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Evaluating Different Agricultural Wastes as Carriers of Hydrocarbon Degrading Bacterial Inoculants

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ABSTRACT

Hydrocarbon contamination is a pressing environmental issue, necessitating sustainable and effective remediation approaches. This study focuses on evaluating three common agricultural wastes using cassava peels, sugarcane bagasse, and rice husk as potential carriers for hydrocarbon degrading bacteria, MS6 (*Klebsiella pneumoniae*), MS17 (*Lactobacillus sp*) and MS19 (*Staphylococcus aureus*) isolated from mangrove sediments from Ogu-Bolu, Rivers State. Immobilization was by direct adsorption of the isolates onto the carrier materials and viability was determined by plate count method. Physicochemical analysis revealed pH of 2.6 and Total Petroleum Hydrocarbon (TPH) of 136.20 mg/Kg, exceeding FEPA and WHO permissible limits of pH (5.5-9.5) and TPH (100 mg/Kg). However, other parameters remained within acceptable ranges. Bacterial counts on carriers like cassava peels, sugarcane bagasse, and rice husk exhibited progressive growth from day 0 to day 14. This study showed that cassava peel supported more growth of these bacteria than sugarcane bagasse and rice husk. The result ranges from 8.70,8.76,8.58 for MS19, 8.80, 8.90 and 8.98 for MS17, and 8.77,8.22 and 8.10 for MS6 respectively. These findings underscore the potential of utilizing locally available farm waste for sustainable oil spill cleanup initiatives in mangrove ecosystems.

1.INTRODUCTION

Large volumes of trash are produced annually by agricultural sectors; if these wastes are released into the environment without following the right disposal protocols, they may pollute the environment and have negative health consequences on both humans and animals [1]. Governments, environmentalists, and other interested parties have therefore continuously sought to convert agricultural waste into valuable commodities using affordable, environmentally responsible, and sustainable methods [2]. According to [3] waste has the potential to significantly contribute to both ecological sustainability and energy security when it is used and managed appropriately. Agricultural waste is generally used as animal feed, fertilizer, soil conditioner, bagasse, bran, husks, peels, leaves, seeds, stems, and stalks, among other forms. Unquestionably, agro-industrial food and crop wastes have several properties with interesting prospective uses, such as high porosity, high surface area, and the presence of several chemical groups, including phosphate, thiol, amino, and carboxyl groups [4-5]. A wide range of microorganisms, including bacteria, fungus, actinomycetes, algae, and protozoa, are discovered in agricultural wastes [2]. These bacteria are essential to the environmentally benign and sustainable breakdown of agricultural waste [4]. When applied to land, livestock wastes—a type of agricultural waste—contain dangerous microorganisms such as bacteria, viruses, and protozoa that can pollute the soil and surrounding environment. Furthermore, from agricultural wastes, a study found 22 bacteria, 7 basidiomycete fungus, and 7 filamentous fungi. Some strains of these fungi, including Pleurotus, Trichoderma, Talaromyces, Bacillus, and Chryseobacterium sp, demonstrated strong lignocellulolytic enzyme activity. These microbes may be used to efficiently pretreat agricultural wastes containing lignocellulose so that fermentation can produce high-value natural products [6]. According to [6], studies is being carried out on isolating specific organisms that can break down constituents of crude oil even the more complex hydrocarbons. Utilizing living things to eliminate or neutralize contaminants from a contaminated area is known as bioremediation. "A treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or nontoxic substances" is what the Environmental protection agency [EPA] defines as bioremediation [7]. The result of bioremediation, which is water and carbon dioxide produced from hydrocarbons, is what makes it so beautiful [8], [9]. Because hydrocarbon compounds are so poorly soluble in water, bioremediation of these chemicals is hampered by this [10] [11]. Agricultural waste has multiple uses that can lower production costs and pollution [12]. Microbes are the source of a wide range of organic materials and products, frequently obtained by cultivation on highly valuable agro-industrial leftovers. Organic substances known as microbial biosurfactants are used extensively in environmental protection, including the biodegradation of oil-contaminated industrial effluents and soils, the prevention of oil spills, and soil detoxification [7]. Using agricultural waste as a carrier for bacterial inoculants can aid in sustainable agriculture and enhance soil fertility and crop productivity [13]. Recent research shows that immobilized hydrocarbon-degrading bacteria are more effective in degrading petroleum hydrocarbons compared to free-living cells [8], sparking increased interest in immobilizing microbial cells for bioremediation. This study therefore examines the viability of a hydrocarbon-degrading bacterial consortium immobilized on various carriers to assess their potential for use in bioremediation efforts.

2. MATERIALS AND METHODS

2.1. Sources of Samples

At a geographic coordinate of a fused location source with a latitude of N 4°39'14.5098" and a longitude of E 7°14' 17.1312 [2]," the mangrove soil samples were aseptically collected from the Ogu-Bolu geographic region, which is situated within River State, Nigeria.

2.2 Isolation of Hydrocarbon-Degrading Bacteria using Dilution Technique

The soil samples were dried for three hours at 40°C in an oven. Before being used, the samples were chilled at 4°C after being sieved using a 2 mm mesh sieve to eliminate undesirable debris. Using a sterile pipette, add 1 g of soil samples aseptically into test tubes containing 9 mL of 0.85% of normal saline solution labeled 10^{-1} to 10^{-5} dilutions to perform a ten-fold serial dilution of the samples. 0.1 mL aliquots of the 10^{-3} and 10^{-5} dilutions were aseptically distributed in duplicate on the solidified media using a glass spreader and another sterile pipette. The 10^{-3} and 10^{-5} dilutions were spread out onto recently made Bushnell Haas agar plates with crude oil added as a supplement.

2.3 Collection and Screening of Carriers

Three (3) agricultural waste products were gathered from vendors in Alakahia, River State: rice husk, sugarcane bagasse, and cassava peel. To lessen the surface area vulnerable to microbial attack, the carriers were accurately weighed and shred to a particle size of 40 mesh. As possible carriers of the bacterial inoculants, the carriers were screened. The purpose of this test was to evaluate the carriers' biocompatibility with the bacterial inoculant.

2.4 Decontamination of the Carriers

With a few minor adjustments, the carrier materials were decontaminated using the technique outlined by [10]. To put it briefly, carriers underwent a 1-hr oven sterilization at 140^{o}C. The items were placed in an autoclave and further sterilized for 30 minutes at 121°C (15 psi) after 1 hr. To guarantee adequate mixing, 0.2 mL of sterile crude oil, or 2% (v/w) of the carrier, was added to the carrier. The mixture was then vortexed.

2.5 Immobilization by Adsorption of Bacterial Inoculants on the Carriers

The individual bacterium was initially grown in a Bushnell Haas Broth medium for 48 hours in a shaker incubator set to 150 revolutions per minute to obtain the bacterial consortium inoculant. The inoculum's turbidity was corrected to 1.0 McFarland standard equivalent after 48 hours. The consortium was homogenized by hand swirling before injection. Then, 50 g of the carrier material and 10 mL of the inoculum were added to a 250 mL Pyrex Erlenmeyer’s flask [16]. The carriers that were inoculated were thoroughly mixed and incubated in a shaker incubator that was set to 30^{o}C and 150 rpm. After that, the immobilization procedure was observed for 14 days. After 48 hours of incubation, the initial sample (marking Day 0) for the bacterial count was collected. This was done to give the bacteria time to adhere to the carrier materials. Counts were then conducted at days 0 through 14.

2.6 Enumeration of Total Viable Counts in the Immobilized Carrier

One gram (1 g) of the carrier material was serially diluted and used to estimate the Total culturable bacterial count (TCBC) using Nutrient agar [1]. A volume of 100 µL each of 10-5 and 10-6 dilutions was spread onto freshly prepared agar plates. The plates were incubated at 30^{o}C for 24 h. After incubation, the plates with discrete colonies ranging between 30 and 300 were selected. Total viable cell (TVC) was calculated in cfu/g using the formula in Equation. [1]

$$\text{TVC (cfu/g)} = \frac{\textit{No of colonies} \times \textit{dilution factor}}{\textit{Volume of inoculum}}$$

2.7 Microbial Identification

Bacterial pathogens were identified using Gram staining and biochemical test.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of the Mangrove Soil

The result of physicochemical component of the mangrove soil sample shows that only pH (2.6-2.9) and TPH (< 0.001-136.20 mg/Kg) ranges were above maximum permissible limits (MPL) stipulated by WHO, [2011] and FEPA, [2012] whereas NO_3^-, PO_4^{3-}, Fe, Cu, Pb and Zn were within acceptable ranges stipulated by WHO and FEPA

Table1: *Physicochemical components of the mangrove sediment samples.*

Parameters	0-15cm Depth	15-30cm Depth	≥ 30cm Depth	Range	WHO MPL	FEPA MPL
pH	2.6	2.9	2.7	2.6-2.9	-	5.5 – 9.5
NO_3^- (mg/Kg)	6.40	4.70	4.0	4.0-6.4	50	
PO_4^{3-} (mg/Kg)	8.90	3.86	0.57	0.57-8.90	50,000	
TPH (mg/Kg)	< 0.001	136.20	< 0.001	< 0.001-136.20	100	
Fe (mg/Kg)	109.55	1989.1	1454.5	109.55-1989.10	50,000	5000
Cu (mg/Kg)	< 0.001	11.39	2.55	< 0.001-11.39	100	50
Pb (mg/Kg)	< 0.001	< 0.001	1.77	< 0.001-1.77	10	420
Zn (mg/Kg)	1.45	3.49	3.46	1.45-3.49	300	750

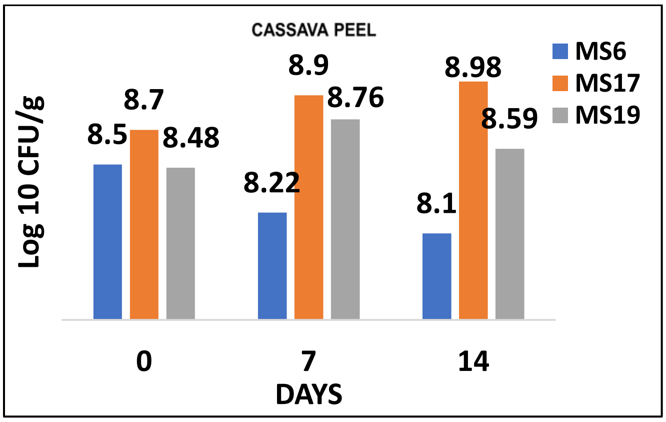

Figure 1: *Growth of the hydrocarbon-degrading bacterial consortium immobilized on cassava peel carrier.*

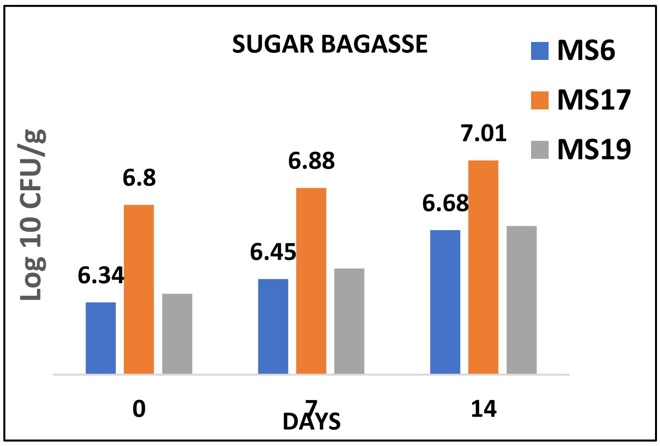

Figure 2: *Growth of the hydrocarbon-degrading bacterial consortium immobilized on sugar bagasse carrier.*

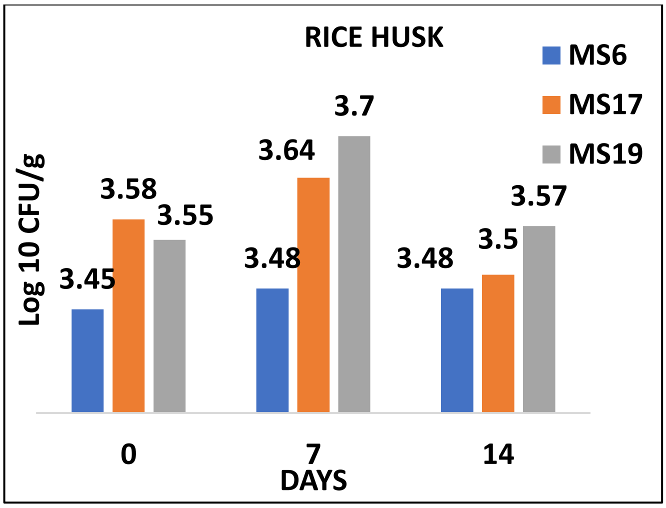

Figure 3: *Growth of the hydrocarbon-degrading bacterial consortium immobilized on Rice husk carrier.*

4. DISCUSSION

The physicochemical components of the mangrove soil samples showed contaminations of heavy metals such as Cu, Fe, Pb and Zn, compounds such as phosphate and nitrate, and mixture of total petroleum hydrocarbons (TPH). This result agrees with reports from studies on mangrove samples in the Niger Delta region of Nigeria [17] [16] [8] [4]. The pH range of the samples were beyond maximum permissible limits (MPL) set by World Health Organization (WHO) and Federal Environmental Protection Agency (FEPA). The high pH might be due to frequent inundation. The range of TPH was also beyond MPL stipulated by WHO, 2011 and FEPA, 2012. This is due to both the legal and illegal oil exploration in the region that is bedeviled with incessant petroleum spills into rivers [18]. The ranges of Cu, Fe, Pb and Zn, phosphate and nitrate were within acceptable ranges. This could be because the source(s) of these contaminants release negligible amounts. This study explored the use of agro-waste materials as carriers for immobilizing the hydrocarbon-utilizing bacteria. The results demonstrated in figures 1-4 above shows that these agro-wastes, specifically cassava peel, sugar cane bagasse, and rice husk, effectively enhanced the viability and activity of the bacteria. Among the tested carriers in the figures 1, 2 and 3, cassava peel exhibited the highest efficacy, followed by sugar cane bagasse and rice husk. The total culturable bacteria counts recorded for the three isolates MS6, MS17 and MS19 showed progressive bacterial growth from day 0 to day 14 on all the carriers (cassava peels, sugarcane bagasse and rice husk). This proved the efficacy of the carriers in supporting the growth of hydrocarbon degrading bacteria. This result agrees with the results from previous studies by [6] and [19] that demonstrated the capacity of the agro-wastes used to effectively retain the viability of hydrocarbon-utilizing bacterial consortium. According to [20], Date molasses was used as a novel substrate and sole source of carbon for biosurfactant production. However, because of their low manufacturing costs, they are more effective in bioremediation processes and increase the amount of bioavailability during the metabolic degradation process. The goal of any bioremediation strategy is to eliminate the pollutant by using readily available and reasonably priced nutrient sources, so obtaining a variety of low-cost agro-industrial substrates is highly prioritized. Findings from this kind of study suggest that readily available solid waste data (SWD) can be a helpful bioremediation tool for clearing environments contaminated with hydrocarbons [21]. To improve petroleum hydrocarbon degradation, low-cost and environmentally friendly solutions are required. [22] noted that compared to physical and chemical methods, the use of microbial technology to remediate petroleum hydrocarbon-contaminated soil and water is an efficient, economical, environmentally friendly, and adaptable method. It has the potential to completely degrade pollutants, have higher safety, and have less interference. According to [23], among microorganisms, bacteria have been identified as the main degraders and the most active substances in the breakdown of petroleum contaminants. Bacteria are the most active microorganisms that survive in extreme environments. The use of bacteria to treat oil pollution has been a promising technology due to its low-cost production, high efficiency, and environmental friendliness [24]. To date, many bacterial species that degrade hydrocarbons have been identified such as, *Bacillus sp., Bacillus pumilus, Pseudomonas aeruginosa and Rhodococcus sp., Pseudomonas sp. and Klebsiella sp* [24]. The oil-degradation efficiency of bacteria is much higher than that of fungi and algae [25]. Mehdi et al. [26] found that *Achromobacter piechaudii* and *Rhodococcus erythropolis* isolated from petroleum reservoir wastewater had a significant degradation rate on hexadecane at 93 % and 84 % respectively [24]. Another research demonstrated that under aerobic conditions, the degradation of n-alkanes by Pseudomonas sp. and Brevibacterium sp. reached 90–95 % [24]. Screened streptomyces strains from plant roots showed significant degradation effects on n-alkanes and PAHs, and the oil removal rate in a liquid medium was as high as 98 % [27]. Hydrocarbon utilization efficiency by

Streptomyces spp. isolated from plants that grew in oil-contaminated soil was high as 98 %. These strains were grown on petroleum as a sole carbon source and it degraded the aromatic, PAHs, and n-alkanes (C6-C30), hydrocarbons at variable levels within 7 days [27]. Similarly, *Sphingomonas sp*. and *Acinetobactersp*. can rapidly degrade oil, and the degradation efficiency of 2 % crude oil reached 70.86 % within 7 days [28]. Bacillus spp., in participation of nitrate and electronic intermediates, not only eliminates the accumulation of nitrite but also improves the degradation efficiency of petroleum hydrocarbons, which is of great significance for the treatment of petroleum-containing sewage [29].According to [30], the bacterial growth and biodegradation efficiency were improved by maize steep liquor (CSL) and solid waste dates (SWD), two naturally occurring, easily accessible, low-cost agro-industrial wastes.. It has a high content of carbs (typically around 50%) along with other beneficial substances like vitamins. One liquid by-product produced by the maize wet milling industry is called corn steep liquor (CSL). It is a valuable source of nitrogen for numerous biotechnological processes due to its richness in vitamins, minerals, amino acids, and proteins [31] [32]. According to [33], these substrates were useful materials that can be employed as culture medium or nutrient enhancers for microbial growth in various industrial fermentation processes because of their high nutritional content, low cost, and availability. In their work, [19] revealed that *P. aeruginosa* strain was able to produce biosurfactants by settling a low-cost culture medium utilizing two agro-industrial by-products, namely corn steep liquor and molasses. This biomolecule, which was identified as a combination of eight distinct rhamnolipid congeners, performed comparably in oil experiments to commercially available chemical surfactants, indicating that it would be a good fit for use in the bioremediation of oil spills.

5. CONCLUSION

The evaluation of cassava peels, rice husk, and sugar bagasse as carriers for hydrocarbon-degrading bacterial inoculants shows substantial potential for bioremediation in hydrocarbon-polluted soils. Cassava peels provide a nutrient-rich environment, rice husk ensures excellent bacterial adherence, and sugar bagasse supports robust bacterial colonization. These agricultural wastes can significantly enhance the efficiency and sustainability of hydrocarbon degradation. Leveraging them as carriers for bacterial inoculants offers a sustainable and eco-friendly approach to managing hydrocarbon pollution, promoting environmental health, and involving local communities in bioremediation projects. Further studies should be carried out to optimize their preparation and validate these findings in real-world conditions.

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Conflict of Interest Declaration

The author(s) declare that for this article, they have no actual, potential, or perceived conflict of interest.

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