PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (2) 381-387 February 2023

Some Physical Properties and Bacteriological Evaluation of Raw Landfill Leachate from Gosa Landfill in the Federal Capital Territory, Abuja, Nigeria

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ABSTRACT: The physical and bacteriological evaluation of raw landfill leachate from Gosa Landfill in FCT, Abuja, Nigeria was carried out in this study using standard procedures. The probable bacteria identified from the biochemical tests of isolated samples were *Streptococcus pneumoniae with Pseudomonas aeruginosa and Staphylococcus aureus*. However, molecular characterization involving sequencing and BLAST further confirmed the bacteria present. This study concludes that the bio filter produced from Coco peat and Ash affects both the physicochemical properties and bacteriological flora of raw landfill leachate through degradation.

DOI: **https://dx.doi.org/10.4314/jasem.v27i2.29**

Cite this paper as: NASIR, Z. J; MOHAMMED, S. S. D; MANGSE, G. (2023). Some Physical Properties and Bacteriological Evaluation of Raw Landfill Leachate from Gosa Landfill in the Federal Capital Territory, Abuja, Nigeria. *J. Appl. Sci. Environ. Manage.* 27 (2) 381-387

Keywords: Landfill leachate; Bacterial isolates; Polymerase Chain Reaction; *Streptococcus pneumonia*

Landfills have been identified as one of the major threats to groundwater resources worldwide (Amadi *et al*., 2012). During rainfall, the dumped solid waste on landfills receives water and the by-product of their decomposition sips into the groundwater through water deposition. The liquid containing innumerable organic and inorganic compounds is called the 'leachate'. This leachate accumulates at the bottom of the landfill, percolates through the soil and reaches the groundwater (Nagarajan *et al*., 2012). The specific composition of leachate depends upon the nature of solid waste buried, chemical and biochemical processes responsible for the decomposition of waste materials, and water content in total waste. (Oyekun and Eludoyin, 2012). Once groundwater becomes contaminated, full restoration of its quality is most likely not possible in some cases (Nicole *et al*., 2019). The continuous degradation of groundwater quality by anthropogenic activities, particularly from landfill leachates will greatly affect its portability. Similarly, physicochemical, bacteriological and heavy metal pollution of groundwater has a direct impact on human health; from poisoning such as heart diseases, teratogenic abnormalities, cancer and water-borne diseases such as typhoid, cholera and dysentery (Amadi *et al*., 2010). Therefore, this paper is aimed at evaluating the physical properties and bacteriological characteristics of leachate obtained from Gosa Landfill in the Federal Capital Territory, Abuja, Nigeria

MATERIAL AND METHODS

Collection of Sample: Leachate samples were collected from Gosa Landfill, located at 28GP+3P8, 900106, Federal Capital Territory, Abuja, during the January dry season using a 5-litre plastic bottle that had been sterilized by soaking in 10 % nitric acid and rinsed with distilled water in order to avoid contamination then allowed to dry before use. On the sampling site, the bottle was rinsed twice with the leachate before the leachate was collected. The sample was then transported to the Nile University Biology laboratory located on university campus at 'Plot 681, Cadastral Zone C-OO, Research & Institution Area Nigeria, Airport Rd, Jabi 900001, Abuja' in an air conditioned system and stored at a temperature of 4 °C. Prior to analysis, the samples was allowed to return to room temperature and measurement for leachate parameters were carried out. All the parameters were measured according to the standard method for the examination of water and wastewater by APHA, 2005 using a spectra AA-20 (Varian) atomic absorption. All experiments were carried out in triplicate and the results were found reproducible within ±3 % error.
Compact coco-peat block Samples were purchased from an agro allied vendor 'dSharon Herb Gardens',

located along airport Rd, Jabi 900001, Abuja. Samples were transported in sterilized package to the Nile University Biology Laboratory, then soaked up to loosen up so as it may be more convenient for the experiment.

Physical analyses of leachate Sample(s): Leachate samples were measured for their temperature using a laboratory thermometer, pH using a pH meter, and their absorption and Concentration using a UV-Spectrophotometer.

Temperature Measurement: Leachate sample was placed in a beaker immediately after arrival to the lab and measured for its temperature using a thermometer and the readings were immediately recorded.

pH Measurement: Leachate sample(s) was transferred into a beaker, pH meter was switched on and its electrodes were removed from their storage solution and rinsed with water. The electrodes were then bloated with soaked tissue paper. The pH meter was standardized with electrodes immersed in a buffer solution (Acetate buffer pH = 4) and were then rinsed, bloated and dried. The pH on the pH meter was confirmed to be neutral (pH = 7). Electrodes were again rinsed, bloated and dried. The pH meter was standardized with electrodes immersed in a buffer solution (Ammonium buffer pH = 10) and the electrodes were rinsed, bloated and dried then checked on the pH meter (pH = 7), it was then dipped in the beaker containing leachate to be tested and pH result was noted. The electrodes were then put back in the storage solution.

Analyses for Absorbance and Concentration: UV-Spectrophotometer was turned on and top door was opened. A blank cuvette (empty or only solvent) was placed into the cuvette holder and the top door was closed. A "Zero" button on the left side of the screen was clicked on. And the absorbance became 0 while a wavelength of 667 was chosen for the analyses according to the standard wavelength of leachate water. A leachate sample was placed inside the machine and ran for reading. The absorbance and concentration of the leachate sample(s) were shown on the screen and were recorded.

Microbial Analyses of Leachates: Media preparation: Nutrient agar was prepared; using standard manufacture instruction. Procedure; 5.8 g of the NA was dissolved into 200 ml of distilled water; this is calculated as follows: $28*2001000=5.6\ g$ The mixture was heated while stirred with a magnetic stirrer to be dissolved completely and the formed media was then sterilized using an autoclave at 15 psi and the temperature of 121°C for about 15 minutes. This was allowed to cool down without solidifying then the media was carefully dispensed into sterile petri dishes as described by Soumya and Jaya (2016).

Isolation, and Identification of bacteria from leachate samples: Isolation of Bacteria from Leachate Samples: EMB culture media was prepared and the leachate samples collected were inoculated using swab stick in a sterilized environment, the standard streak method was adopted and the media was incubated at 37 °C for a period of 24hours. For microbial growth, discrete colonies on plate were carefully examined for their physical features such as shape and pigment, however, isolated colonies were further sub cultured on the nutrient agar slant. And this was used for further biochemical tests (Parajuli *et al*., 2014). Leachate water samples were each inoculated into the culture and left to incubate for about 24 hours at 37 °C temperature. The physical characteristic of the growth on the culture gives an idea of what kind of bacteria it is. To get a pure culture of the samples, a loop of the microorganism grown in EMB and Endo agar culture media were inoculated into a nutrient agar and incubated for 24hours at 37 °C. Culture media of nutrient agar was prepared and the poured into a mc cockney bottle then slanted, the growth from the cultured media was then inoculated into the media and allowed to incubate for 24 hours at 36 °C.

Identification of Bacteria Isolate from leachate samples a) Gram Staining: A sterilized grease free slide was used. A loop of the inoculum was homogenized on the slide with distilled water to make a smear, this was then air dried and heat fixed then stained with crystal violet solution for one minute, the slide was then washed with water and flooded with iodine solution for 30mins, washed then decolorized with an acidic alcohol. The slide run over tap water then counter stained with safranin for 60 secs. It was then screened for the presence of inflammatory cell and microbes, using oil immersion objective lens of 100x magnification, this revealed the stain absorbing ability of the organism in question (gram + or gram -) which appear either purple or pink/red (Chesbrough *et al*., 2006).

Biochemical Characterization of Bacteria Isolates from Leachates: a) Catalase Test: Two drop of physiological saline were used on a clean glass slide which has been divided into two parts with a grease pencil, the inoculum was carefully emulsified on each drop of saline and 2-3 drops of hydrogen peroxide was added to each of the colony then observed for the presence of bubbles or effervescence. Active bubbles or effervescence indicate positive catalase tests while

the presence of no bubbles or no effervescence indicate negative catalase test (Ochei *et al*., 2007).

b) Coagulase Test: Two drops of physiological saline were added about 2 cm apart on a sterilized grease free microscopic slide which was divided into two sides with grease pencil, a colony of the test organism was emulsified in each of the drop to make a thick suspension and mixed gently for one minute. Clumping of cell was apparent in the bacterial suspension mix with the plasma, i.e. culture under test is coagulase positive, Clumping of cells- coagulase positive, no clumping of all- coagulase negative (Ochei *et al*., 2007).

c) Urease Test: A loop of isolated was added to a slant of urease agar and incubated at 37 °C for 72 hours. Result; yellow indicates a negative result while pink/red indicates a positive result (Ochei *et al*., 2007).

d) Methyl Red Test (MR-VP): A pure culture was inoculated into 5 ml of peptone-broth and incubated for 72hrs at 37 °C, after which 1ml of the cultured broth was added into serological tube and 3-3 drops of methyl red solution was added, Result; red indicated a positive result while an absence of change in colour indicates a negative result (Oyelele and Manga., 2008).

e) Citrate test: A pure culture was inoculated with a sterile wire loop into a slant tube of Simone Citrate agar and incubated for 24 hours. Result; A deep blue colour indicates a positive reaction while a green colour indicates a negative reaction (Oyeleke and Manga, 2012)

f) Indole Test: The isolate was inoculated using a sterile wire loop into a 5 ml sample of peptone water an incubated for 24 hours. After incubation 0.2 ml of kovacs reagent was added to a test tube containing 1 ml of the growth medium and shaken gently. Result; the appearance of a red colour in the reagent layer above the bottle within a minute indicates a positive reaction while the appearance of a yellow pigment indicates a negative reaction (Oyeleke and Manga, 2012).

g) Sugar utilization/Fermentation Test: Culture media was allowed at warm to room temperature prior to inoculation. Phenol red carbohydrate broth (sucrose, glucose, fructose, lactose and maltose) was inoculated with isolated colonies from a 24-hour pure culture of the organism. Control tube of Purple Broth Base in parallel with the carbohydrate based media was inoculated. The inoculated media was then incubated aerobically at 35-37 ºC. for 3-5 days (Sagar, 2019).

Molecular Characterization of Bacteria Isolates from Leachates: Extraction of genomic DNA from the industrial Landfill leachate was conducted using a FastDNATM tool. Universal primers were used for amplification of V4 region of the bacterial 16S gene. Polymerase Chain Reaction (PCR) products were purified using a EDVOTEKTM purification kit and DNA samples were then subjected to gel electrophoresis and sequenced in a DNA laboratory located in Kaduna, Nigeria.

DNA Extraction: Proteinase K was completely dissolved in 1,250 µl of nuclease free water and RNase A was completely dissolved in 600 µl of nuclease free water. A correct amount of absolute ethanol was then added to WA1 buffer. The cultured cells (104 – 106) were centrifuged for 5 min at 300 x g thereafter, the supernatant was discarded carefully without disturbing the pallets. The pallet was then re-suspend in 200 µl of 1 x PBS and 20 µl of proteinase K was added, 10 µl of RNase A was also added and mixed thoroughly then the tubes were incubated for 2 min at room temperature. 200 µl of GB buffer was added to the sample and mixed immediately by vortex mixer and the sample was then incubated at 60 °C for 10min and 400 µl of absolute ethanol was added and mixed well by pipetting. The lysate was carefully transferred into the upper reservoir of the binding column tube (fit in the collection tube without wetting the rim) and the tube was closed and centrifuge at 8000 rpm for 1 min, the collection was discarded from the collection tube and the collection tube was reused. 500 µl of WA1 buffer was then added without wetting the rim, the tube was closed and centrifuged at 8000 rpm for 1min. The solution was discarded from the collection tube and the collection tube was reused. Another 500 µl of W2 buffer was added without wetting the rim, the tube was closed and centrifuged at 8000 rpm for 1 min. The solution was then discarded from the collection tube and the collection tube was reused. Once more, the tube was centrifuged at 13000 rpm for 1 min to completely remove ethanol, then it was confirmed that there was no droplet clinging to the bottom of the binding column tube. The binding column tube was then transferred to a new 1.5 ml tube for elusion, and 50- 200 µl of EA Buffer was added onto binding column tube for at least 1min at RT (15-20 °C) the sample was then centrifuged at 8000 rpm for 1min to elude.

Amplification by PCR: The DNA fragment is terminally labelled with fluorescence-labelled nucleotides by PCR by adding 18mm of nucleus free water to the negative control and 16mm of the water to all samples. Afterwards, 2 mm of primer (1 mm

forward primer and 1mm reverse primer) was added to all the samples including the negative control, 2 mm of DNA extract was then added to all the samples and then run in a PCR machine.

Gel Electrophoresis: The amplified DNA products extracted from leachate samples were analysed with electrophoresis on 1.2 % agarose w/v gels stained with Ethidium bromide stain. Five microliters (5 µ) of PCR product were loaded on to agarose gel. A 100 bp DNA ladder was used as a marker for PCR product. A difference of electric potential of 110 V was applied to each gel and thereafter visualized by UV illumination. The PCR products positive for each of the 5 genes were further run through gel extraction kit to obtain the elute of the positive bands. These elutes were sent to a DNA laboratory for sequencing.

Sequencing of the DNA Extracted from the Leachate Sample(s): Sequencing as a process involves the determination of a nucleotide sequence of a particular DNA fragment. This process used four types of fluorescence-labelled nucleotides, and they are dideoxynucleotides (ddNTPs). ddNTPs lack a 3′ OH group to which the phosphate group of the incoming nucleotide is attached. Therefore, ddNTP was added to the growing chain, there was no further addition of nucleotides at the 3′ end of the chain. That means the addition of a ddNTP into the growing chain terminated the chain growth. Since ddNTPs were added to the PCR mixture in low concentrations, each growing chain was terminated at different levels. The emitting fluorescence was detected to determine the nucleotide sequence of the DNA fragment at the end of the PCR.

BLAST of the DNA Query: A BLAST program was selected and the file containing sequence was uploaded to the program then a database for the search was selected and the algorithm and parameters of the algorithm for the search were selected. The BLAST program was then run on the program for similar sequences.

RESULT AND DISCUSSION

Physical Analyses: Figure 1a, Figure. 1b and Figure. 1c show the absorbance and concentration and pH of the leachate samples in a raw state and after treatment with the five different concentrations of the filter mixture which were represented in a graph. Where the concentrations were directly equal to the absorbance of the leachate samples after treatment (Figure 1a). Raw leachate having a higher concentration and absorbance than the treated leachate samples (Figure 1b). The pH of raw leachate being lower than the treated leachate samples and the increase in pH of the leachate samples after treatment and was directly proportional to the concentration of ash in the bio filter sample (Figure 1c).

Biochemical Analyses: The biochemical analyses for all leachate samples were carried out meticulously in the biological laboratory (Table 1). The table shows the individual results for each biochemical test carried out for the various Leachate samples. The gram staining, upon viewing under a light microscope showed Gram positive bacteria (red colour) presumed to be S. pneumonia, P. aeruginosa and S. aureus. After further biochemical tests.

Gel Electrophoresis: Figure 2 identifies the result for gel electrophoresis of DNA extracted from possible bacteria present in all the leachate samples. Sample 1,2,4 and 5 appeared on the band.

4 Sequencing for Bacteria DNA: Figure 3a and Figure 3b shows the DNA nucleotide sequencing for the bacteria culture with the most visible growths which are sample 2 (representing landfill leachate filtrate after treatment with 40:60 concentrations in ratio if coco peat to ash) and the sample 6 (representing the raw leachate sample which is untreated). ACTG represent Adenine, Guanine, Cytosine and Thymine on both 16s rRNA gene sequences.

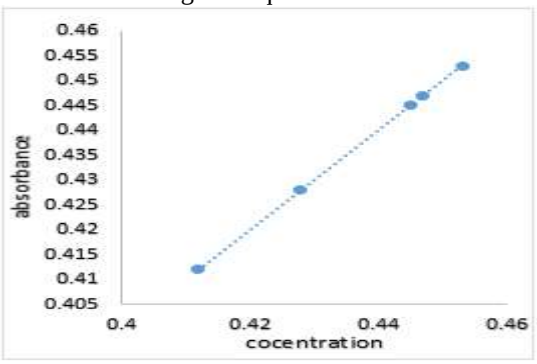

Fig 1a: Absorbance of Treated Leachate Samples against Concentration

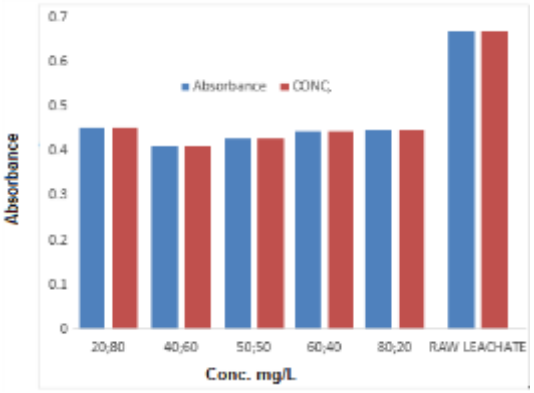

Figure 1b: The Absorbance and Concentration of Raw Leachate and Treated Leachate Sample

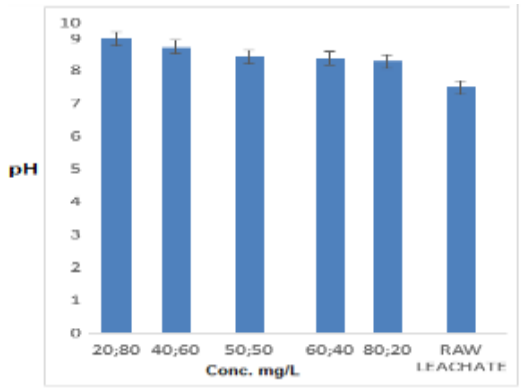

Fig 1c: The pH levels of the various Leachate Samples

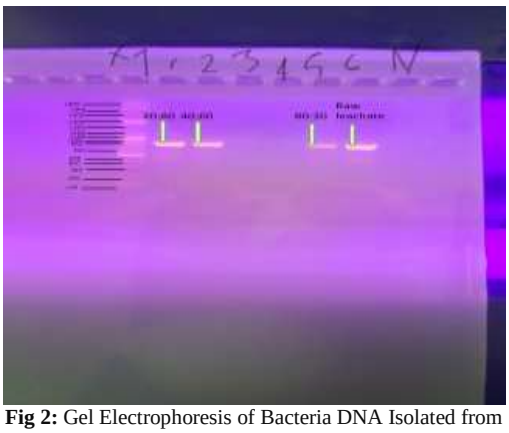

Fig 2: Gel Electrophoresis of Bacteria DNA Isolated from Leachate Samples
Where: M- Molecular Marker; 1- 20:80; 2- 40:60; 3- 50:50; 4- 60:40; 5- 80:20; 6- Raw Leachate and N – Negative Control.

Sequencing for Bacterial DNA

GTCGTTTTACCTGCAAGTCGAACGGCAGCACGGCTTCGG
TCTGGGGCGAGTGCGACGGAGTAATGTATCGAACGGCCC
AGTAGCGGGGGAAACTACGCGAACGGAGCAACCGGTAC
GATCTTGGGGAAAAGCAGGGGACTCGATACATGTTATTG
GAGGGCGATGCGGAGAGGGGGAGGCCGGGATCGGGGGG
CAAATTGGTGGTAGAAAAGTGCCGGGCCTGGTAGGGAA
GGTCTACGGAGTTGGKGGATATGCACGGGAACCTTTCTA
AGATTCGGTGGTAAAAGCTTGGGTGGAAAGTCCTGCAAG
GAAAACCGGTAAACCCGGAGCGGACTGAGAAAGACGGT
TCATTGAGAGGGGGGTTTGGGGGGGAAGTTCGGATATGG
GGAAGGTGGGAGGGGTTGGAAGAAAAGAATCCGGCCAA
CTTGGACTTGTTTTAATAAGGGTAAGGGTGTCAGAGGGG
AGGGAATCTCCGGGTAGGTGAAAACGTAAATATGCGGA
GGAAACCCATGSGAAAGGACCCCTGGGAAAACTGACTCT
TTCAAAAAAAGGGAGAAAAGGTTAGTCTTCAAA

Fig 3a: Sample 2

TTGTTACACATGCAGTCGTACGGCAGCAAGAGCTTGCTC
TCTGGGGGCGAGTGGCGAGAAAAAGGAATACATCGGAA
TCTACCTCGGGGATAAAAGAGGGAAACTTACGCTAATAC
CGCATACGACCTACGGGGAAGCGGGGACCTTCGGGCCTT
GCAGTTGTATGAGCCGATGTCGGATTAGCTAGTTGGCGG
GTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGCTGAG
AGGATGACAGCCACACTGAACTGAGACACGGTCCAGACT
CCTACGGGAGGCAGCAGTGTGGAATATTGGACAATGGGC
GCACGCSTGATCCAGCCTTCCCGCGGGGCAAGGSCTTTCG
TTGTATTGCCCTTTTGTGAAAATTCAAATCTGCTGCCTAT
ACCCGGTTGGGACGACGGCTCAAAAGAGAGTACTGGTTA
CTTCTCGCACGAGCTACCGATAGTCGAAGGGTGAACCTT
TACTCAAACTATCGACTCAGAGAGCGCGTCGTTGAACCT
TTCAACCTCGGACCCCTGCTTTTCACCTTGGACTGGGTGC
TGAGACTTTCGAGGTGTTAGAACGCGCAATTCCGTAGAT
TGTGAATTCCCGGCCACGGACCCGGAGCGCCTCCCGACC
ACTGGACT

Fig 3b: Sample 6

Table 1: Biochemical Characterization of Bacteria Isolates from Leachates

Sample	Desc	Gra	Citr	MR-VP	Indo	Cata	Sucr	Xyl	Mal	Lac	Fruc	Cell	Predic
Raw	Cocci	+	+	-,-	+	+	+	+	+	+	-	+	*P. aeruginosa*
20:80	Cocci	+	+	+,-	+	-	+	-	+	-	-	+	unclear
40:60	Cocci	+	+	+,+	+	+	+	-	+	+	+	+	*S.aureus*
50:50	Cocci	+	+	+,-	+	-	+	+	+	+	-	+	*S.pneumonae*
60:40	Cocci	+	+	+,-	+	-	+	+	+	+	-	-	*S.pneumonae*
80:20	Cocci	+	+	+,-	+	-	+	+	+	+	+	-	*S.pneumonae*

BLAST
Sample 2

```
Query: None Query ID: lcl|Query_57983 Length: 573

>Limnohabitans sp. USH12 gene for 16S ribosomal RNA, partial sequence
Sequence ID: AB599889.1 Length: 1413
Range 1: 3 to 100

Score:100 bits(54), Expect:1e-16,
Identities:85/98(87%),  Gaps:10/98(10%), Strand: Plus/Plus

Query  6   TTTACCTGCAAGTCGAACGGCAGCACGG-CTTCGGTCTGG-GGCGAGTG-CGA-CG---G  58
           |||||||||||||||||||||||||||| |||||||||| |||||||| ||| ||   |
Sbjct  3   TTTACCTGCAAGTCGAACGGCAGCACGGACTTCGGTCTGGTGGCGAGTGGCGAACGGGTG  62

Query  59  AGTAATGTATCG-AACG-GCCCAGTAGCGGGGGA-AAC  93
           |||||| ||||| |||| |||||||| | |||||| |||
Sbjct  63  AGTAATATATCGGAACGTGCCCAGTCGTGGGGGATAAC  100
```

The result of the leachate analyses before and after treatment by the bio filter showed that, the concentrations were directly equal to the absorbance of the leachate samples, with that of the raw leachate being higher than the other leachate samples and this could be due to the absence of pre-treatment in the raw leachate. This phenomenon could be seen in another research by Kõiv *et al*., (2006) where, the purification

rate of the landfill leachate used depended on the contamination rate – the outflow results were better with pre-treated leachate, the results also improved due to the lowering of the flow rate (on average by 60 times); because of this, during the treatment process in this thesis, hydraulic loading of the leachate unto the filter was ensured to be slow and steady to enhance the absorbance, adsorption capacity and the overall efficiency of the bio filter samples used. Another study by Chen and Li (2020) also supports these findings The pH level of the leachate sample was neutral (7.52) and in line with the WHO standards for potable water in the raw leachate while after treatment with varying concentrations of coco-peat - ash, the alkalinity level was directly proportional to the concentration of Ash in the bio filter mixtures but does not exceed the WHO limits for water standards. The increase in pH could be attributed to the bacterial count in the culturing sample plates which shows a significant rise after treatment using the bio filter. This bacterial growth increase could be due to biological activity (biodegradation) during treatment which would have led to the breakdown of compounds and elements in the leachate samples; biodegrading alkalinophiles are bacteria that biodegrade in an alkaline environment. A research by Shugen Liu *et al*., (2019) shows how pH affects biodegradation by bacteria.

Sample 6

```
Query: None Query ID: lcl|Query_57733 Length: 634

>Stenotrophomonas maltophilia strain C1A 16S ribosomal RNA gene, partial sequence
Sequence ID: KJ466103.1 Length: 1446
Range 1: 13 to 447

Score:453 bits(245), Expect:9e-123,
Identities:377/436(86%),  Gaps:32/436(7%), Strand: Plus/Plus

Query  5    TACACATGCAGTCGTACGGCAGCA----AGAGCTTGCTCTCTGGG-GGCGAGTGGC-GA-  57
            |||||||||||||| |||||||||    |||||||||||||||||| |||||||||| ||
Sbjct  13   TACACATGCAGTCGAACGGCAGCACAGGAGAGCTTGCTCTCTGGGTGGCGAGTGGCGGAC  72

Query  58   GAAAAAGGAATACATCGGAATCTAC-C--TC---GGGGATAAAAGAGGGAAACTTACGCT  111
            |    |||||||||||||||||||| |  ||   ||||||||   ||||||||||||||||
Sbjct  73   GGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTACGCT  132

Query  112  AATACCGCATACGACCTACGGG-G-AAGC-GGGGACCTTCGGGCCTTGC-AG-TTGTATG  166
            ||||||||||||||||||||||| | |||| ||||||||||||||||||  | ||| |||
Sbjct  133  AATACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCGGGCCTTGCGCGATTGAATG  192

Query  167  AGCCGATGTCGGATTAGCTAGTTGGC-GGGTAAAGGCCCACCAAGGCGACGATCCGTAGC  225
            |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  193  AGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGACGATCCGTAGC  252

Query  226  TGG-CTGAGAGGATGA-CAGCCACACT-GAACTGAGACACGGTCCAGACTCCTACGGGAG  282
            ||| |||||||||||| |||||||||| ||||||||||||||||||||||||||||||||
Sbjct  253  TGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG  312

Query  283  GCAGCAGTGTGGAATATTGGACAATGGGCGCACGCSTGATCCAGCCTTCCCGCG-GGGC-  340
            ||||||||| ||||||||||||||||||||| || |||||||||| | ||||| |||
Sbjct  313  GCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTG  372

Query  341  AAG--GSCTTTC--GTTGTATTGCCCTTTTGT-GAAAATTCAAATCT-GCTGCCT-ATAC  393
            |||  | | |||  ||||||  |||||||||| |  ||   |||||  |||| || ||||
Sbjct  373  AAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAG-AAATCCAGCTGGCTAATAC  431

Query  394  CCGGTTGGGACGACGG  409
            |||||||||| |||||
Sbjct  432  CCGGTTGGGATGACGG  447
```

Many bacteria and Fungi are already proven to have high removal capacity of leachate parameters like BOD, COD, Nitrite, Sulphate and even heavy metals. In recent years, microorganisms able to remediate heavy metals, xenobiotic and even plastics have been discovered. The isolated and presumptively identified bacteria(s) were all gram positive in nature when viewed under a light microscope and the structure and morphology of the bacterial cells closely resembled that of S. pneumonia on study. However, *S. pneumoniae*, *P. aeruginosa* and *S. aureus* were further presumed through a series of other biochemical tests carried out and this is supported by the research findings by Khan *et al* (2019). On further confirmation through 16S rRNA gene synthesis, two samples out of the six samples (where sample 2 was of a treated leachate and sample 6, of raw leachate) were analysed; sample 2 query corresponded with Limnohabitans sp. strain subject while sample 6 corresponded with *Stenotrophomonas maltophilia* strain subject. The

presence of *Limnohabitans sp*. in treated landfill leachate could be attributed to the abundance of its class (Betaproteobacteria) in freshwater lakes, and its ability to be easily to cultured; and this makes it the best-studied group in freshwater lakes (Ruben and Vincent., 2020). *Stenotrophomonas maltophilia* on the other hand is an infectious group of bacteria that live in wet environments; in a hospital setting, they are able to survive and multiply in fluids such as mucus of the respiratory system, urine, intravenous (IV) fluids, and irrigation fluids used to clean wounds or body cavities such as the ear canal or bladder. *S. maltophilia* can also grow in medical devices exposed to these fluids including urinary catheters, IV lines, and breathing machines (ventilators) (Cunha, 2017; Lewis and Zaas, 2018). This may indicate that medical waste was present in the sample collection site of the landfill leachate and further explains that coco peat-ash bio filter could be effective in degrading bacteria in waste water.

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