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Biodegradability of polystyrene plastics by bacterial isolates from plastic composted waste soil and molecular characterization of plastic degrading bacterial isolates

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This study examined the biodegradability of polystyrene (PS) plastics. Soil samples were collected from Oluku Community in Egor Local Government Area, Benin City, Edo State, Nigeria. Heterotrophic bacteria were enumerated and screened for PS degradation potential. Plastics degrading potential of the isolates was determined by Shake Flask method, degradation of PS plastics was determined by analyzing the formulated PS plastic solution for its additive concentration before and after the degradation process using gas chromatograph with mass spectrometry. Identified bacterial isolates were further characterized using the 16S ribosomal RNA gene. The results from all the parameters indicate that there was active utilization of oxygen and other nutrients available in the test system which is an evidence of PS degradation. The pH had values ranging from 6.5 and 7.4. It was observed that the nutrients and the biochemical oxygen demand decreased considerably with time. There was a reduction in the concentration of bisphenol A (BPA) contingents recorded before (37.04 mg/kg) and after (1.19 mg/kg) the degradation process. The bacterial isolates with codes B1 and B3 belonging to *Bacillus* while B2 belong to *Pseudomonas* genera were identified. Two isolates had 99% similarity with *Bacillus subtilis* strain BS3902 and EU047884.1 respectively, while the third isolate had 100% similarity with *Pseudomonas aeruginosa* strain KAVKOI. This results shows that the strains have the ability and are able to degrade PS plastics.

Key words: Polystyrene plastics, plastic composted soil, biodegradability, heterotrophic bacteria, molecular characterization.

INTRODUCTION

The term “plastics” includes materials composed of various elements such as carbon, hydrogen, oxygen, nitrogen, chlorine, and sulphur. They are produced by the conversion of natural products or by the synthesis from primary chemicals generally coming from crude oil, natural gas, or coal (Coors et al., 2003; Jonsson et al.,

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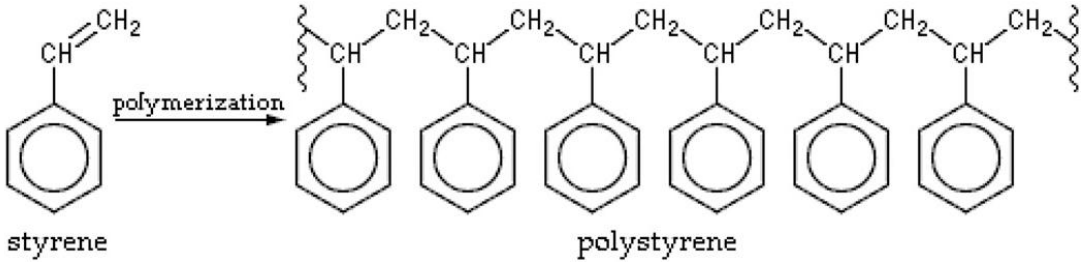

Figure 1. Chemical formula of polystyrene (Ho et al., 2018).

2003). The increased use of plastics in day to day consumer applications has resulted in municipal solid waste containing an ever growing fraction of plastic material used for a short time and discarded (Ho et al., 2018). Plastics have taken centre stage in daily life due to its qualities like low weight, durability and low cost as compared to other materials types (Andrady and Neal, 2009). Polystyrene (PS) is a synthetic aromatic polymer with high molecular weight (formula (C8H8)n) made from the monomer styrene (Figure 1) (Ho et al., 2018). Like other plastics, PS is widely used because of its good mechanical properties and relatively low cost (Ho et al., 2018). PS is widely used in construction materials (insulation), packaging foam, food containers, disposable cups, plates, cutleries, cassette boxes, and compact disks (Ho et al., 2018). There is about 21 million tons of PS produced in the world in 2013 (Yang et al., 2015). As a result of such wide use, plastics including PS have accumulated in the environment, causing environmental pollution, human health problems, and ecosystem changes due to their toxicity and recalcitrant compounds. PS materials can be recycled; however, most PS foam ends in landfill (Ho et al., 2018). Plastic pollution affects soil aeration, soil fertility, soil pH, nitrification and the activities of soil fauna and soil flora which act as sentinels in the soil (Atuanya et al., 2016).

Biodegradation of plastics is the process in which microorganisms (fungi, bacteria, and archaea) degrade them by their extracellular or intracellular enzymes and use the plastics as a substrate for growth (Adamcova and Vaverkova, 2014; Himani et al., 2013; Zheng et al., 2005). PS biodegradation starts when microorganisms begin growing on the surface of PS and secrete their enzymes to degrade the polymer into smaller molecular fragments called oligomer and maybe monomeric units (Zheng et al., 2005). Styrene itself is able to be used as a carbon source for growth by some microorganisms. *Rhodococcus ruber* has been shown to form biofilms on PS and partially degrade it (Mor and Sivan, 2008). A biofilter consisting of *Brevibacillus* species has been shown to remove 3 kg of styrene in a day (Motta et al., 2009). The biodegradation rate depends on the thickness and the molecular weight of the plastic (Hwang et al., 2008). In fact, a large number of microorganisms can bring about styrene biodegradation (Baggi et al., 1983). There are several ways of styrene catabolism; however, a predominant pathway involves the oxidation of styrene to phenylacetate, which is then converted via the TCA cycle (Mor and Sivan, 2008). This pathway is as shown in Figure 2.

Biodegradation of PS has been reported in some previous studies. In the literature, few reports describe the microbial utilization of PS as a carbon source (Kaplan et al., 1979; Sielicki et al., 1978). However, there are few reports of microbes degrading PS in the real environment such as landfill, soil, etc. Oikawa et al. (2003) isolated and identified *Pseudomonas* and *Bacillus* species for styrene degradation; also *Xanthomonas* and *Sphingobacterium* species for PS decomposition by 16 S ribosomal DNA analysis from soil (Sielicki et al., 1978). Four microbial strains have been isolated from garden soil after 8-month buried samples of PS and EPS solution (2%) in chloroform. They were identified as *Microbacterium* species NA23, *Paenibacillus urinalis* NA26, *Bacillus* spp. NB6, and *Pseudomonas aeruginosa* NB26. They were able to extract some carbon from the complex molecules of PS but the process was very slow and caused no significant chemical changes on the surface (Atiq et al., 2010). Therefore, this study examined biodegradability of polystyrene plastics by bacterial isolates from Plastic Composted waste Soil and Molecular Characterization of Plastic Degrading Bacterial Isolates.

MATERIALS AND METHODS

Sample collection

Soil samples (500 g) were collected from different locations within the waste management landfill site located at Oluku Community, Benin City, Edo State, Nigeria at a depth of 0 to 10 cm with a standard soil auger in plastic bags. The soil samples were homogenized and kept on the laboratory bench to air dry (Atuanya et al., 2012). The soil sample was used for the isolation and enumeration of total heterotrophic bacteria.

Isolation and enumeration of heterotrophic bacteria

Serial dilution of soil sample was made to form 10^{-4}, 10^{-5} and 10^{-6}

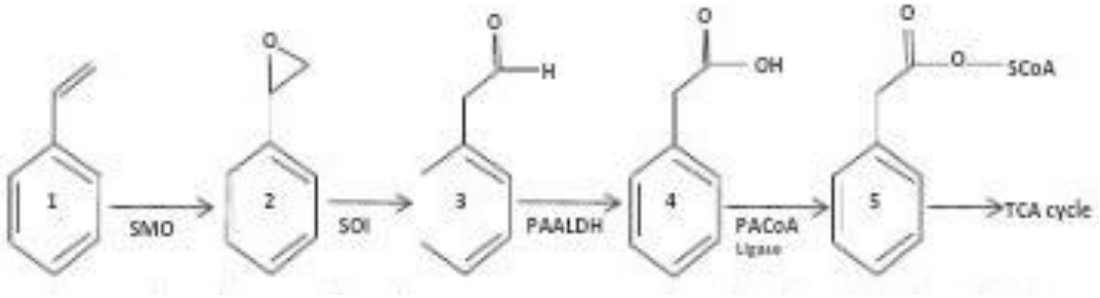

Figure 2. Degradation pathway for styrene (Tischler et al., 2009; Mooney et al., 2006). 1-styrene, 2-styrene oxide, 3-phenyl acetaldehyde, 4-phenylacetic acid, 5-phenylacetyl coenzyme A SMO: styrene monooxygenase, SOI: styrene oxide isomerase, PAALDH: phenylacetaldehyde dehydrogenase, PACoA ligase: phenylacetyl coenzyme A ligase.

dilutions using normal saline. Total viable heterotrophic bacterial counts were determined. Nutrient agar plates were prepared; the plates were inoculated and were incubated at 37°C for 24 h. Colony counts were taken after incubation and biochemical tests were carried out (Burkhard et al., 2001).

Collection and preparation of polystyrene plastic granules

Waste polystyrene plastics were collected and blended into powder using an industrial grinding machine. The plastic granules was weighed and kept in small white polyethylene bags. This polystyrene granule was used to formulate different polystyrene plastic concentration in a mineral salt medium which was used for biodegradation test (Atuanya et al., 2016).

Screening test for biodegradation potential of polystyrene plastics

Bacterial isolates were screened for the ability to degrade polystyrene plastics using mineral salt medium. 9 ml of the mineral salt medium was dispensed into seven test tubes and sterilized. In each of the test tubes, 0.1 g of plastic at 20 ppm was added to serve as the only source of carbon and energy (Atuanya et al., 2011). Thereafter, all the test tubes were inoculated with two drops of cell suspension of an isolate previously grown in mineral salt medium. The cell suspension was prepared by suspending a loopful of the bacterial isolate from nutrient agar plate into two (2 ml) mineral salt medium. Among the tubes, there was a control which was not inoculated. All the tubes were incubated at room temperature (28±2°C) for 7 days after which the tubes were checked for turbidity which indicated the ability of the isolates to utilize PS plastics as growth source (Ferrara et al., 2006).

Determination of plastics degrading potential of the isolates by shake flask method

A known volume of 150 ml of the mineral salt medium was dispensed into 250 ml conical flask and the test polystyrene (PS) plastic granules were introduced separately into the conical flask after sterilization (Nishida and Tokiwa, 1994). Overnight, broth culture of each isolate was seeded into each flask and incubated on the laboratory bench. The utilization of PS plastics was monitored at two days interval for 10 days by monitoring the bacterial growth measured by viable counts on nutrients agar. The optical density was determined at 620 nm wavelength using Comspec Visible Spectrophotometer, changes in ionic concentration and pH were determined with pH meter (Model Hanna microprocessor P211 pH meter, India) and temperature using temperature meter. Physicochemical analyses were carried out such as pH, total organic carbon, biochemical oxygen demand (BOD), alkalinity analysis, sulphate content, nitrate content and phosphate content to determine the rate of degradability of PS plastic (Brulle et al., 2010).

Determination of plastic degradation

Degradation of the PS plastic granules and the level of degradation was determined using Hewlett Packard HP 5890 series II Gas chromatograph with Mass Spectrometry before and after the degradation process.

Instrumentation and conditions

Hewlett Packard HP 5890 series II Gas chromatograph equipped with an Agilent 7683B injector (Agilent Technologies Santa Clara, CA, USA), A 30 m, 0.25 mm i.d. HP-5MS capillary column (Hewlett – Packard, Palo Alto, CA, USA) coated with 5% phenyl-methylsiloxane (film thickness 0.25 m) and an Agilent 5975 mass selective detector (MSD) was used to separate and quantify the BPA compounds. The samples were injected in the split less mode at an injection temperature of 300°C. The transfer line and ion source temperature was 280 and 200°C. The column temperature was initially held at 40°C for 1 min, raised to 120°C at the rate of 25°C/min, then to 160°C at the rate of 10°C min^{-1} and finally to 300°C at 5°C min^{-1}, held at final temperature for 15 min. Detector temperature was kept at 280°C. Helium was used as a carries gas at a constant flow rate of ml/min. Mass spectrometry was acquired using the electron ionization (EI) and selective ion monitoring (SIM) mode. A PerkinElmer Gas Chromatograph model Autosystem XL, with Flame Ionization Detector was used for identification of BPA, phthalate, organotin, alkyl phenol and other plastic components by comparison between the retention times of the BPA sample peak and the standard compound. The quantification was done by the internal normalization method. An Elite-5 fused silica capillary column (30 m × 0.25 mm i.d. crossbond 5% diphenyl 95% dimethyl polysiloxane, 0.25 µm film thickness) was used for the GC separation using the following oven temperature program: 150°C (5 min hold) heating to 250°C at 3°C min^{-1} and heating to 300°C at 10°C min^{-1} (5 min hold). The injector temperature was 250°C. The injection volume was 1.0 µL (n=3) in the split

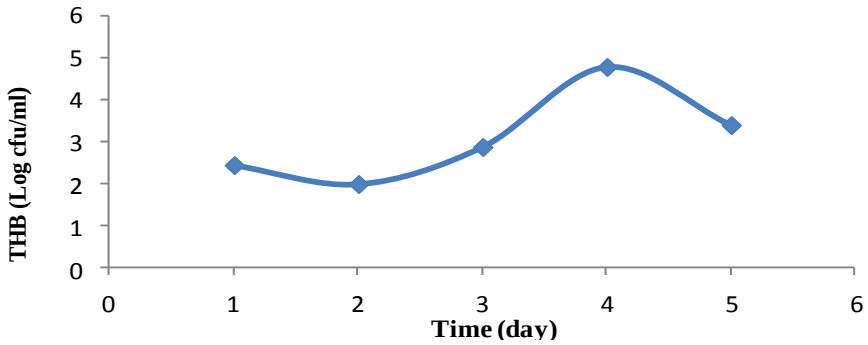

Figure 3. Change in total heterotrophic bacterial count (Log cfu/g) of the test system.

mode (1:50) (Burkhard et al., 2001).

Molecular characterization of plastic degrading bacterial isolates

DNA extraction

Bacteria in saline were added to 1.5 ml micro centrifuge tube. 450 µl of a 240 mM NaOH, 2.7 mM EDTA, and 74% ethanol solution were added to the tube and mixed gently to give final concentrations of 200 mM NaOH, 2.25 mM EDTA, 61% ethanol. The tube was then heated to 80°C for 10 min and centrifuged at 16,060 ×g for 10 min. The supernatant was removed, and 100 µL of an optimized suspension solution containing 0.1 mM EDTA, 50 Mm Tris-HCl, Ph 8.0, 1% Triton-X-100, and 0.5% Tween-20 was added to solubilize the denatured DNA. DNA was collected by centrifugation at 7200×g for 10 min, washed with 500 µl of 70% ethanol, air dried at room temperature for approximately 3 h and finally dissolved in 50 µl of TE buffer (Brosius et al., 1981).

Polymerase chain reaction procedure

The PCR consist of final volume of 50 µl which included 8 µl DNA and 42 µl reaction cocktail consisting of 5x GoTaq green reaction, 10 Mm of each dNTPs, 10 pmol each 27F: 5´-AGAGTTTGATCMTGGCTCAG-3´ and 1525 R: 5´-AAGGAGGTGWTCCARCC-3´ specific for ~ 800 bp conserved domain of the 16S rRNA polymerase. PCR was carried out using the following thermal cycles regime; an initial denaturation at 94°C for 1 min, this was followed by 29 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min and an extension at 72°C for 1.5 min, a final extension at 72°C for 5 min ended the PCR experiment (Brosius et al., 1981).

Agarose gel electrophoresis

Agarose gel was prepared and buffered with 1.5 ml of 0.5x TAE. 10 ml of ethidium bromide was added, mixed and then poured into electrophoretic tank with the comb in place to obtain a gel thickness of about 4 to 5 mm. 10 µl of sample was mixed with 1 µl of the 10x loading dye. DNA samples were loaded and ran. The DNA was viewed using a UV-trans-illuminator (Opere et al., 2013).

Sequencing of the 16S rDNA gene

The purified DNA samples were sequenced at the Bioscience Laboratory, International institute for tropical Agriculture (I.I.T.A), Ibadan, Oyo State with an automated DNA sequencing analyzer (ABI 3730x) using 27F and 1492R primers. Sequence assembly and alignment were carried out using CLC bio software, followed by searching the homology in the Gene Bank using Basic Local Alignment Search Tool (BLAST) program of CLC bio software.

RESULTS AND DISCUSSION

The results from this research showed evidence of polystyrene plastic degradation. All the parameters (Figures 3 to 10) indicate that there was active utilization of oxygen and other nutrients available in the test system. The pH profile obtained generally fell between the optimum range of 6.5 and 7.4 which favors most of the heterotrophic bacterial though the values did not follow a consistent trend as for the other parameters; it was observed that the metabolic products produced by PS plastic utilizing bacteria must have contributed to the fluctuation of the pH readings near neutrality. It was also observed that the nutrients (sulphate, phosphate and nitrate) decreased considerably with time. The decrease is understandable as they are used in the metabolism of microorganism in building biomass. There is correspondence in the utilization of phosphate, sulphate and nitrate indicating their relative importance in cell metabolism as stated by Odum's combine law. The biochemical oxygen demand (BOD) of the media was also decreasing as the study progressed indicating that the oxygen content in the medium is been utilized by the aerobic bacteria. There was evidence of degradation of polystyrene plastics from the concentration of Bisphenol A (BPA) contingents recorded before (37.04 mg/kg) and after (1.19 mg/kg) the degradation process shown in Table 2. Although there was no complete degradation of the polystyrene plastic, but there was a considerable reduction in the concentration of the BPA contingents, TOC, nitrate, phosphate, and sulphate in the test system (Odokuma and Okpokwasili, 1993).

There were three major plastic degrading bacterial isolates of which two were identified as *Bacillus* spp. and one as *Pseudomonas* spp. (Table 1) which was further

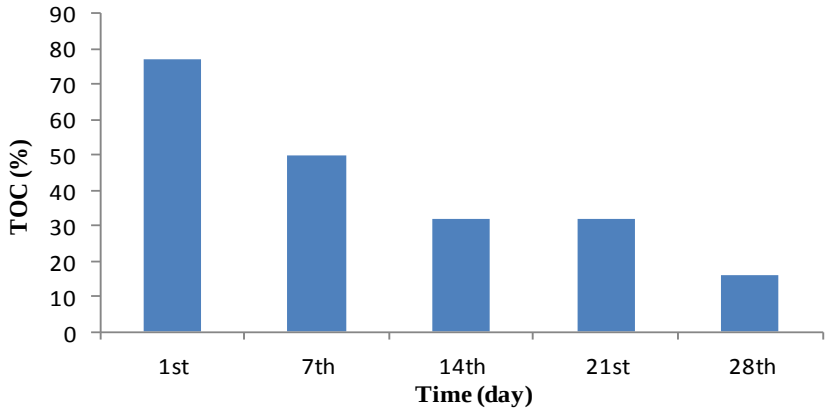

Figure 4. Change in percentage of total organic carbon of the test system.

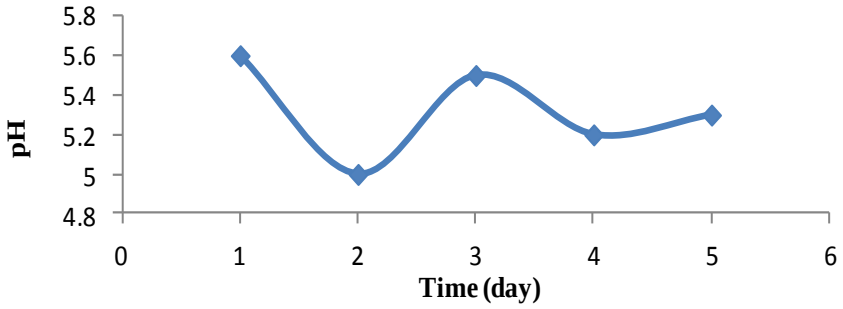

Figure 5. Change in pH of the medium for the test systems.

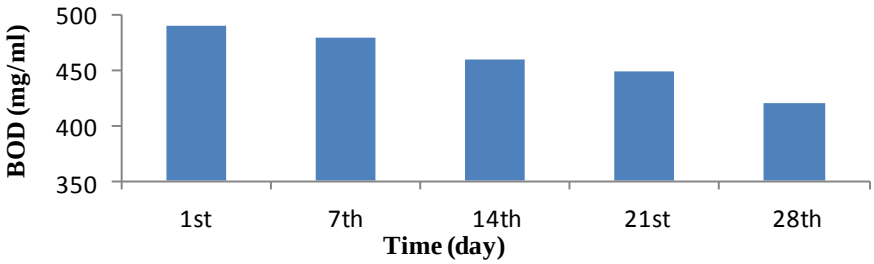

Figure 6. Change in BOD OF the test systems.

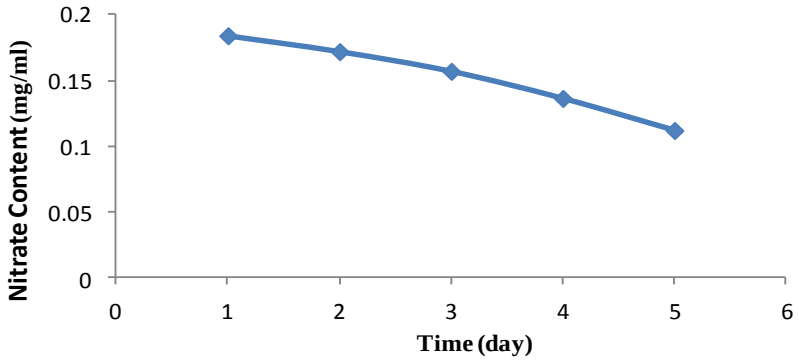

Figure 7. Change in concentration of nitrate content for the test systems.

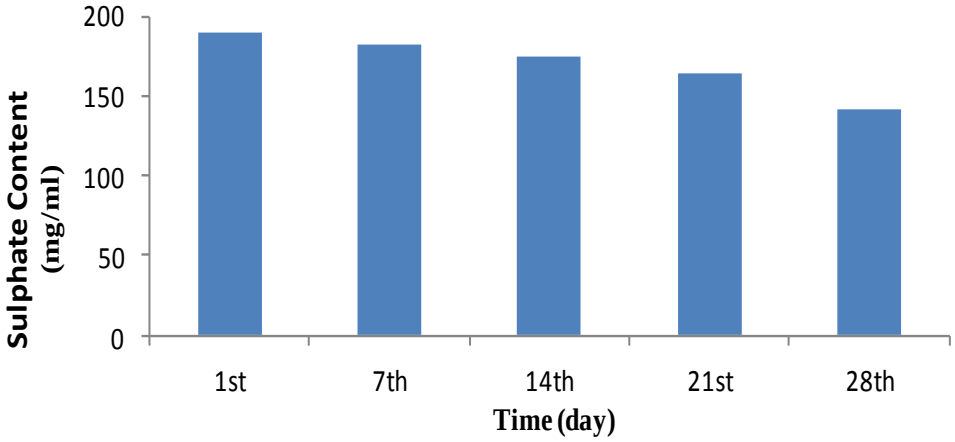

Figure 8. Change in the concentration of sulphate content of the test systems.

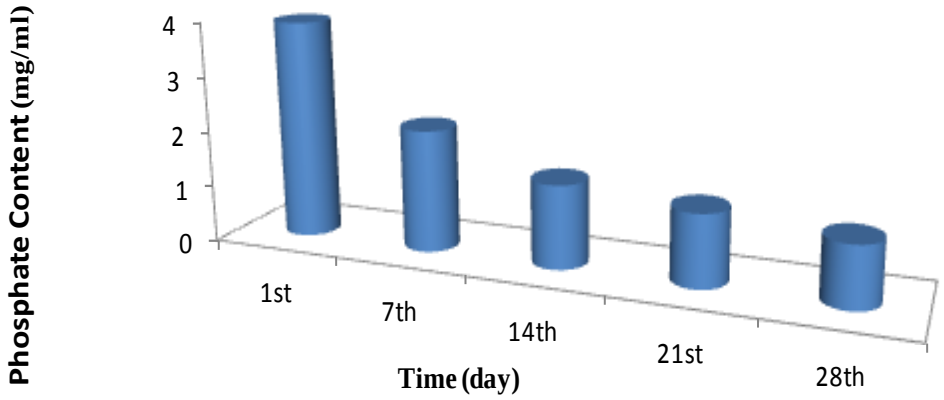

Figure 9. Change in the concentration of phosphate content of the test systems.

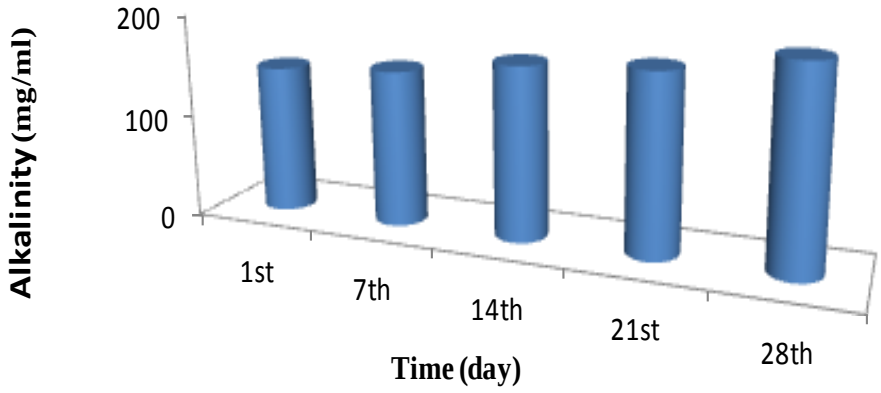

Figure 10. Change in alkalinity of the test systems.

characterized using the 16S ribosomal RNA gene (Table 3). PCR amplification using 16S rRNA gene universal primer set generated amplicons of around 500 bp fragments. This is in line with the results of previous

Table 1. Cultural, morphological and biochemical test of bacterial isolates.

Characteristics	1	2	3
Cultural			
Shape	Circular	Circular	Irregular
Elevation	Low convex	Convex	Flat
Margin	Entire	Undulated	Undulated
Wetness/dryness	Wet	Dry	Wet
Transparency	Opaque	Opaque	Opaque
Colour	Green	Cream	Cream
Size	Medium	Medium	Large
Morphological			
Gram staining	-	+	+
Cell type	Rod	Rod	Rod
Cell arrangement	Single	Chains	Large
Biochemical			
Catalase	+	+	+
Oxidase	+	-	-
Coagulase	-	-	-
Urease	-	+	+
Indole	-	-	-
Citrate	+	+	+
Sugar fermentation			
Glucose	+	+	+
Lactose	-	-	-
Possible isolates	*Pseudomonas* spp.	*Bacillus* spp.	*Bacillus* spp.

Table 2. Degradation of polystyrene plastics and the bisphenol A contingence found in the plastic composted soil sample.

Parameter	Before degradation	After degradation
Methylene	17.45	0.54
Hexane	10.05	0.26
Chloroform	1.56	0.31
Toluene	5.87	0.07
Tetrachloroethylene	1.48	0.01
Chlorobenzene	0.37	0.00
Dichlorobenzene	0.15	0.00
Benzene	0.11	0.00
Total	37.04 mg/kg	1.19 mg/kg

study as theoretically predicted for bacterial family (Opere et al., 2013). Amplicon from the first round of PCR were thereafter used as templates to run a bacterial species level, which generated PCR products of about 600 bp (Plate 1) and 550 bp (Plate 2) in size as predicted for *Bacillus* and *Pseudomonas* spp., respectively. BLAST results of the sequences obtained in this study showed an identity query coverage length of 1533, 1532 and

Table 3. 16S rRNA sequence of the plastic degrading bacterial isolates.

Isolate code	Sequence blast	Ascension no.	Sequence identity	Query coverage length	Score bits (%)
B1	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTT GCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAA CCGGGGCTAATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATG GACCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTG ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACG AAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAG TACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT AATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGA AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCAC GTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG AGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTT AGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAA CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTG TCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAG TTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG ACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAAATC TGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCC GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAG GTAACCTTTTAGGAGCCAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGA AGGTGCGGCTGGATCACCTCCTT	EU047884.1	*Bacillus subtilis* strain BS3902	1533	1539/1542 (99)
B2	GGCTACACATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAG GAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGG GGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCG ACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG CAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTA AAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGC TAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTA GGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTAC GGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGA CCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC CGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGG GGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTC GAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACT CAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCC TTGTCCTTAGTTACCAGCACCTCGGGTGCGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT GACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCC GCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAAT CGCTAGTAATCGTCAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGG AGTGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCAAGGGG	GQ865644.1	*Pseudomonas aeruginosa* strain KAVKOI	2595	1405/1405 (100)

Table 3. Contd.

B3	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGATGGGAGCT TGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGA AACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGA TGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGG TGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGA CGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACA AGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATG TGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTACAGAAGAGGAGAGTGGAATTC CACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGAC GCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGA CTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAA CCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGC ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCA GCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGC CCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCC CACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCA GCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGT CGGTGAGGTAACCTTTTAGGAGCCAGCCGCCAAAGGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAGCC GTATCGGAAGGTGCGGCTGGATCACCTCCT	KR967375.1	*Bacillus subtilis* strain AER111-2	1532	1538/1541 (99)

Genome DNA of the isolates was extracted using QIAamp DNA Mini kit (250) cat no. 51306 with quagen DNA extraction protocol. Extracted DNA templates were subjected to PCR using set (Forward and Reverse) universal primers 16SF-AGAGTTTGATCMTGGCTCAG and 16SR-AAGGAGGTGWTCCARCCGCA, the primers allowed amplification of the 16Srna genes of the isolates. The base was edited with BioEditR software. The edited sequences were then used for similarity searches using Base Local Aligment Search Tool (BLAST) program in the NCBI GenBank which is a DNA database for identify bacterial strains. B1: *Bacillus subtilis* strain BS3902; B2: *Pseudomonas aeruginosa* strain KAVKOI; B3:.*Bacillus.subtilis.*strain.AER111-2.

2595. It was observed that the isolates from plastic composted soil with codes B1 and B3 belong to *Bacillus,* while B2 belong to *Pseudomonas* genera. Two isolates with accession number EU047884.1 and KR967375.1 had 99% similarity with *Bacillus subtilis* strain BS3902 and *B. subtilis* strain AER111-2, respectively, while the third isolate had 100% similarity with *P. aeruginosa* strain KAVKOI with accession number GQ865644.1. It was observed that these strains were able to degrade polystyrene plastics (Opere et al., 2013).

Polystyrene plastics have been found to be susceptible to microbial attack and hence biodegradation or even biodeterioration of these plastics can occur (Okpokwasili and Okorie, 1991). Researchers have reported that *P. aeruginosa* (Hill, 1978) as the predominant species in petroleum product which is in accordance with this research. This is expected because the genus is commonly found everywhere especially in hydrocarbon polluted area (Fought and Westlake, 1988). The total heterotrophic bacteria differ from those of the hydrocarbon utilizing bacteria when compared. This is due to the ability of the heterotrophic bacteria to withstand stress with time and have resided in the water phase where little nutrient is available. Though there were appropriate bacterial population in the samples, plastic degradation is near impossible if necessary nutrients were not available.

Conclusion

The results of the research have shown evidence of polystyrene plastic degradation which is in accordance with previous researches. Time series degradation processes by indigenous microorganisms from the soil have shown to be relatively efficient in the breaking down of plastics products as evidently indicated by the physicochemical analysis.

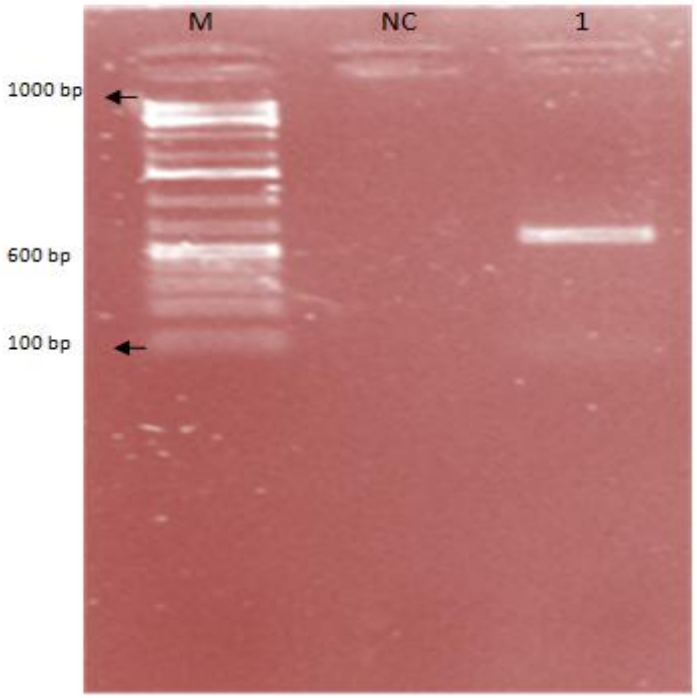

Plate 1. Polymerase chain reaction results for bacterial isolate analyzed with 1.5% agarose gel electrophoresis. M is 100 bp-1 kb DNA ladder (molecular marker). Lane 1 is positive for *Bacillus subtilis* with band at 600 bp. NC is a no DNA template control.

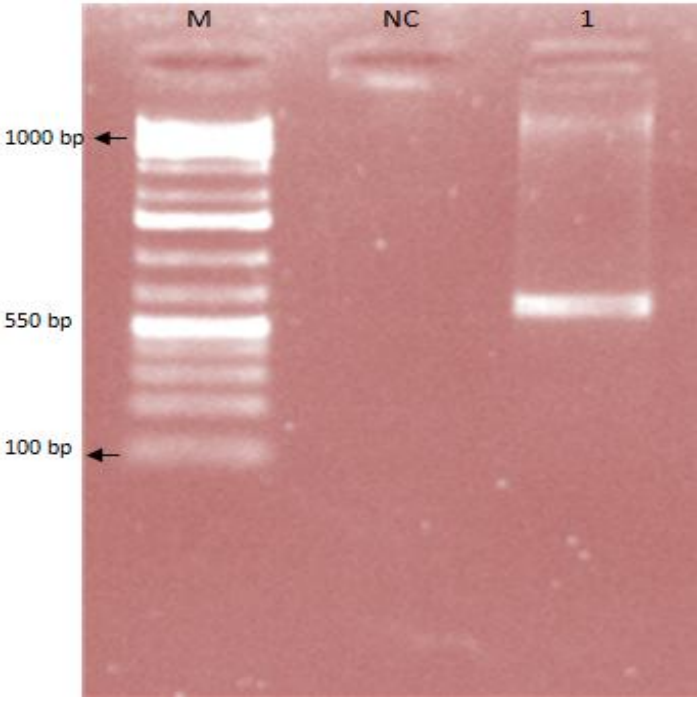

Plate 2. Polymerase chain reaction results for bacterial isolates analyzed with 1.5% agarose gel electrophoresis. M is 100bp-1kb DNA ladder (molecular marker). Lane 1 is positive for *Pseudomonas aeruginosa* with band at 550bp. NC is a no DNA template control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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