# *Original Research Article*

**Viability of hydrocarbon-degrading bacterial consortium immobilized on different carriers**


## Abstract

**Aim:** Viability of hydrocarbon-degrading bacterial consortium immobilized on different carriers was studied. **Methodology:** Hydrocarbon-degrading bacteria were isolated from crude oil contaminated sites in Gio and K-Dera, Rivers State, Nigeria using enrichment method. Proximate analyses were carried out on the best carrier materials. Immobilization was by direct adsorption of the isolates onto the carrier materials and viability was determined by plate count method. The carrier materials tested included soya bran, sugarcane bagasse, corn cob, brown saw dust, white saw dust, cassava peel and red mud (bentonite). **Results:** The bacterial isolates demonstrated varied degradation capacity. The best carrier material was saw dust (103.6% survival) and corn cob (103.6% survival) followed by soya bran (94.4% survival rate) and cassava peel (94.4% survival rate). The saw dust had moisture content, 5.92%; ash content, 7.49%; crude protein, 2.2%; volatile matter, 74.28; and fixed carbon, 12.34%; whereas, the percentage chemical composition observed for soya bran were 10.11, 4.08, 5.22, 42.61, 18.37 and 8.89 for moisture content, ash content, crude fibre, crude protein, crude fat and carbohydrate, respectively. There was significant difference (p=0.05) between viability rate observed with the different carrier materials. **Conclusion:** This study showed that the agro-wastes used in this study can effectively retain the viability of hydrocarbon-utilizing bacterial. The result is significant as it shows the possibility of using these carrier materials for biodegradation of hydrocarbon contaminated media.



**Keywords:** Carrier materials; hydrocarbon-utilizing bacteria; immobilization; viability; saw dust and soya bran.


## 1.0 INTRODUCTION

Immobilization refers to the process of limiting the mobility of microbial cells or their enzymes in order to preserve their viability and and/or catalytic function [1-4]. Not all materials qualify as carrier or support materials for immobilization. A material is considered suitable for immobilization, when the material is partly insoluble in water, cost-effective, readily available, non-toxic to the environment and the material being immobilized, stable and fit for regeneration. Immobilization of microorganisms on suitable carriers is widely acknowledged to be beneficial for the maintenance of long-term viability.

Different immobilization techniques exist and these techniques are employed based on certain pre-determined criteria [5]. Amongst the many criteria to consider in choosing a suitable carrier for use in immobilization is the property of the carrier material. The aim of immobilization is another important factor to consider; for instance, carriers for bioaugmentation should be readily biodegradable. A few of the characteristics of a carrier material that influences the immobilization technique to be employed is its porosity and surface area. These features are especially important in adsorption technique. Kariminiaae-Hamedaani *et al*. [6], Martin *et al*. [7] and Bayat *et*

*al*. [8] noted that carriers used for binding or adsorption on the surface must possess high porosity so that the immobilized material and the carrier will have large enough contact area.

Carrier materials to be used for immobilization may be classified as either organic and inorganic or natural and synthetic. Natural organic carriers possess several functional groups which aid biocatalysts' stabilization. Examples of natural organic carriers frequently employed in immobilization process include bagasse [9], rice [8], corn cob [10], saw dust [11], straw [12], charcoal [13], plant fibres [14], alginate [15], diatomite [16] etc. The features of these materials that have encouraged their use as carriers for immobilization include their hydrophilic nature, biodegradability, cost effectiveness and biocompatibility.

Several studies have reported enhanced petroleum hydrocarbon degradation by immobilized hydrocarbon-degrading bacteria compared to free-living cells [17,18]. This observation has increase the interest in immobilization of microbial cells for bioremediation purposes. This study investigated the viability of hydrocarbon-degrading bacterial consortium immobilized on different carriers. This is important in determining the suitability of these carrier materials for use in bioremediation.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Hydrocarbon-Degrading Bacteria

Soil samples were collected from K-Dere and Gio local government areas in Rivers State, Nigeria and processed by drying in an oven at 40 $^{\circ}$C for 3 h, sieving with a 2 mm mesh sieve to remove unwanted debris and refrigerating at 4 $^{\circ}$C prior to use. Hydrocarbon-degrading bacteria were isolated by enrichment method as described by [19] and [20] with slight modification. In brief, 10 g of the processed soil was dissolved in 250 mL Erlenmeyer's flask, containing 100 mL of sterilized normal saline. The flask was votexed at maximum speed for 2 min and the suspension allowed settling for 5 min. A volume of 5 mL of the supernatant was used to inoculate another separate flask containing 100 mL Bushnell Haas Broth (BHB) (Hi Media, India) (containing in g/L: 0.2 $MgSO_4.7H_20$; 0.02 $CaCl_2.2H_2O$; 1 $KH_2PO_4$; 1 $K_2HPO_4$; 1 $NH_4NO_3$; 0.05 $FeCl_3$; nystatin- 0.1g; and pH 7.0) supplemented with crude oil (1%, v/v) as the sole carbon and energy source. Tween 80 (0.05% v/v) was added to the broth to enhance hydrocarbon degradation. The procedure was repeated for each of the soil samples and the set-up performed in duplicate. The flasks were incubated in a rotary shaker incubator at 150 rpm for 7 days. At the end of 7 days, successive sub-culturing were done by transferring 5 mL of BHB culture into fresh BHB medium supplemented with crude oil (1%, v/v). The sub-culturing ensured isolation of only oil-tolerant and oil-degrading bacteria [21].

After three sub-culturing stages, 1 mL of the broth was pipetted aseptically and serially diluted to make $10^{-1}$ to $10^{-6}$ dilutions. The $10^{-3}$, $10^{-5}$ and $10^{-6}$ dilutions were plated out on freshly prepared Bushnell Haas agar plates supplemented with crude oil (1% v/v). The inoculated plates were incubated for 4 days at 30 $^{\circ}$C. Discrete colonies on the plates were picked and purified by repeated streaking on Bushnell Haas Agar supplemented with crude oil (1%, v/v). The pure isolates were further purified on nutrient agar and stored in Bushnell Haas Agar slants supplemented with 1% (v/v) crude oil.

### 2.2 Screening of Carriers

Seven (7) agro-wastes materials (corn cob, sugarcane bagasse, white saw dust, soya bran, brown saw dust, cassava peel, and white mud (bentonite)) were screened as potential carriers of the bacterial consortium inoculant. This test was carried out to determine the biocompatibility of the carriers with bacterial consortium inoculant. Prior to screening the carrier were grinded and sieved to a 40 Mesh particle size.

### 2.2.1 Decontamination of carriers

Prior to inoculation with the bacterial consortium, carrier materials were decontaminated following slight modification of the method described by [22]. In brief, carriers were first oven-sterilized at 140 °C for 1 h. After 1 h, the materials were transferred into an autoclave and sterilized further at 121 °C (15 psi) for 30 min. A volume of 0.2 mL sterile crude oil (corresponding to 2% (v/w) of the carrier) was added to the carrier and the mixture votexed to ensure proper mixing. To confirm the absence of viable cells in the carriers, 1 g of each of decontaminated carriers was dissolved in 10 mL of normal saline and 0.1 mL spread-plated on fresh nutrient agar plates. The plates were incubated at 30 °C for 48 h and thereafter observed for growth. Absence of growth confirmed successful decontamination.

### 2.2.2 Immobilization (by adsorption) of bacterial consortium on the carriers

The bacterial consortium inoculant was obtained by first growing the individual bacterium in a Bushnell Haas Broth medium for 48 h in a shaker incubator set at 150 rpm [23]. After 48 h, the turbidity of the inoculum was adjusted to 1.0 McFarland standard equivalent. Prior to inoculation, the consortium was homogenised by swirling with hand. Ten millilitres (10 mL) of the inoculum was then dispensed into a 250-mL Erlenmeyer's flask containing 50 g of the carrier material [24]. The inoculated carriers were mixed properly and incubated in a shaker incubator set at 150 rpm and 30 °C. The immobilization process was thereafter monitored for 21 days. The first sample (representing Day 0) for bacterial count was taken after 48 hours of incubation; this was done to allow the bacteria adsorb onto the carrier materials. Subsequent counts were carried out at days 7, 14 and 21.

## 2.3 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 [25]. A volume of 100 µL each of $10^{-3}$, $10^{-5}$, and $10^{-6}$ dilutions was spread onto freshly prepared agar plates. The plates were incubated at 30 °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 Eq. 1.

$$\text{TVC (cfu/g)} = \frac{\text{No of colonies} \times \text{dilution factor}}{\text{Volume of inoculum}} \qquad \text{Eq. 1}$$

## 2.4 Proximate Analysis of Agro-Waste Materials

Chemical characteristics of the agro-waste carrier materials used in the study were determined using standard methods. Crude protein, crude fat, crude fibre, total carbohydrate, moisture content and ash content were determined using the methods described by [26] and [27].

# 3. RESULTS

## 3.1 Hydrocarbon-utilizing bacteria

Eight (8) hydrocarbon-utilizing bacteria were used in the study. The bacterial isolates all grew on Bushnell Haas agar amended with 1% crude oil. The isolates also showed high turbidity when subjected to biodegradability test using turbidometric technique.

## 3.2 Chemical composition of the carrier materials

Table 1 shows the chemical composition of the different carrier materials used in the study. Soya bran had the highest crude protein (42.61±2.1%). Sugarcane bagasse had the highest carbohydrate content.

**Table 1. Chemical composition of carrier materials used in this study**

| Parameters | Saw Dust | Soya Bran | Cassava peel | Corn cob | Bagasse |
|---|---|---|---|---|---|
| Moisture content (%) | 5.92±0.09 | 10.11±0.11 | 8.03±0.89 | 5.11±0.05 | 6.34±0.08 |
| Ash content (%) | 7.49±0.08 | 4.08±0.05 | 9.5±0.7 | 7.2±0.6 | 4.3±0.5 |
| Volatile matter (%) | 74.28±2.02 | ND | ND | ND | ND |
| Fix carbon (%) | 12.34±1.1 | ND | ND | ND | ND |
| Crude fibre | ND | 5.22±0.07 | ND | ND | ND |
| Crude protein (%) | 2.2±0.09 | 42.61±2.1 | 4.8±0.3 | 4.3±0.3 | 3.8±0.1 |
| Crude fat | ND | 18.37±1.01 | 0.9±0.02 | 0.7±0.04 | 0.6±0.04 |
| Carbohydrate | ND | 8.89±0.09 | 69.6±1.2 | 68.0±2.3 | 70.3±1.9 |

**ND: not determined**

### 3.3 Viability of the bacterial consortium on different carrier materials

Figure 1 shows the growth of the bacterial consortium (Log10 cfu/g) on different carrier materials over a period of 21 days. The growth of the consortium with almost all the carrier materials peaked at day 7. Figure 2 shows the survival rate of the bacterial consortium on different carrier materials. From the figures, best carrier materials were saw dust (103.6% survival) and corn cob (103.6% survival) followed by soya bran (94.4% survival rate) and cassava peel (94.4% survival rate).

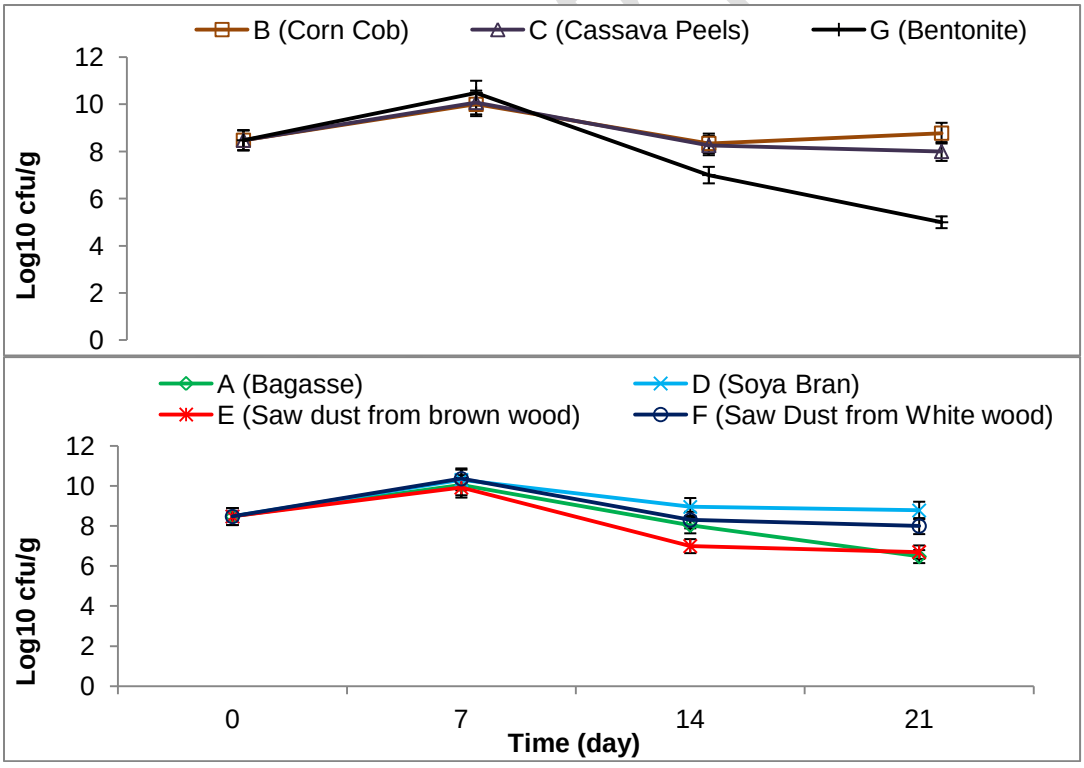


**Figure 1: Growth of the hydrocarbon-degrading bacterial consortium immobilized on different carriers**

### 3.3 Growth of the free living bacterial consortium on crude oil

The growth of the free living bacterial consortium with hydrocarbon as the sole carbon and energy source is presented in Figure 2.

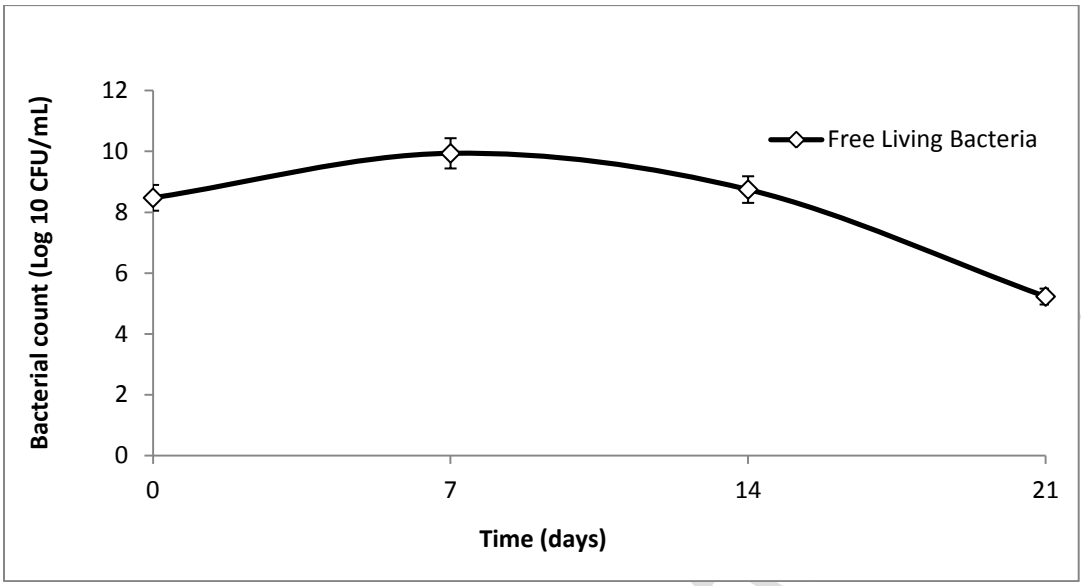


**Figure 2: Growth of the free hydrocarbon-utilizing bacterial consortium with hydrocarbon as the sole energy and carbon source.**

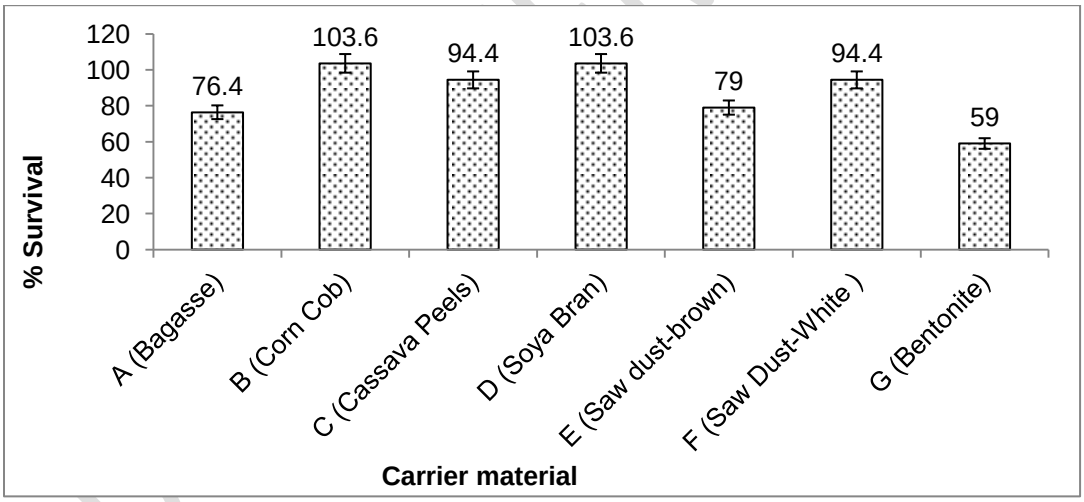


**Figure 3: Percentage survival of the bacterial consortium immobilized on the different carrier materials**

## 4. DISCUSSION

This study investigated the viability of hydrocarbon-utilizing bacterial consortium immobilized on different carrier materials. Immobilization enhances the capacity of bacterial isolates to survive and degrade organic contaminants. According to Martin [28] immobilization provides high biomass, provides cell reuse and reduces the costly processes of cell recovery and cell recycle, eliminates cell washout problems at high dilution rates, high flow rates allowing high volumetric

productivities, provides suitable micro environmental conditions, improves genetic stability, protects cells against shear damage and improves resistance to toxic chemicals, pH, temperature, solvents and heavy metals. In this study 7 different carrier materials were tested for use as carrier materials. The result obtained showed that the carriers enhanced the survival of the bacterial consortium over a period of 21 days compared to the free-living bacterial consortium. This finding therefore corroborates the earlier statement that immobilization "enhances the capacity of bacterial isolates to survive and degrade organic contaminants."

Immobilization of bacterial cultures has been generally reported to increase their viability than when left in free-living form. Obuekwe and Al-Muttawa [29] immobilized *Arthrobacter* sp., a Gram-negative bacillus isolated from Kuwait oil lakes, using sawdust, Styrofoam or wheat bran, as carriers, under low nutrient conditions and reported the production of stable exopolysaccharide. When they investigated the ability of the immobilized cells to survive and degrade hydrocarbons for 6 weeks at 45 °C against free suspensions of the same bacterium they discovered that the immobilized cells performed better than the freely suspended cells. Similarly, Quek *et al.* [30] reported the immobilization and performance of *Rhodococcus* sp. F92 on polyurethane foam (PUF) in the bioremediation of petroleum hydrocarbons. They discovered that the immobilized cells were able to degrade a variety of petroleum products such as Arabian light crude, Al-Shaheen crude, diesel and oil slops more than the freely suspended cells. The result obtained from this study showed that the survival rate of the free-living bacterial culture in hydrocarbon over 21 days was 38.3%. This value is lower than the least percentage (59%) obtained from immobilized cells.

This study employed the following carrier materials for the immobilization process: Bagasse, cassava peels, corn cob, saw dust, soya bran and benthonite. These carrier materials have been used by different researchers at different times to immobilize bacteria with outstanding result. Most of the reports focussed on single organisms but in this study bacterial consortium were immobilized using these different carrier materials. Bagasse has been used as a carrier for the immobilization of bacterial isolates. Mohammadi and Nasernejad [9] studied the enzymatic degradation of anthracene by the white rot fungus *Phanerochaete chrysosporium* immobilized on sugarcane bagasse. The result was compared with the unimmobilized cell with the immobilized resulting in higher degradation than the unimmobilized cell. Liu *et al*. [31] in another study investigated the efficiency of sugarcane bagasse as support material for the immobilization of *Bacillus pumilus* HZ-2 and thereafter applied the immobilized cells in the bioremediation of mesotrione contaminated soils. The result showed a better degradation of the contaminant with immobilized cells than the freely suspended cells.

Rivelli *et al*. [32] studied persistence and degrading activity of free and immobilised allochthonous bacteria during bioremediation of hydrocarbon-contaminated soils and reported better degradation with immobilized cells than freely suspended cells. Paliwal *et al*. [33] successfully immobilized bacterial consortium on corn cob with slightly enhanced biodegradation of chlorophenol when immobilized cells were used compared to the freely suspended cells.

The saw dust used as a carrier material in this study showed one of the highest survival rates. Obuekwe and Al-Muttawa [29] immobilized bacterial consortium on saw dust and reported enhanced petroleum hydrocarbon degradation by the immobilized cells compared to the freely suspended cells. Similarly, Hazaimeh *et al*. [34] enhanced crude oil hydrocarbon degradation by self-immobilized bacterial consortium culture on sawdust and oil palm empty fruit bunch. From the discussion, it is obvious that immobilization enhances bacterial survival rate compared to when the bacterial isolates are freely suspended in media. This is important in bioremediation of

hydrocarbon contaminated soil as the longer the bacterial cultures survive in the media the higher their chances of degrading the contaminant.

The differences observed in the viability of the bacterial consortium immobilized on the different carrier material may be ascribed to the difference in the carrier composition and probably structure. Another possibility may be the ease in which the bacteria detach from the immobilized carrier. If the bacterial cells are strongly attached to the matrix of the carrier there is possibility that they may remain fixed even after votexing thus yield false viability count. However, the findings of this study shows the potentials of these carrier material as suitable carriers for bacterial immobilization.

## 5. CONCLUSION

This study has demonstrated the capacity of the agro-wastes used to effectively retain the viability of hydrocarbon-utilizing bacterial consortium. The study showed that saw dust, corn cob, soya bran and cassava peels can effectively be used as carrier agents in immobilizing hydrocarbon-utilizing bacteria. The result is significant as it shows the possibility of using these carrier materials for biodegradation of hydrocarbon contaminated media. These wastes are easily available and besides their use may help mitigate the cost involved in managing them thereby reducing pollution. Further study on the effectiveness of these immobilized cells in bioremediation of different hydrocarbon contaminants should be carried out.

## Conflict of interest

The authors declare that they have no conflicts of interest to this work.

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