Pseudomas Aeruginosa Isolated from Vermicompost in the Degradation of Varying Concentration of Polyvinyl Chloride (PVC) and Polyvinyl Alcohol (PVA)

Uwanta L.I.[1*], Orji. M.U [1], Agu K.C [1], Udenweze E.C1, Umeoduagu N. D[2], Egurefa, S. O[2], Awari, V. G[2]

[1]Department of Applied Microbiology, Nnamdi Azikiwe University, PMB 5025, Awka
[2]Department of Microbiology, Tansian University, Umunya, Nigeria.
Email: kc.agu@unizik.edu.ng

ABSTRACT

This study investigated the degradation of polyvinyl chloride (PVC) and polyvinyl alcohol (PVA) by the bacterial species *Pseudomonas aeruginosa* isolated from a 60-day vermicompost, in a range of concentrations. The objectives of this study were to determine the optimal concentrations of PVC and PVA for *Pseudomonas aeruginosa* to biodegrade, and to determine the most efficient degradation process for these polymers using microbiological and spectrophotometric analysis. The experiments were conducted by incubating the bacterial species in a Mineral Salt Vitamins Medium (MSVM) containing varying concentrations (100mg/L, 300mg/L and 500mg/L) of PVC and PVA. After every 10 days interval for 40-day period, the samples were harvested and evaluated for biodegradation using mass spectroscopy at 680nm wavelength. The results were compared between the different concentrations to determine the optimal concentration and degradation time. The results of the experiments indicated that *Pseudomonas aeruginosa* was able to degrade both PVC and PVA with varying efficiencies at different concentrations. The highest degradation efficiency was observed at a concentration of 0.3% PVC and 0.5% PVA. At this concentration, the maximum degradation was achieved at 40 days period.

Key word: *Pseudomonas aeruginosa*, Polyvinyl Alcohol, Polyvinyl Chloride and Vermicompost

Introduction

Bioremediation is an environmental clean-up technique involving the use of naturally occurring microorganisms in the decontamination process. While biodegradation connotes a process of converting organic waste into nutrients through the biological action of live microorganisms (Orji et al., 2014; Ifediegwu et al., 2015; Agu et al., 2017; Ojiagu et al., 2018).

Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer that is commercially prepared by the hydrolysis of vinyl acetate units followed by removal of an acetate group through a hydroxyl group (Hamad *et al*., 2016). PVA has been widely used in paper coatings, adhesives, textiles, and biodegradable polymers (Larking, *et al.,* 1999) and enters wastewater from different sources, resulting in serious environmental problems for industrial wastewater treatment (Chiellini, *et al.,* 1999).

Polyvinyl Chloride (PVC) is a white, brittle solid with the chemical formula $[C_2H_3Cl]_n$, which contains 56.77% (w/w) chlorine element, and is insoluble in water and alcohol but soluble in tetrahydrofuran (THF). PVC is commercially applied in rigid form and flexible form. The rigid form is used in pipe construction and in profile applications (doors and windows), packing (non-food packaging and food-covering sheets), and cards (bank or membership cards). The flexible form is made by the addition of plasticizers and used in plumbing, imitation leather, and many other applications such as in replacement of rubber and canvas making (PlasticsEurope, 2019; Ceresana, 2014).

The early usage of crude oil was primarily for lighting as it conveniently replaced whale oil which was expensive; nonetheless, today, it is used as fuel and it is at present Nigeria's and indeed, the world's leading energy source (Agu *et al.,* 2014; Orji *et al.,* 2014; Anaukwu *et al.,* 2016; Agu and Odibo, 2021). In Nigeria, oil producing areas have witnessed more than 5 decades of crude oil exploration and production (Kadafa, 2012; Anaukwu *et al.,* 2016; Agu and Odibo, 2021). The Niger Delta is among the top 10 most important wetlands and marine ecosystems on earth, whose ecosystem has been brutally devastated by petroleum contamination due largely to poor petroleum exploration (Mbachu*et al.,* 2014). the Niger Delta have borne the brunt of **oil spill**age on both the aquatic and terrestrial environments for more

Vermicomposting is a biological method for the conversion of organic wastes into compost by earthworms (COMMUNITIES COTE 2006). This industry originates from Canada, but currently, vermicompost facilities are in operation in several countries such as Canada, the USA, Italy, and Japan (in both

home and industrial areas) (Ghosh 2004). Earthworms, which are the important factor in mixing materials in compost stacks, play a significant role in the collection of nutrients in their bodies, while they can eat equal to the amount of their weight each day (Chaoui *et al*. 2003). *Eisenia fetida* is the most common type of earthworms for vermicomposting due to their considerable ability to change the physical, chemical, and biological features in natural materials (Garg *et al*. 2006).

The aim of this study is to estimate the degradation potential of *P. putida* isolated from a 60-day vermicompost on varying concentration of the synthetic plastic (polyvinyl chloride and polyvinyl alcohol) over a 40-day through mineral salt vitamins medium optimization procedures.

Methodology

Sampling sites

This study will be conducted on sludges from Ezenwa Plastic Industry Limited plant located in Awada-Obosi in Onitsha South Local Government Area, Anambra State, Nigeria. It is located between 6°07'31.6"N and 6°48'29.8"N of the equator. Ezenwa is a Polyvinyl Chloride processing plant that manufactures industrials and household plastic wares.

Production of Vermicompost (60 Days)

Two parallel vermicomposting reactors will be set-up, and 2 kg sludge obtained from sampling site without synthetic plastics will be put into each reactor. 200g of vegetable waste (egg shells and kitchen waste), 20g of cow-dung, 20g of synthetic plastics will be added into two vermicomposting reactors of PVA and PVC, respectively. The two reactors will be named PVAX and PVCX. Finally, 50 adult earthworms weighing about 0.6g will be randomly selected and inoculated into the vermicomposting reactor. All reactors covered with dark shading cloth to prevent earthworms from escaping and allowing maximum nutrient conversion by the earthworms. The experiment will be conducted at room temperature (22°C - 34°C), and distilled water sprayed three times a week to keep the humidity around 65%. To make the vermicomposting reaction under the condition of sufficient oxygen, every week, the reactors will be turned over under the environment to allow proper mixture and percolation.

Preliminary Screening of PVC and PVA degrading Bacteria from Vermicompost:

Two gram (2g) each from of the homogenized vermicompost samples PVCX and PVAX will be weighed out aseptically and introduced into 100ml of sterile Enriched Mineral Salt Vitamin Broth medium containing 5g/L of PVC and PVA powder respectively for the screening of PVC and PVA degrading bacteria. The set-up will be placed on a rotary shaker at a revolution speed of 200rpm for 10 days.

Preliminary Isolation of *Pseudomonas aeruginosa* from Vermicompost:

One gram (1g) of the vermicompost samples were weighed out aseptically and introduced into 10ml of sterile peptone water for *P. aeruginosa*, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using peptone water as the diluents. 0.1ml of appropriate dilutions (10^{-2}) of the sample were pour plated in sterile plates of Nutrient, Cetrimide Agar. The culture plates were incubated at 37°C aerobically for 24-48hours for bacteria. Developing green colonies on Nutrient agar were counted to obtain total *Pseudomonas aeruginosa* count. Discrete colonies for the bacteria were obtained by sub culturing into Nutrient agar plates and were subsequently identified using standard methods**.**

TpaC = $\dfrac{(\text{N})}{\text{VD}}$

Where TpaC: Total *Pseudomonas aeruginosa* Count   on Centrimide Agar

V: Volume plated

D: Dilution Factor

MICROBIOLOGICAL ANALYSES OF SAMPLE

Total PVA and PVC Degraders Counts:

A 10-fold serial dilution of each of the samples (vermicompost) in the rotary shaker will be carried out using peptone water as the diluents. 0.1ml of appropriate dilutions (10^{-2}) of the samples will be pour plated in sterile plates of enriched mineral salt vitamins medium (MSVM) plates for the culture of PVA and PVC degrading bacteria. The culture plates will be incubated at 37°C aerobically for 5days in duplicates. Developing colonies on MSVM agar were counted to obtain total PVA and PVC degrading bacteria count. Discrete colonies of the bacteria will be obtained by sub culturing into Nutrient agar plates and will be subsequently identified using biochemical and molecular identification methods**.**

Total PVA and PVC degrading bacteria Count on MSVM is estimated by

TPDBC = $\dfrac{(\text{N})}{\text{VD}}$

Where TPDBC: Total Plastic Degrading Bacterial Count

V: Volume plated

D: Dilution Factor

N**:** Total count on MSVM plate

Total Heterotrophic Bacteria (THB)

THB count will be determined by pour plate method. Serial dilution will be carried out on homogenized (vermicompost) sample collected from each plot and 0.1ml of the aliquot from each of the dilution will be inoculated by pour plate method onto Nutrient Agar plates in duplicates. The plates will be incubated at 37^0C. THB counts will be determined after 24hrs of incubation.

Total Heterotrophic Bacterial Count on Nutrient Agar is estimated by

TBC = (N)

VD

Where THB: Total Heterotrophic Bacterial Count

V: Volume plated

D: Dilution Factor

N: Total bacteria count on nutrient agar plate

PVA/PVC Mineral Salt Vitamins Medium Agar composition (gL^{-1})

PVA/PVC, 5.0 g; $(NH_4)2SO_4$, 1.0 g; KH_2PO4, 1.0 g; K_2HPO_4, 8.0 g; $MgSO_4.7H_2O$, 0.2 g; NaCl, 0.1 g; $CaCl_2.2H_2O$, 0.02 g; $FeSO_4$, 0.01 g; $Na_2MoO_4.2H_2O$, 0.5 mg; MnSO4, 0.5 mg; Inositol, 0.2 mg; p-amino benzoic acid, 0.2 mg; pyridoxine, 0.4 mg; thiamine, 2.0 µg; biotine, 2.0 µg; vitamin B, 120.5 µg; DW, 1000 ml; pH 7. The medium used will be the same one that was used previously by Suzuki *et al.* (1973). A solid medium was prepared by adding 20 g of agar agar powder to 1000 ml of the MSV medium before autoclaving.

PVA/PVC Mineral Salt Vitamins Medium Broth composition (gL^{-1})

PVA/PVC, 5.0 g; $(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 1.0 g; K_2HPO_4, 8.0 g; $MgSO_4.2H_2O$, 0.2 g; NaCl, 0.1 g; $CaCl_2.2H_2O$, 0.02 g; $FeSO_4$, 0.01 g; $Na_2MoO_4.2H_2O$, 0.5 mg; $MnSO_4$, 0.5 mg; Inositol, 0.2 mg; p-amino benzoic acid, 0.2 mg; pyridoxine, 0.4 mg; thiamine, 2.0 µg; biotine, 2.0 µg; vitamin B, 120.5 µg; DW, 1000 ml; pH 7. The medium used will be the same one that was used previously by Suzuki *et al.* (1973).

Determination of Synthetic Plastic (POLYVINYL CHLORIDE & POLYVINYL ALCOHOL) degradation by spectrophotometric assay

The process will be a modification of Patil and Bagde (2014).The kinetics of PVA and PVC degradation by the selected isolated strain of bacteria was studied by determining the residual concentration of PVA and PVC left in the growth media at 10 days interval of time using spectrophotometric assay. The test will be performed in a 100 ml Erlenmeyer flask containing 50 ml of MSVM-PVA media containing varying concentration of the synthetic plastic (1%, 3% and 5%) of PVA and PVC. 0.5ml of 0.5 McFarland standard of microbial cell suspension (approximately 1×10^8 cells/ml) will be used as an inoculum for the degradation assay. The test flasks will be incubated at room temperature in a shaker incubator at 180 rpm along with control flasks. The control will be maintained, MSVM-PVA and MSVM-PVC medium without test cultures. At each test interval of 10days, the cell growth will be estimated by reading the optical density at 680 nm. Simultaneously, 10 ml of the sample will be taken from each culture flask for analysis of residual PVA concentration. Controls will be treated in the same way. After appropriate dilution, residual PVA concentration in the culture filtrate will be estimated according to Finley (1961) using spectrophotometric assay. All the measurements will be made in triplicate.

Characterization and Identification of Bacteria Isolates

Identification of the bacterial isolates will be accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Chessbrough, 1984). The characterization of the isolates will be performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, Methyl Red and Voges proskauer test as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (1994).

Gram Reaction

Thin smear of the isolate will be made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear will be flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet will be washed off with gentle running water. Again, the slide will be flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide will be counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope ($\times$ 100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms.

Catalase test

Exactly 3ml of 3% solution of hydrogen peroxide (H_2O_2) will be transferred into a sterile test tube. Then, 3 loopful of a 24 hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

Motility test (Hanging Drop Method)

A loopful of 18-24 hour broth culture of the test bacteria will be placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip will be inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement will be observed for motility at X100 magnification on a compound microscope. Care will be taken to not interpret "drift" or "Brownian motion" as motility. Results will be recorded as motile or non-motile.

Oxidase Test

All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

Whatmann No.1 filter paper will be soaked with the substrate tetramethyl-p-phenylenediaminedihydrochloride. The filter paper will be moistened with sterile distilled water. Then the test colony will be picked with wooden or platinum loop and smeared in the filter paper. The inoculum will be observe the area around the inoculated paper for a color change to deep blue or purple within 10-30 seconds. Positive and negative quality controls will also be set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*).**Positive** was indicated by development of dark purple color (indophenols) within 10 seconds. **Negative**: Absence of color.

Urease Test.

Heavy inoculum from an 18- to 24-hour pure culture will be used to streak the entire **Christensen's Urea Agar** slant surface. Adequate care will be taken not to stab the butt as it will serve as a colour control. The tubes will be incubated loosened caps at 35℃. The slants were observed for a colour change at 6 hours, 24 hours, and every day for up to 6 days. Urease production will be indicated by a bright pink (fuchsia) colour on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea will also be set up. Rapidly urease-positive *Proteeae* (*Proteus spp.*, *Morganella morganii,* and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish colour if the organism is urease negative

Indole Test

A loopful of an 18-24 hour culture will be used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation will be done at 35–37℃ first for 24 hours and further for up to 48 hours. Test for indole will be done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

Methyl Red test

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl Red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

Voges-Prausker test

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR-VP broth will be asceptically transferred into a sterile test tube. Then 0.6ml of 5% α-naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that this reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test will be indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

Citrate test

A 24h old culture will be inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test will be indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

Sugar Fermentation Test

Each of the isolate will be tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes will be incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

RESULTS

Table 1: Morphological Identifications of the Bacterial Isolates.

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity
W	Irregular	Glistening	Cream	irregular	Flat	Opaque

Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Oxi	Ure	Identity
-Rod	+	+	-	-	-	+	-	-	-	-	-	+	-	*Pseudomonas aeruginosa*

Table 2: Biochemical Identifications of the Bacterial Isolates.

Table 3: Biodegradation pattern of Polyvinyl Alcohol (PVA)

	Day 0			Day 10			Day 20			Day 30			Day 40		
	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L
P. aeruginosa	0.165	0.187	0.210	0.145	0.164	0.201	0.128	0.149	0.191	0.111	0.140	0.184	0.089	0.127	0.159

Table 4: Biodegradation pattern of Polyvinyl chloride (PVC)

	Day 0			Day 10			Day 20			Day 30			Day 40		
	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L	100 mg/L	300 mg/L	500 mg/L
P. aeruginosa	0.205	0.298	0.380	0.194	0.287	0.374	0.189	0.275	0.371	0.181	0.268	0.363	0.174	0.261	0.360

Table 5: Table showing Total Heterotrophic Bacteria Count (THB), Total *Pseudomonas Putida* Count on Centrimide Agar Base (TpdC), Total PVA Degraders Counts (TpvaDC) and Total PVC Degraders Counts (TpvcDC)

	THB (10^{-4})	TpaC (10^{-4})	TpvaDBC (10^{-4})	TpvcDBC(10^{-4})
PVAX	247	TFTC	48	35
PVCX	201	TFTC	32	TFTC

Where;

TFTC: Too Few To Count

TpvaDBC: Total polyvinyl alcohol degrading bacteria count

THB: Total heterotrophic bacteria control

TpvcDC: **Total polyvinyl chloride degrading bacteria count**

TpaC: Total *Pseudomonas aeruginosa* Count   on Centrimide Agar Base

DISCUSSION

The present study showed that several *Pseudomonas spp* isolates present a in 60-day vermiculture   produced from sludge gotten from a plastic manufacturing site possess the capacity for synthetic plastic (polyvinyl alcohol and polyvinyl chloride) degradation. Some researchers have reported polyvinyl alcohol and polyvinyl chloride degradation by various *Pseudomonas spp* and posited that they have the capacity to not just degrade plastics but also play a key role in xenobiotics and reclaiming many habitats polluted with microplastics and nanoplastics.

Evaluation of PVA and PVC degradation capacity of the screened isolates showed that *Pseudomonas aeruginosa* were very potent degraders of Polyvinyl alcohol (PVA) as compared to the polyvinyl chloride (PVC) counterpart based off on the preliminary screening for degradation. This research finding

corresponds with that of El-Naas *et al.* (2008) which focused on batch experiments carried out to evaluate the biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel pellets in a bubble column bioreactor at different conditions. The bacteria were activated and gradually acclimatized to high concentrations of phenol of up to 300 mg/l. The experimental results indicated that the biodegradation capabilities of *P. putida* are highly affected by temperature, pH, initial phenol concentration and the abundance of the biomass. The biodegradation rate is optimized at 30 ∘C, a pH of 7 and phenol concentration of 75 mg/l. Higher phenol concentrations inhibited the biomass and reduced the biodegradation rate. At high phenol concentration, the PVA particle size was found to have negligible effect on the biodegradation rate.

The results also agrees with the research of Giacomucci *et al.* (2019) These results confirmed the effectiveness of a PVC biodegradation process by *Pseudomonas citronellolis* even using PVC wastes (sterilized and unsterilized films) showed higher weight losses compared to sterilized films and *Pseudomonas citronellolis* was not outcompeted by microbial contaminants. Specifically, waste PVC films showed a gravimetric weight loss of up to $18.58 \pm 0.01\%$ compared to virgin PVC films, which exhibited up to $13.90 \pm 6.84\%$.

CONCLUSION

The accumulation of high amounts of petroleum-derived plastics in the environment has raised ecological and health concerns on a global scale. The need for environmentally friendly approach in reclaiming both terrestrial and water bodies polluted with synthetic plastics through biodegradation processes using novel bacterial isolates. The study examines degradation of PVA and PVC using *Pseudomonas aeruginosa* strain isolated from a 60-day vermicompost and from observation PVA appeared to be more biodegradable as compared to PVC at both lower (100mg/L) and higher (500mg/L) concentration owing to the alcohol group attached to its molecule making it prone to microbial degradation.

REFERENCES

Agu, K.C., Nmecha, C.O., Nwaiwu, M.O., Ikedinma, J.C., Awah, N.S., Eneite, H.C., Victor- Aduloju A.T., Umeoduagu N., Onwuatuegwu, J.T.C. (2017). Isolation and Characterization of Halotolerant Bacteria from Ezzu River Amansea, Awka, Anambra State. *Bioengineering and Bioscience*, 5 (4): 86-90. DOI: 10.13189/bb.2017.050303

Agu, K.C. and Odibo, F.J.C. (2021). Biodegradation Potentials of *Aspergillus flavipes* Isolated from Uburu and Okposi Salt Lakes. International Journal of Trend in Scientific Research and Development, 5 (5): 1160-1170. URL: www.ijtsrd.com/papers/ijtsrd44949.pdf

Agu, K.C., Bassey, E.E., Iloanusi, C.A., Awah, N.S., Okeke, B.C., Anaukwu, C.G., Ezenwa, C.U., Orji, M.U. and Okafor, A.C. (2015). Isolation and Characterization of Microorganisms from Oil Polluted Soil in *Kwata*, Awka South, Nigeria. *American Journal of Current Microbiology*, **3**: 46-59. www.ijtsrd.com/papers/ijtsrd44949.pdf

Agu, K.C., Orji, M.U., Ikele, M.O., Uwanta, L.I. and Onyeneho, V.I. (2022). Hydrocarbon Biodegradation Potential of Cyanobacteria in Oil Polluted Soil. International Journal of Trend in Scientific Research and Development, **6** (7): 733-737. URL: www.ijtsrd.com/papers/ijtsrd52397.pdf

Agu, K.C., Orji, M.U., Onuorah, S.C., Egurefa, S.O., Anaukwu, C.G., Okafor, U.C., Awah, N.S., Okafor, O.I., Mbachu, A.E. and Anyaegbunam, B.C. (2014). Influence of Solid Waste Dumps Leachate on Bacteriological and Heavy Metals Contamination of Ground Water in Awka. *American Journal of Life Science Researches*, **2** (4): 450-457.

Anaukwu, C.G*., Ezemba, C.C., Anakwenze, V.N., Agu, K.C., Okeke, B.C., Awah N.S., Ekwealor, I.A. (2016). Effect of biosurfactant produced by Citrobacter murliniae AF025369 and a synthetic surfactant on degradation of crude oil. *Edorium Journal of Microbiology,* **2**: 1–6

Anaukwu, C.G.*, Ezemba, C.C., Anakwenze, V.N., Agu, K.C., Amechi, S.N., Okeke, B.C., Awah, N.S. (2016). Influence of Anionic, Cationic And Non-Ionic Surfactants on Growth of Hydrocarbon Utilizing Bacteria. *American Journal of Current Microbiology*, **4**:10-16.

Ceresana Research Institute (2014). Global demand for PVC to rise by about 3.2%/year to 2021. *Polymer*. 11: 10–11.

Chaoui H. I, Zibilske L. M., and Ohno, T. (2003). Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. *Soil Biology and Biochemistry*. 35:295–302.

Chiellini, E., Corti, A., D'Antone S, Solora, R. (1999). Biodegradation of PVA based formulations. *Macromolecular Symposia*. 144:127–139.

COMMUNITIES COTE (2006) Commission of the European on implementation of the community waste legislation

El-Naas, M. H., Al-Muhtaseb, S. A. Makhlouf, S. (2009). Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *Journal of Hazardous Materials*. 164(2–3):720-72

Garg. P, Gupta A., and Satya, S. (2006) Vermicomposting of different types of waste using *Eisenia foetida*: A comparative study. *Bioresources Technology*. **97**:391–395.

Giacomucci, L., Raddadi, N., Soccio, M, Lotti, N and Fava, F. (2019). Polyvinyl chloride biodegradation by *Pseudomonas citronellolis* and *Bacillus flexus*. *New Biotechnology*. 52:35-41.

Hamad, D., Dhib, R., and Mehrvar M. (2016). Effects of hydrogen peroxide feeding strategies on the photochemical degradation of polyvinyl alcohol. *Environmental Technology*. **37**: 2731–2742.

Ifediegwu, M.C., Agu, K.C., Awah, N.S., Mbachu, A.E., Okeke, C.B.,Anaukwu, C.G., Uba, P.O., Ngenegbo, U.C., Nwankwo, C.M. (2015). Isolation, Growth and Identification of Chlorpyrifos Degrading Bacteria from Agricultural Soil in Anambra State, Nigeria. *Universal Journal of Microbiology Research* **3** (4): 46-52

Larking, D. M., Crawford, R. J., Christie, G. B. Y., and Lonergan G. T., (1999). Enhanced degradation of polyvinyl alcohol by *Pycnoporus cinnabarinus* after pretreatment with Fenton's reagent. *Applied Environmental Microbiology*. **65**:1798–1800.

Mbachu, A.E., Onochie, C.C., Agu, K.C., Okafor, O.I. and Awah, N.S. (2014). Hydrocarbon Degrading Potentials of Indigenous Bacteria Isolated from Auto-Mechanic Workshops at *Mgbuka*-Nkpor, Nigeria, *Journal of Global Biosciences,* **3** (1): 321-326

Ojiagu, K.D., Odibo, F.J.C, Ojiagu, N.C., Agu, K.C., Okafor, A.C. (2018). Biosorption of Hexavalent Chromium by *Pseudomonas aeruginosa* Strain ANSC: Equilibria Isothermic, Kinetic and Thermodynamic Studies. *Bioengineering and Bioscience*, **6**(1): 1-10. DOI: 10.13189/bb.2018.060101

Orji, M.U., Ezenekwe, F.N., Odibo, F.J.C., Agu, K.C**.** and Mbachu, A.E. (2014). Utilization of Used Global System for Mobile Communication (GSM) Recharge Cards for Cellulase Production by *Penicillium* Species and *Aspergillus* Species. *American Journal of Life Science Researches*, **2** (4): 548-556.

Orji, M.U., Agu, K.C., Ikele, M.O., Uwanta, L.I. and Ugwuoke, G. (2022). Bioremediation of Glyphosate Polluted Soil using Fungal Species" Published in International Journal of Trend in Scientific Research and Development, **6 (**7): 726-732, URL: www.ijtsrd.com/papers/ijtsrd52396.pdf

Patil, R., and Bagde, U. S. (2015). Enrichment and isolation of microbial strains degrading bioplastic polyvinyl alcohol and time course study of their degradation potential. *African Journal of Biotechnology*. **14**(27): 2216-2226

Plastics Europe (2019). Plastics—the facts 2019. An analysis of European plastics production, demand and waste data. https://www.plasticseurope.org/application/fi les/1115/7236/4388/FINAL_web_version_Plastics_the_facts2019_14102019.pdf (accessed May 01, 2020).

Suzuki, T., Ichihara, Y., Yamada, M., Tonomura, K. (1973). Some characteristic of *Pseudomonas* O-3 which utilizes polyvinyl alcohol. *Agricultural and Biological Chemistry* **37**:747-756