mixture was heated until a colorless solution is obtained.
- The sample was put into a 100 ml vol. flask and rinsed 5 times with water, each time adding the washing water to the flask to make up the volume.

- Blank samples were prepared using the same procedure without any sample.
- The filtrates of the samples were analyse for heavy metals using AAS

A B

Figure 1 a) Dried Sample, b) Mortar and Pestle used to Grind Sample

2.6. Isolation of Bacteria

- Sterilization of Materials
- Preparation of Media
- Serial Dilution of Samples
- Identification of Selected Bacterial and fungal Isolates
- Inoculation of Samples/Plating (Pour Plate Technique)
- Formulation of Consortia

2.7. Bioremediation By Using Selected Consortium

Cultures were prepared according to the procedure mentioned above. 1 ml of Bacteria (*Bacillus spp*) and Fungi (*Penicellium spp*) respectively. Consortium was inoculated in the sample of about 99 ml in a conical flask to make a 1 percent concentration, and sample conical flasks were kept in shaking incubator at 180–200 rpm and 32–37 °C for 7 days. After incubation, the percentage heavy metal removal was estimated using an atomic absorption spectroscopy according to the procedure mentioned in standard methods (APHA).

2.8. Effect of Contact Time

The kinetic studies were performed to determine the adsorption rates of the *Bacillus spp* and *Penicellium spp* and the minimum contact time for adsorption. In this experiment, an aliquot was taken for analysis to determine the overall effects of contact time on the adsorption process.

2.9. Adsorption Efficiency

The impact of adsorption process was studied by monitoring the overall effects of adsorption on the following indices;

- Impact on conductivity
- Amount of metal ion removed

Conductivity levels were monitored before and after adsorption to evaluate the impact of bioremediation process. The amount of heavy metal ions removed during the series of batch investigation was determined using the mass balance equation of the form (Raghuvanshi et al, 2004).

$$q = \frac{v}{m}(Co - Ce) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Were,
q defines the metal uptake [mg/g],
Co and C are the initial and equilibrium metal ion concentration in the sludge [mg/kg] respectively,
V is the sludge sample volume (ml) and
M is the mass of selected Consortium used [g].

The efficiency of metal ion removal (%) was calculated using the following mass balance equation of the form (Badmus et al, 2007).

$$\text{Removal Efficiency (\%)} = \left[\frac{(Co - Ce)}{Co}\right] \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(2)}$$

Where Co and Ce are the metal ion concentrations (mg/kg) in the water sample before and after treatment, respectively.

2.10. Optimization of Parameters

After the whole experiment, the different parameters like bacterial biomass, shaking speed, temperature, and so forth need optimization. Various parameters (temperature and agitation) were standardized in order to get efficient treatment in less duration.

3. Result

3.1. Physical Property of Digestate (Cattle Rumen and Food waste)

This chapter reflects the result obtained from all the studies done in this project. Presented are the results of digestate characteristics, effects of Contact Time in the bioremediation processes. Also presented are the results of adsorption kinetic models.

Table 1 Physical Property of Digestate

S/n	Parameter	Units	Mixture (20:80)	Mixture (40:60)
1	PH		4.20	4.10
2	EC	µS/cm	3,555.00	8,334.00
3	Mn	mg/kg	113.86	351.49
4	Zn	mg/kg	100.00	408.87
5	Cu	mg/kg	7.00	7.00
6	Pb	mg/kg	1.82	1.46
7	Organic matter	g/100g	61.97	59.20
8	Moisture content	g/100g	94.81	94.14
9	TS	g/100g	5.19	5.86
10	Crude Protein	g/100g	13.33	5.15
11	Fat Content	g/100g	18.20	5.80
12	Ash Content	g/100g	22.00	22.00
13	C/N Ratio	g/100g	16.37	38.90

3.2. Parameters of heavy Metals Removal

Table 2 Parameters of Metals Removal by Bacteria (1% *Bacillus spp*) CR: FW, RATIO 20:80

Time (Days)	Residual concentration (mg/kg)				PH
	Pb	Mn	Zn	Cu	
0	1.82	113.86	100.00	7.00	4.20
7	0.85	70.25	21.05	3.36	4.30
14	0.50	42.95	19.05	2.22	4.40
21	0.25	42.45	15.05	1.85	4.50
28	0.10	41.85	14.05	1.48	4.50
35	0.00	40.95	9.05	1.48	4.50

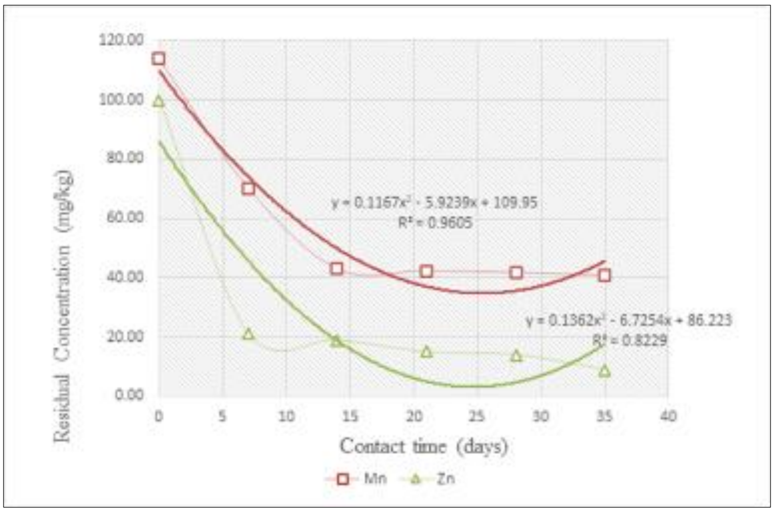

Figure 2 Impact of Bacteria (1% *Bacillus spp*) on Removal of Mn and Zn from Digestate, Cattle Rumen: Food waste Ratio 20:80

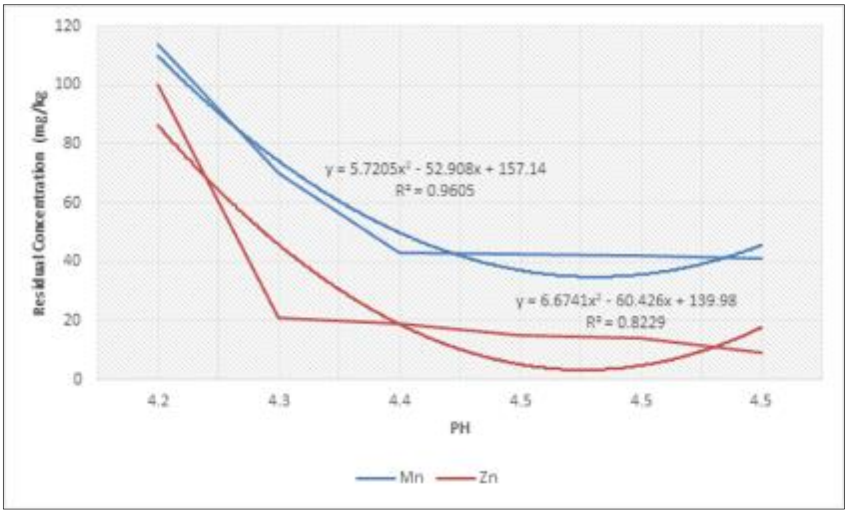

Figure 3 Impact of PH on Removal of Mn and Zn from Digestate by Bacteria (1% *Bacillus spp*). Cattle Rumen: Food waste Ratio 20:80

Table 2 presents the results of Metal (parameters) Removal by Bacteria (1% *Bacillus spp*) with a CR:FW, ratio of 20:80. The results as shown in Figures 3 and 4 show that the amount of metal parameters (Pb, Mn, Zn and Cu) removed by Bacteria (1% *Bacillus spp*) of ratio 20:80 increases with time (days) and PH.

Table 3 Parameters of Metals Removal by Fungi (1% *Penicellium spp*) CR: FW, RATIO 20:80

Time (Days)	Residual concentration (mg/kg)				PH
	Pb	Mn	Zn	Cu	
0	1.82	113.86	100.00	7.00	4.20
7	0.98	53.80	24.05	4.32	4.30
14	0.62	47.45	20.05	2.59	4.40
21	0.30	47.30	20.05	1.48	4.40
28	0.20	46.40	17.05	1.85	4.50
35	0.00	44.55	9.00	0.74	4.60

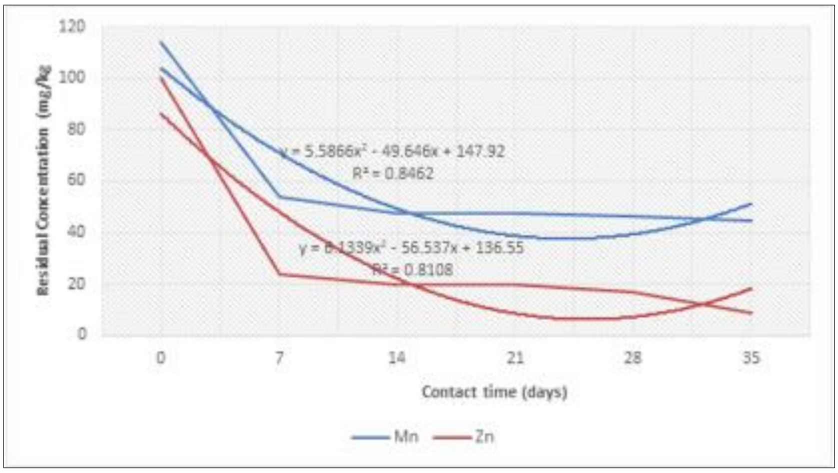

Figure 4 Impact of Fungi (1% *Penicellium spp*) on Removal of Mn and Zn from Digestate. Cattle Rumen: Food waste Ratio 20:80

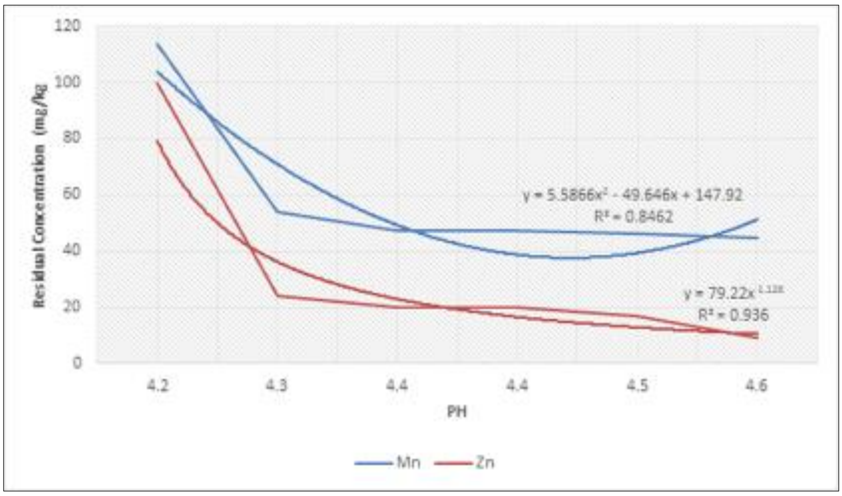

Figure 5 Impact of PH on Removal of Mn and Zn from Digestate by Fungi (1% *Penicellium spp*). Cattle Rumen: Food waste Ratio 20:80

Table 3 presents the result of Metal (parameters) Removal by Fungi (1% *Penicellium spp*) with CR: FW, ratio of 20:80. Figure 4 and figure 5 show that the amount of metal parameters (Pb, Mn, Zn, and Cu) removed by Fungi (1% *Penicellium Spp*) increased with time (days) and PH.

3.3. Effects of Contact Time

Table 4 Effects of contact time on bioremediation of Mn from digestate using Bacteria (1% *Bacillus spp*)

Cattle Rumen: Food waste Ratio 20:80						
S/No	**Contact Time (days)**	**C0 (mg/kg)**	**Ce (mg/kg)**	**C0 - Ce (mg/kg)**	**Efficiency (%)**	**q(mg/g)**
1	-	113.86	113.86	-	-	-
2	7.00	113.86	70.25	43.61	38.30	440.51
3	14.00	113.86	42.95	70.91	62.28	716.26
4	21.00	113.86	42.45	71.41	62.72	721.31
5	28.00	113.86	41.85	72.01	63.24	727.37
6	35.00	113.86	40.95	72.91	64.03	736.46

Table 5 Effects of contact time on bioremediation of Zn from digestate using Bacteria (1% *Bacillus spp*)

Cattle Rumen: Food waste Ratio 20:80						
S/No	**Contact Time (days)**	**C0 (mg/kg)**	**Ce (mg/kg)**	**C0 - Ce (mg/kg)**	**Efficiency (%)**	**q(mg/g)**
1	-	100.00	100.00	-	-	-
2	7.00	100.00	21.05	78.95	78.95	797.47
3	14.00	100.00	19.05	80.95	80.95	817.68
4	21.00	100.00	15.05	84.95	84.95	858.08
5	28.00	100.00	14.05	85.95	85.95	868.18
6	35.00	100.00	9.05	90.95	90.95	918.69

Table 4 and Table 5 show the efficiency of Bacteria (1% *Bacillus spp*) with CR:FW ratio of 20:80. Figure 6 shows that the efficiency of Bacteria (1% *Bacillus spp*) to remove metals increased with time (days) with total efficiency of 100%, 64.03%, 90.95% and 78.86 % at 35days for 20:80 Cattle rumen and Food waste ratio

Table 6 Effects of contact time on bioremediation of Mn from digestate using Fungi (1% *Penicellium Spp*)

Cattle Rumen: Food waste Ratio 20:80						
S/No	**Contact Time (days)**	**C0 (mg/kg)**	**Ce (mg/kg)**	**C0 - Ce (mg/kg)**	**Efficiency (%)**	**q(mg/g)**
1	-	113.86	113.86	-	-	-
2	7.00	113.86	53.8	60.06	52.75	606.67
3	14.00	113.86	47.45	66.41	58.33	670.81
4	21.00	113.86	47.30	66.56	58.46	672.32
5	28.00	113.86	46.40	67.46	59.25	681.41
6	35.00	113.86	44.55	69.31	60.87	700.01

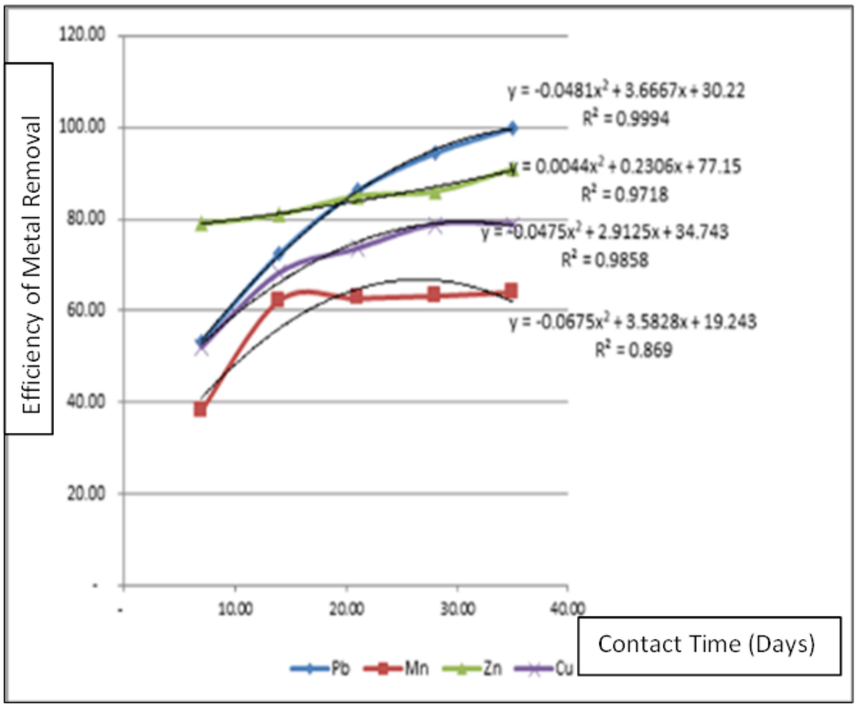

Figure 6 Effect of Bacteria (1% *Bacillus spp*) for Removal of Pb, Mn, Zn and Cu from Digestate, Cattle Rumen: Food waste Ratio 20:80

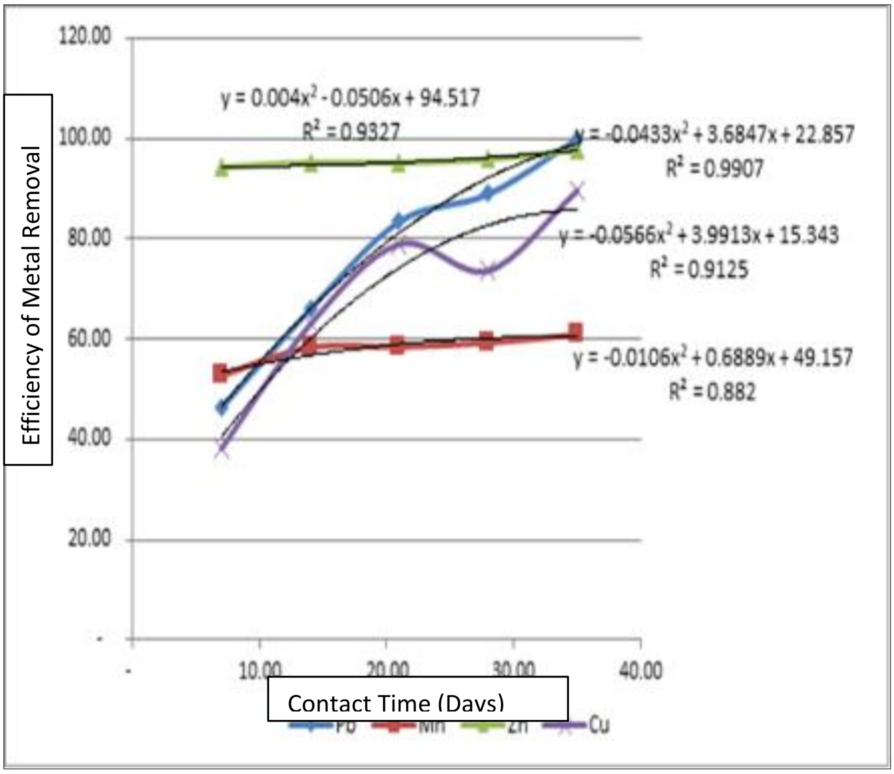

Figure 7 Effect of Fungi (1% *Penicellium Spp*) for Removal of Pb, Mn, Zn and Cu from Digestate, Cattle Rumen: Food waste Ratio 20:80

Table 6 and Table 7 show the efficiency of Fungi (1% *Penicellium spp*) for CR:FW ratio of 20:80. Figure 7 shows that the efficiency of Fungi (1% *Penicellium spp*)) to remove metals increased with time (days) with total efficiency of 100%, 92.66%, 84.80%, and 63.00% at 35days for 20:80 Cattle rumen and Food waste ratio.

Table 7 Effects of contact time on bioremediation of Zn from digestate using Fungi (1% *Penicellium Spp*)

Cattle Rumen: Food waste Ratio 20:80						
S/No	**Contact Time (days)**	**C0 (mg/kg)**	**Ce (mg/kg)**	**C0 - Ce (mg/kg)**	**Efficiency (%)**	**q(mg/g)**
1	-	408.87	408.87	-	-	-
2	7.00	408.87	24.05	384.82	94.12	3887.07
3	14.00	408.87	20.05	388.82	95.10	3927.47
4	21.00	408.87	20.05	388.82	95.10	3927.47
5	28.00	408.87	17.05	391.82	95.83	3957.78
6	35.00	408.87	9.00	399.87	97.80	4039.09

4. Discussion

The plots of the results shown in Figure 2 to Figure 7 have their respective equations and R-squared values. The equations can be used to predict the dependent functions (percentage removed, efficiency and so on) for given range of values of the indepent variable of interest. The R-squared values of each equation show how well the equation (graph) represents the information graphed. R-square value ranges from 0 to 1 (0% to 100). The closer the value to 1 the more efficient the plot is.

5. Conclusion

From the results, Bacteria (1% *Bacillus spp*) has an efficiency of 100%, 64.03%, 90.95%, and 78.86% on 20:80 (cattle rumen to food waste ratio) at 35days for Pb, Mn, Zn, and Cu, respectively. Fungi (1% *Penicellium spp*) has an efficiency of 100%, 92.66%, 84.80%, and 63.00% at 20:80 (cattle rumen to food waste ratio) at 35days for Pb, Mn, Zn and Cu, respectively. Therefore, bioremediation is an efficient means of removing heavy metals from AD sludge.

The action of indigenous microbes (Bacteria and Fungi) in bioremediation of AD sludge reduces the number of metals (Pb, Mn, Zn, and Cu) with time and increases the PH of the digestate. The removal of heavy metals (Pb, Mn, Zn and Cu) from an Anaerobic digestate (AD) by Indigenous microbes (Bacteria and Fungi) is gradual and continuous over a period (not spontaneous).

Fungi and bacteria have the same efficiency to remove lead (Pb) at all ratios of cattle rumen and food waste. Bacteria has a better performance in the removal of Zinc and copper at 20:80. Fungi has a better performance in the removal of magnesium at 20:80 cattle rumen and food waste ratio.

The R-squared values of the data presented in this project ranges from 0.82 (82%) to 0.99 (99%). This implies that the plots and the presentation of the results obtained from this research have been effectively represented.

Compliance with ethical standards

Acknowledgements

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Disclosure of conflicts of interest

The authors declare that they have no conflicts of interest.

Statement of ethical approval

This research involving human participants was approved by the Department of at Civil Engineering at Delta State University, Nigeria.

Statement of Informed consent

Informed consent was obtained from all individual participants included in the study.

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Authors short Biography

Augustine Ojumah is a highly motivated Civil Engineering graduate from Delta State University, Nigeria. He graduated with a second class upper division. He has developed strong communication, time management, and teamwork skills through active participation in workshops. He enjoys collaborating as part of a team on field projects and is actively looking for ways to improve his research abilities through different workshops.