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# Isolation and Screening for the Degradative Potential of Beneficial Microbes on Different Solid Waste Substrates Generated at Ihiala, Anambra State, Nigeria

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**Abstract:** Several environmental researchers have widely documented microorganisms' role in restoring the environment's natural state. This study investigated the isolation and screening for the degradative potentials of beneficial microbes on different solid waste substrates generated at Ihiala, Anambra State, Nigeria. Soil and root soybean samples were collected at a garden located at Chukwuemeka Odumegwu Ojukwu University, Uli Campus. Ripe queen pineapple (Ananas comosus) specimens were bought from Nkwo Ogbe Market, Ihiala, Anambra State. The samples were screened for beneficial microorganisms using a composed medium for bacteria and yeast extract agar for yeasts. The ability of the microbial isolates to degrade complex environmental wastes was investigated using starch, fat, protein, lignin, and cellulose-containing media, which also enabled clear zones and enzymatic indexes to be measured using Congo red stain. The results revealed that 13 microorganisms were isolated from the cultured substrates, and 3 out of the 13 isolates exhibited high degradative potential, as seen in the value of the enzymatic index. The ability of the microbial isolates to degrade starch, lipid, protein, lignin, and cellulose in the composed media showed that the isolates synthesized amylase, lipase, proteinase, ligninase, and cellulase, respectively. This study has revealed that degradative microorganisms are found in the environment, and the ability of the isolated microorganisms to break down complex organic compounds into simpler ones shows that they could break down the components of solid wastes, mainly carbohydrates, protein, cellulose, lignin, and lipids.

**Keywords**: Isolation, Screening, Degradation, Microbes, Solid Waste.



## 1.0 Introduction

Solid wastes are debris generated within the environment due to human activities (AbMuttalib et al., 2016). These wastes could emanate from household items such as remains of food, pieces of paper, remains of plants and animals, etc. The generation of solid wastes in the environment cannot be prevented, though research has shown that it can be minimized (Abu-Zahra et al., 2014). The presence of solid wastes in the environment is a source of environmental deterioration, especially when indiscriminate dumping is practiced. Air pollution emerges when the solid wastes dumped openly decay, posing a threat to the health of mankind (Ali et al., 2013; Ajmal et al., 2020a).

In the quest to curtail the havoc created by indiscriminate solid waste disposal, humans have devised means of treating wastes such as burning, which is not eco-friendly, and continuous burning of solid wastes is capable of causing devastating effects on the ecosystem, as documented by Anukam et al. (2020a). Globally, solid waste management has been prioritized by both individuals and governmental organizations due to the adverse effect of its negligence. One of the targets of environmental researchers is to develop a method of solid waste management that is eco-friendly and affordable (Abu-Zahra et al., 2014; AbMuttalib et al., 2016).

Industrial growth, urbanization, and man-made problems stemming from population growth are major drivers of environmental pollution, a critical hazard in both developing and developed nations (Ikele *et al.*, 2022; Umeoduagu *et al.*, 2022). Research has revealed that microorganisms degrade solid wastes naturally in the environment (Awasthi et al., 2015; Ajmal et al., 2020a). These microorganisms are mostly found in the habitats where solid wastes are dumped. Research has also shown that microbes can synthesize degradative enzymes such as amylase, cellulases, etc., which enable them to break down organic matter in the waste to generate carbon and energy necessary for metabolism. Some of these microorganisms have been identified as Gram-negative bacteria such as Pseudomonas species, Klebsiella species, Escherichia coli, and Gram-positive bacteria such as Bacillus species, Micrococcus species, etc. (Ajmal et al., 2020a). The recent surge of interest in organismal peroxidases stems from their compelling catalytic advantages, such as their high biocompatibility, biodegradability, biodigestibility, simple preparation, and stability under a wide array of physicochemical

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conditions (Oparaji et al., 2024). Inappropriate dumping of solid waste has significantly contributed to water scarcity worldwide (Oriyomi *et al.*, 2022; Oriyomi *et al.*, 2023).

Environmental cleanup through bioremediation relies on the action of naturally occurring microorganisms for decontamination. Biodegradation, as defined by (Orji et al., 2014; Ifediegwu et al., 2015; Agu et al., 2017; Ojiagu et al., 2018), is the biological process by which living microorganisms convert organic waste into nutrients. Several researchers have worked on microorganisms that are capable of degrading solid wastes, such as Ali et al. (2013), Abu-Zahra et al. (2014), AbMuttalib et al. (2016), and Ajmal et al. (2020a), but few studies are available on the isolation and screening for microbes that have degradative potential on different solid wastes generated at Ihiala, Anambra State, Nigeria. Hence, this study aims to isolate and screen for microbes that have degradative potential on different solid wastes generated at Ihiala, Anambra State, Nigeria. The outcome of this study would contribute immensely to the appropriate waste management strategy globally.

## 2.0 Materials and Methods

**Sample Collection**

Rhizosphere soil samples and roots of soybeans were aseptically collected with a sterile hand trowel and knife from the school garden (coordinates describe using hand-held GPS) within premises of Chukwuemeka Odumegwu Ojukwu, Uli Campus, Ihiala Local Government Area, Anambra State. Ripe queen pineapple (Ananas comosus) specimens were bought from Nkwo Ogbe Market, Ihiala, Anambra State. All the samples were placed into sterile polyethylene bags and transported on ice to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University Uli Campus, Nigeria, for further analysis.

**Isolation of Rhizosphere Bacterial (RB) Species**

According to the method of Ogbo and Okonkwo (2012), the washed roots of soybean plants were cut into 3 cm sections and cleaned by soaking in 70% ethanol for 5 min and in 6.25 % sodium hypochlorite for 10 min, taken after a few flushes in sterile refined water. Intact root pieces (0.5 to 1.0 cm) were at that point put into tubes of semisolid (0.05%) nitrogen-free biotin medium (NFb) and incubated without shaking for 5 days at 30℃. This medium was composed (g/L) of:

DL-malic corrosive 5; KOH 4; K2HPO4 0.5; MgSO4·7H2O 0.2; CaCl2 0.02; NaCl 0.1; FeSO4·7H2O 0.5; Agar 5 g and (mg/L) of: NaMoO4·2H2O 2; MnSO4·H2O 10. The medium, moreover, contained 2 mL of a 0.5% arrangement of bromothymol blue in 95% ethanol and had a pH of 6.8. After incubation, the white pellicles from tubes that showed this characteristic feature were sub-cultured onto a strong semi-Congo red-NFb medium. The appearance of a red to scarlet colony suggestive of the rhizosphere bacterium was selected and filtered by repeated streaking on the same medium.

**Isolation of Phosphate Solubilizing Bacteria (PSB)**

Phosphate-solubilizing microbes were isolated by suspending 10 g of the rhizosphere soil in 100 mL of refined water in a conical flask. High-impact spore formers were pasteurized in a diluted soil test at 80 °C for 10–15 min, and serial dilutions were made from soil suspension. At that point, 100 µL of soil suspension at diverse dilutions was spread onto the surface of synthetic Pikovaskaya (PVK) agar medium (10.0 g glucose, 5.0 g Ca3(PO4)2, 0.5 g NH4) 2SO4, 0.1 g MgSO4.7H2O, 0.02 g NaCl, 0.02 g KCl, 0.003 g FeSO4.7H2O, 0.003 g MnSO4·H2O, 0.5 g yeast extract, 15.0 g agar, and 1000 mL refined water), which was sterilized at 121 °C and 105 kPa for 15 min and incubated at 28 °C. After initial incubation at 48 h, single colonies that delivered halo zones were more than once streaked on PVK agar plates until purified cultures were obtained. Purified cultures were preserved in nutrient agar slant in a Bijou bottle and stored at 4 °C refrigeration temperature (Jahangir et al., 2019).

**Isolation of Yeast**

The fruits were washed with sterile water containing disinfectant, drained, peeled, and cut into little pieces. The juices were aseptically extracted using a hand juice extractor, sifted with a muslin cloth, and the filtrates were collected in a sterile plastic holder as depicted by Umeh et al. (2019). For the confinement of the yeasts, 100 mL of Yeast Extract Dextrose Peptone Broth (40 g of peptone water, 10 g of yeast extract, and 20 g of dextrose sugar (sucrose) in 1 L of refined water) was included in 250 mL conical flask containing 100 g of the 48-h aging pulverized pineapple separately and were hatched for 2–5 days at 30 °C to enhance microbial growth. An aliquot of 0.1 mL of the YEDP broth containing the pineapple juice was inoculated onto Potato Dextrose Agar (PDA) medium (fifty (50) mg/L of tetracycline and 0.05 mg/L of gentamycin were included in the PDA medium to hinder bacterial growth) in duplicates using a glass spreader. The plates were incubated at 30 °C for 72 h. The colonies on the plates were further subcultured and incubated for another 48 h at 30 °C to get pure cultures. The selected yeast isolates were obtained, and the pure cultures of the isolates were put away in 10% glycerol at 4 °C in Bijou bottles (Alabere et al. 2020).

**Purification and Maintenance of Bacteria and Yeast**

Each isolated bacteria (48 h old surface film) single colony was streaked on their selective media; the growth was observed depending on the bacteria type after 24–48 h and yeast isolates after 3–7 days at 28 °C of incubation. The well-separated and uncontaminated colonies on the plates were streaked on agar medium, and plating and picking were repeated at least 4-5 times (Talabani et al. 2019). The cultures were maintained for a short time at a slant medium and for a long time without losing their activity in 20% glycerol and stored at -70 °C.

**Screening for Strains with High Degradative Activity**

According to the methods of Zhao et al. (2017) and Limaye et al. (2017), the prevailing strains were inoculated (100 µL) into starch, fat, protein, lignin, and cellulose-containing media. The starch medium (1000 mL) comprised dissolvable starch 2 %, NaCl 0.5 %, peptone 0.5 %, and agar 2 %; the fat medium (1000 mL) comprised peptone 1 %, NaCl 1 %, CaCl2·7H2O 0.01 %, Tween-80 1 %, and agar 2 %, pH 7.4–7.8; the protein medium (1000 mL) comprised non-fat dried milk 5 % and agar 1.8 %; lignin medium (1000 mL) comprised (NH4)2SO4 0.2 %, K2HPO4 0.1 %, KH2PO4 0.05 %, MgSO4 0.02 %, yeast extract 0.1 %, maltose 0.02 %, asparagine 0.02 %, agar 2.3 % and 0.01 % guaiacol dissolved in ethanol. Sterile trace solution (CuSO4·7H2O − 0.01 %, ZnSO4·7H2O 0.03 %, MnCl2·5H2O 0.02 %, MgSO4·7H2O 0.25 %, CaCl2·2H2O

0.15 %, FeSO4·7H2O 0.03 %, and COCl2·6H2O 0.01%) 0.1 % v/v was added to the lignin medium after autoclaving, and the cellulose medium (1000 mL) comprised K2HPO4 0.1 %, carboxyl methyl cellulose 0.5 %, MgSO4 0.05 %, glucose 0.1 %, yeast extract 0.05 %, NaNO3 0.1 %, KCl 0.1 %, and agar-agar 2.2 %. All the plates were incubated for 5 days at room temperature. The presence of "clear zones" encompassing the colonies was taken to demonstrate degradative activity. Additionally, iodine was added to the starch medium and an unbiased red color to the fat medium to increase the color differentiation. The lignin medium was secured with a 0.5% methyl orange color arrangement, whereas the cellulose agar surface was secured with a 0.1% Congo red solution for 15 minutes. Excess Congo red was poured off, and the plate was overlaid with 1 M NaCl for 1 h. The destructive movement was measured in triplicate, and the mean along these lines was calculated. Strains with the most prominent degradative action were chosen to comprise the consortium (successful organisms).

**Biostatistical Analysis**

Data were analyzed utilizing GraphPad Prism Version 8.0.2. Descriptive statistics were performed to summarize the data in mean and standard deviation. The two followed a combined T-test and two-factor analysis of change (ANOVA) taken after Dunnett's different comparison test was embraced in comparing the decay and expulsion efficiencies of the consortium, successful microorganisms, and magnesium oxide nanocomposite concerning their controls at a 95% confidence interval, and P values below 0.05 were considered noteworthy. A direct relapse investigation was embraced to decide the relationship coefficient R2 of natural matter decay models and whether the test information fitted the response to begin with, demonstrated, or not.

## 3.0 Results

The results of colony diameter, clearance zone, and enzymatic action records of the screened separates are presented in Tables 1–5. Among the thirteen (13) isolates that Were screened to be specific RB1, RB2, RB3, PSB1a, PSB1b, PSB1c, PSB1d, PSB2a, PSB2b, PSB2c, Y1, Y2, Y3; Y3 had the most elevated enzymatic activity index of 3.79 units taken after by strains RB1 and PSB1b with the enzymatic activity indices of 2.75 and 2.74 units, individually and in this way chosen for characterization and composting inoculum. The findings revealed that three strains, specifically RB1, PSB2c, and Y3, out of the thirteen useful microbial strains, demonstrated dormant amylase, protease, cellulase, ligninase, and lipase enzymatic catalytic activities on different substrates.

Table 1: Amylase activity of the microbial isolates

| Isolated code | Colony diameter (mm) | Clearance diameter (mm) | Amylase activity index |
|---|---|---|---|
| RB1 | 13.00 ±0.47 | 0 | 1.00 |
| RB2 | 0 | 0 | 0 |
| RB3 | 10. 00 ±0.82 | 0 | 1.00 |
| PSB1a | 0 | 0 | 0 |
| PSB1b | 0 | 0 | 0 |
| PSB1c | 11.00 ±1.41 | 0 | 1 |
| PSB1d | 0 | 0 | 0 |
| PSB2a | 21.30 ±2.90 | 28.30 ±2.50 | 2.33 |
| PSB2b | 0 | 0 | 0 |
| PSB2c | 0 | 0 | 0 |
| Y1 | 0 | 0 | 0 |
| Y2 | 15.70 ±0.47 | 0 | 1 |
| Y3 | 11.00 ±2.16 | 13.00 ±2.16 | 2.18 |

Key: ± = Standard deviation

Table 2: Protease activity of the microbial isolates

| Isolated code | Colony diameter (mm) | Clearance diameter (mm) | Protease activity index |
|---|---|---|---|
| RB1 | 0 | 0 | 0 |
| RB2 | 0 | 0 | 0 |
| RB3 | 0 | 0 | 0 |
| PSB1a | 44.33 ± 3.65 | 49.00 ± 3.00 | 2.11 |
| PSB1b | 0 | 0 | 0 |
| PSB1c | 0 | 0 | 0 |
| PSB1d | 0 | 0 | 0 |
| PSB2a | 25.67 ± 1.53 | 32.67 ± 2.52 | 2.27 |
| PSB2b | 26.67 ± 6.66 | 30.00 ± 6.00 | 2.12 |
| PSB2c | 16.67 ± 5.86 | 26.33 ± 5.69 | 2.58 |
| Y1 | 0 | 0 | 0 |
| Y2 | 0 | 0 | 0 |
| Y3 | 0 | 0 | 0 |

Key: ± = Standard deviation

Table 3: Cellulase activity of the microbial isolates

| Isolated code | Colony diameter (mm) | Clearance diameter (mm) | Cellulase activity index |
|---|---|---|---|
| RB1 | **5.33 ± 1.15** | **9.33 ± 0.58** | **2.75** |
| RB2 | 0 | 0 | 0 |
| RB3 | 10.00 ± 5.29 | 15.67 ± 3.06 | 2.57 |
| PSB1a | 0 | 0 | 0 |
| PSB1b | **9.00 ± 1.00** | **15.70 ± 3.05** | **2.74** |
| PSB1c | 23.60 ± 0.56 | 26.60 ± 1.15 | 2.13 |
| PSB1d | 7.30 ± 0.58 | 9.33 ± 0.58 | 2.28 |
| PSB2a | 7.33 ± 0.58 | 11.33 ± 0.58 | 2.55 |
| PSB2b | 0 | 0 | 0 |
| PSB2c | 0 | 0 | 0 |
| Y1 | 0 | 0 | 0 |
| Y2 | 0 | 0 | 0 |
| Y3 | 0 | 0 | 0 |

Key: ± = Standard deviation

Table 4: Ligninase activity of the microbial isolates

| Isolated code | Colony diameter (mm) | Clearance diameter (mm) | Ligninase activity index |
|---|---|---|---|
| RB1 | 0 | 0 | 0 |
| RB2 | 0 | 0 | 0 |
| RB3 | 0 | 0 | 0 |
| PSB1a | 0 | 0 | 0 |
| PSB1b | 9.67 ± 0.58 | 16.33 ± 0.58 | 2.69 |
| PSB1c | 0 | 0 | 0 |
| PSB1d | 13.67 ± 3.21 | 21.33 ± 1.15 | 2.56 |
| PSB2a | 18.67 ± 0.58 | 21.33 ± 0.58 | 2.14 |
| PSB2b | 0 | 0 | 0 |
| PSB2c | 0 | 0 | 0 |
| Y1 | 0 | 0 | 0 |
| Y2 | 0 | 0 | 0 |
| Y3 | 9.67 ± 2.08 | 27.00 ± 3.79 | 3.79 |

Key: ± = Standard deviation

Table 5: Lipase activity of the microbial isolates

| Isolated code | Colony diameter (mm) | Clearance diameter (mm) | Lipase activity index |
|---|---|---|---|
| RB1 | 20.67 ± 2.89 | 0 | 1 |
| RB2 | 24.67 ± 7.57 | 0 | 1 |
| RB3 | 16.67 ± 2.89 | 0 | 1 |
| PSB1a | 23.00 ± 2.64 | 0 | 1 |
| PSB1b | 17.33 ± 6.65 | 20.33 ± 5.77 | 2.17 |
| PSB1c | 9.00 ± 1.00 | 0 | 1 |
| PSB1d | 15.67 ± 1.15 | 0 | 1 |
| PSB2a | 21.67±6.02 | 0 | 1 |
| PSB2b | 0 | 0 | 0 |
| PSB2c | 19.67±0.58 | 0 | 0 |
| Y1 | 0 | 0 | 0 |
| Y2 | 14.33 ± 4.93 | 15.00 ± 1.00 | 2.05 |
| Y3 | 18.67 ± 3.67 | 30.67 ± 5.13 | 2.64 |

Key: ± = Standard deviation

## 4.0 Discussion

The role of microorganisms in reestablishing the common state of the environment has been broadly reported by a few environmental microbiologists (Ali et al., 2013; Abu-Zahra et al., 2014; Awasthi et al., 2015; AbMuttalib et al., 2016). The results obtained in this consideration uncovered those three strains, specifically RB1, PSB2c, and Y3, out of the thirteen advantageous microbial strains illustrated torpid amylase, protease, cellulose, ligninase, and lipase enzymatic catalytic activities on distinctive substrates and were at long last chosen as viable microorganisms or microbial consortiums for this consideration. Viable organisms (EM) are a blend of groups of life forms that have a resuscitating activity on human beings, creatures, and the characteristic environment and have been depicted as a multi-culture of coexisting anaerobic and oxygen-consuming advantageous microorganisms (Pushpa et al., 2016).

The diverse metabolic capacities of these three microbial strains on carbohydrate, protein, cellulose, lignin, and lipid substrates uncovered that these strains seem to act as great decomposers, viable organisms, and microbially added substances in any composting innovation. By and large, carbohydrates and celluloses are required for cell development, digestion systems, and carbon cycle advancement, and a few things have appeared that proficient protein corruption contributed to nitrogen circulation in compost frameworks and advanced compost aging (Zhao et al., 2017).

In a related study, Limaye et al. (2017) detailed that they chose 8 strains of actinomycetes out of 45 strains since the thermotolerant and alkali-tolerant strains produced chemicals (xylanase, cellulase, and laccase).

## Conclusion

This study showed that degradative microorganisms are found within the environment, degrading carbohydrates, proteins, cellulose, lignin, and lipids. The capacity of the confined microorganisms to break down complex natural compounds to less complex ones was credited to the protein blend, and it appeared that they seemed to break down the components of strong wastes, which are carbohydrates, protein, cellulose, lignin, and lipids. In addition, advance consideration is required to characterize the useful organisms.

## References

1. Ab Muttalib, S.A., Ismail, S.N.S. and Praveena, S.M. (2016). Application of effective microorganism (EM) in food waste composting: A review. *Asia Pacific Environmental and Occupational Health Journal*, **2** (2): 37 – 47.

2. Abu-Zahra, R. T., Ta'any, A. R. and Arabiyyat, A. R. (2014). Changes in compost physical and chemical properties during aerobic decomposition. *International Journal of Current Microbiology and Applied Sciences*, **3** (10):479 – 486.

3. 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

4. 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

5. 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

6. 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

7. 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.

8. Ajmal, M., Aiping, S., Awais, M., Ullah, M.S., Saeed, R., Uddin, S., Ahmad, I., Zhou, B. and Zihao, X. (2020a). Optimization of pilot-scale in-vessel composting process for various agricultural wastes on elevated temperature by using the Taguchi technique and compost quality assessment. *Process Safety and Environmental Protection*, **140**, 34–45.

9. Ali, U., Khalid, A., Mahmood, T. and Aziz, I. (2013). Accelerated biodegradation of solid organic waste through biostimulation. *Proceedings of the Pakistan Academy of Sciences*, **50** (1): 37– 46.

10. 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

11. 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 NonIonic Surfactants on Growth of Hydrocarbon Utilizing Bacteria. *American Journal of Current Microbiology*, *4*:10-16.

12. Anukam, N. B., Alisa, O. C., Ogukwe, N. C., Chinwuba, J. A., Uba, O. B. and Ogukwe, E. C. (2020a). Physico–chemical evaluation of agro–waste formulated compost from five different waste sources. *American Journal of Applied Chemistry*, **8** (6): 130 – 134.

13. Alabere, A., Ogbonna, D. N. and Williams, J.O. (2020). Screening of yeast cells for the production of wine from banana and pineapple substrates. *Journal of Advances in Microbiology*, **20** (7): 38 – 55.

14. Awasthi, M.K., Pandey, A.K., Bundela, P.S. and Khan, J. (2015). Co-composting of organic fraction of municipal solid waste mixed with different bulking waste: Characterization of

physicochemical parameters and microbial enzymatic dynamic. *Bioresource Technology*, **182**: 200 – 207.

15. Ikele, C.B., Ikele, O.M., Obiezue, R.N., Agu, K. (2022). Utilization of natural carbon sources in bioflocculation using plastic bio-reactors: A trial experiment. *Nigerian journal of fisheries*, *19*(1): 2428-2440.
16. Jahangir, G. Z., Arshad, Q.U.A., Shah, A., Younas, A., Naz, S. and Ali, Q. (2019). Bio-fertilizing efficiency of phosphate solubilizing bacteria in natural environment: a trial field study on stress-tolerant potato (*Solanum tuberosum*l.). *Applied Ecology and Environmental Research*, **17** (5): 10845 – 10859.
17. Limaye, L., Patil, R., Ranadive, P. and Kamath, G. (2017). Application of potent actinomycete strains for bio-degradation of domestic agro-waste by composting and treatment of pulp-paper mill effluent. *Advances in Microbiology*, **7**: 94 – 108.
18. Ogbo, F. and Okonkwo, J. (2012). Some characteristics of a plant growth-promoting *Enterobacter* sp. isolated from the roots of maize. *Advances in Microbiology*, **2**: 368 – 374.
19. Oparaji, E. H., Ogana, J., Ngwu, R. O., Agu, K. C. and Ezenwelu, C. O. (2024). Studies on Kinetic Properties of Aspergillus Producing Peroxidase from Petroleum Hydrocarbon Spilled Soil. *Rafidain Journal of Science*, **33** (1): 49-67. https://rsci.mosuljournals.com
20. Oriyomi Opetubo, Ayotunde Idris Ibitoye, Sunday Temitope Oyinbo, Tien-Chien Jen (2022). Analysis of Hydrogen embrittlement in Palladium-copper alloys membrane from first principal method using density functional theory. Vacuum, 205: 111439.
21. Oriyomi Rasak Opetubo, Ricin Kitalu, Peter Ozaveshe Oviroh, Sunday Temitope Oyinbo, Patrick Ehi Imoisili, Tien-Chien Jen. (2023). A mini-review on MoS2 membrane for water desalination: Recent development and changes. *Nanotechnology Reviews*, *12*(1): 20220563.
22. Pushpa, T.B., Sekaran, V., Basha, S.J.S. and Jegan, J. (2016). Investigation on preparation, characterization, and application of effective microorganisms (EM) based composts: An eco-friendly solution. *Nature Environment and Pollution Technology*, **15** (1): 153 – 158.
23. Talabani, S.H.K., Fattah, O. A. and Khider, A. K. (2019). Classical and molecular approaches for identification of *Rhizobium leguminosarium*, *Azotobacter chroococcum*, and *Bacillus megaterium. Applied Ecology and Environmental Research*, **17** (5):12491 – 12506.
24. Umeh, S. O., Okpalla, J. and Okafor, J. N. C. (2019). Novel sources of *Saccharomyces* species as a leavening agent in bread making. *International Journal of Trend in Scientific Research and Development*, **3** (2): 827 – 831.
25. Umeoduagu, N.D., Odibo, F.J.C and Ikele, M.O. (2022). Studies on the use of Cassava Effluent as Substrate for Biomass and Amylase Production from Aspergillus nomius. *Global Journal of Applied, Management and Social Sciences*, *24*:172-182.
26. Zhao, K., Xua, R., Zhanga, Y., Tanga, H., Zhou, C., Caob, A., Zhao, G. And Guoa, H. (2017). Development of a novel compound microbial agent for the degradation of kitchen waste. *Brazilian Journal of Microbiology*, **48**: 442 – 450.