Monoaromatic Hydrocarbon Bioremediation of Hydrocarbon-contaminated Soil Using HBB5 Biosurfactant Produced by Pseudomonas xiamenensis

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

ABSTRACT

Biodegradation of benzene toluene ethylbenzene and xylenes (BTEX) is a slow and complex process. However, many microbial organisms have been shown to possess the capacity to biodegrade various components of a hydrocarbon. This study was aimed at investigating the role of biosurfactant on soil polluted with these monoaromatics. Samples were collected and analyzed using standard techniques. The biodegradation set up was carried out using five earthen pots; each containing unpolluted soil, polluted soil alone, polluted soil + poultry wastes, polluted soil + HBB5 biosurfactant and polluted soil + poultry wastes + HBB5 biosurfactant. The biodegradation of BTEX were periodically monitored every seven days for 28 days using gas chromatograph-mass spectrometer coupled with head space (GC-MS-HS). The respective initial and final concentrations of BTEX (ppm) were as follows: 0.7936 and 0.2063, 0.9733 and 0.0231, 0.9526 and <0.0001, 0.9241 and <0.0001 with degradation efficiencies of 74.0%, 97.6%, 100% and 100% for polluted soil alone, polluted soil + poultry wastes, polluted soil + HBB5 biosurfactant and polluted soil + poultry wastes + HBB5 biosurfactant respectively. The microbial counts increased...
greatly, and the concentrations of the limiting nutrients reduced during the experimental period. The effective treatments for bioremediation increased in the following order: polluted soil alone < polluted soil + poultry waste < polluted soil + HBB5 biosurfactant < polluted soil + poultry waste + HBB5 biosurfactant. Results clearly showed that application of HBB5 biosurfactant only or in combination with poultry wastes has the ability to degrade ethylbenzene and xylenes (BTEX) and thus, can be employed in the clean-up of crude oil contaminated soil.

Keywords: Biosurfactants; hydrocarbon; bioremediation; Pseudomonas; contamination; soil.

1. INTRODUCTION

In the last few decades, different organic pollutants which include petroleum fuels, pesticides, solvents, pharmaceuticals, and other organic chemicals have been produced industrially and released intentionally or unintentionally during their transports or storages [1,2]. These organic pollutants are highly persistent, degrade slowly, and can become trapped for long periods of time in the soil minerals of contaminated sites, and subsequently bioaccumulated, due to their hydrophobic and stable chemical properties [3,4,5]. Due to the potentially adverse effects on human health and multiple environments (water, soil, and air), contamination by these organic pollutants is a cause of great environmental concern, which necessitates the need to restore contaminated sites [1,6,7]. Benzene, toluene, ethylbenzene and xylene (BTEX) compounds are mostly found together in crude oil and petroleum products. They are of major concerns due largely to their toxicity and carcinogenicity, even at low concentrations, thus, the United States EPA classifies them as environmental priority pollutants, making their removal from polluted environments critical [7]. Their high water solubility, unlike the other fuel components, contributes to their mobility, and enables them to migrate in the subsurface and contaminate drinking water supplies [8]. The benzene, toluene, ethylbenzene, and o-, m-, and p-xylene (BTEX) compound is one of the most common groundwater and soil contaminants [9]. Bioremediation is considered an environmentally friendly and cost effective response to oil pollution. Three principal approaches of this technique: natural attenuation (reliance on natural biodegradation activities and rates), which is sometimes called intrinsic bioremediation; biostimulation (stimulation of natural activities by environmental modifications such as fertilizer addition to increase biomass, and thus, rates of biodegradation); and bioaugmentation (addition of exogenous microorganisms to supplant the natural degradative capacity of the hydrocarbon-impacted ecosystem) for in situ biodegradation have been applied several times at pilot and field scale trials with varying degrees of successes [10,11,12,13].

Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats [14]. Studies have shown that crude oil biodegradation can be increased by the addition of nitrogen- and phosphorus-containing fertilizers (organic or inorganic) in aquatic, terrestrial or swampy soil environments [15]. It is frequently reported that various types of microorganisms can produce biosurfactants and this capability is often intensified with their growth on nearly insoluble hydrocarbons as the sole carbon source [16]. In many cases, biosurfactants have certain advantages over their chemical counterparts such as lower toxicity, biodegradability and higher activity at extreme conditions, turning them into a potentially effective agent to be used in bioremediation processes [17]. A number of surfactants have been isolated from microbial cultures following growth of bacteria and fungi on a variety of aliphatic and aromatic hydrocarbons [18]. These biosurfactants are extracellular, and may be relatively simple glycolipids or complex high molecular weight substances. The objectives of the present study were to investigate the comparative degradability of BTEX-contaminated soil using biologically produced surfactants and organic nutrients.

2. MATERIALS AND METHODS

2.1 Collection of Soil and Poultry waste Samples

The soil samples were collected from hydrocarbon impacted and non-impacted sites at Idu, Onelga Local Government area of Rivers State, Nigeria. Soil samples were collected at a depth of 0-15 cm with a clean hand auger into sterile polythene bags and stored in an ice packed cooler. Poultry wastes were collected...
from a poultry farm in Port Harcourt, Rivers State, Nigeria.

The samples were aseptically collected using standard methods and immediately transported to the laboratory for analysis within 24 hours [19].

2.2 Biosurfactant

The biosurfactant used was HBB5 biosurfactant produced from *Pseudomonas xiamenesis* that was isolated from Amadi-Ama creek in Port Harcourt, Rivers state. The biosurfactant is an anionic naphthalene sulfonate produced with slop oil carbon substrate at standard conditions using response surface methodology (RSM).

2.3 Physicochemical Analysis of Different Treated Soil Samples

Physicochemical analyses of the soil samples were conducted according to standard procedures of APHA [20] and ASTM [21]. The physicochemical parameters determined include pH, nitrate, phosphate and total organic carbon.

2.4 Determination of Total Heterotrophic Bacterial (Thb) And Hydrocarbon-Utilizing Bacterial (Hub) Counts in Soils with Different Treatment Options

The total heterotrophic bacterial (THB) count was determined using the nutrient agar and spread plate technique as described by Prescott [22]. An aliquot (0.1ml) of each serially diluted sample using dilution factors of $10^{-4}$ for all the treated soil samples were separately inoculated onto different sterile nutrient agar plates in triplicates. The plates were incubated at 37°C in an inverted position for 24 hours. After incubation, colonies that developed on the plates were counted and only counts of between 30 and 300 were recorded. The average values of replicate plates were calculated and expressed as colony-forming units per gram (CFU/g). The populations of the hydrocarbon-utilizing bacteria of treated soil samples were determined by inoculating 0.1ml aliquot of the serially diluted ($10^{-3}$ and $10^{-4}$) samples of treated soil onto mineral salt agar media using the spread plate technique [19]. The vapour Phase Transfer method was adopted by the use of sterile filter paper discs that was soaked in filter-sterilized crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were aseptically transferred to the inside covers of the inoculated Petri dishes and incubated for 5 days at room temperature [19]. Colonies that developed were counted, and average of duplicate plates counted as colony forming units per gram of soil was calculated.

2.5 Determination of Total Fungi and Hydrocarbon Utilizing Fungal Count of Soils with Different Treatment Options

The total counts of fungi in the samples were also determined by the spread plate technique. An aliquot (0.1ml) of serially diluted volumes ($10^{-2}$) of each of the various samples was plated onto separate Potato dextrose agar plates to which 0.1 ml of streptomycin solution was incorporated to suppress bacterial growth. The plates were incubated at 28°C for 5-7 days and the discrete colonies that developed were enumerated as the viable counts (CFU) of fungi in the soil samples [23]. Hydrocarbon-utilizing fungal count of soil samples was determined by inoculating 0.1ml of the serially diluted samples ($10^{-1}$) on mineral salt agar. The mineral salt medium was supplemented with streptomycin (0.1ml) to suppress bacterial growth [19]. The Vapour Phase Transfer method [19] was also adopted.

2.6 Biodegradation Test

The experiment was designed to study the relative roles of poultry waste and HBB5 biosurfactant in the degradation of soil polluted with BTEX [3].

2.7 Composition of Biodegradation Set up

Five earthen pots were used for the degradation experiment and were properly labeled. The five earthen pots contained 2kg of soil, one of which contained unpolluted soil and another polluted soil alone, while the other four contained in addition to polluted soil; 200 ml of the poultry waste, 100ml of HBB5 biosurfactant and 200ml of the poultry waste with 100ml of HBB5 biosurfactant. The experimental set ups were presented in Table 1.
### Table 1. Test set ups

| Set up | Content |
|--------|---------|
| A      | Unpolluted soil only |
| B      | Polluted soil alone |
| C      | Polluted soil + poultry wastes |
| D      | Polluted soil + HBB5 Biosurfactant |
| E      | Polluted soil + poultry wastes + HBB5 Biosurfactant |

#### 2.8 Bioremediation Procedure

Each of the bioremediation experimental set-ups was incubated at room temperature. The set-ups containing the soil samples and various treatments were thoroughly mixed. The test set ups were analyzed for pH, nitrate, phosphate, total organic carbon, BTEX, total heterotrophic bacterial count, hydrocarbon-utilizing bacterial count, total fungal count and hydrocarbon-utilizing fungal count on the first day, and at weekly intervals for 28 days. The pH was determined using electrometric method, while nitrate and phosphate concentrations were determined using spectrophotometry as described in APHA 4500. Similarly, total organic carbon concentrations were determined titrimetrically using the Walkley Blacky method [24]. The concentrations for benzene toluene ethylbenzene xylene (BTEX) were determined using gas chromatography following methanol extraction, wherein the methanol-phase extract was analyzed using gas chromatograph-mass spectrometer coupled with head space (GC-MS-HS).

#### 3. RESULTS

The results of this study are presented in Tables 1 to 6 and in Figs. 1 to 5. Results of pH in the various treatments are as shown in Fig. 1. At 0.05 confidence limit, the polluted and unpolluted soils without treatments recorded no significant change in value, while the other setup with treatments increased significantly with time. The poultry waste-amended polluted soil recorded a higher rate of increase in pH within the first twenty one days, whereas its amendment in isolation and with the HBB5 biosurfactant recorded the highest rate of pH increase between the twenty first and twenty eighth days. Nitrate showed highest value (in ppm) of 1.142, 1.193, 4.771, 1.127 and 4.762 for the various amendments on day 1, with the highest value of 4.762 ppm recorded for the polluted soil + poultry waste + HBB5 biosurfactant. These values reduced over time, with nitrate concentrations (in ppm) of 1.126, 1.163, 3.818, 0.218 and 1.1254 on the twenty eighth day. This accounted for approximately 76% nitrate reduction in the option with poultry waste and HBB5 biosurfactant treatments. In a similar trend, phosphate recorded the highest concentrations (in ppm) of 0.467, 0.147, 1.812, 0.142 and 1.936 for the various amendments on the first day, with the highest value of 1.936 ppm recorded for the polluted soil with poultry waste and HBB5 biosurfactant amendments. The total organic carbon concentrations for the various treatments recorded varying percentage losses ranging from 1.7% to 64.9% after twenty eight days. The treatment with a combination of HBB5 Biosurfactant and poultry wastes recorded the highest organic carbon loss, while the polluted soil without amendment recorded the least organic carbon loss. The percentage loss of monoaromatic hydrocarbons measured as BTEX increased in this order: polluted soil alone (73.8%) < polluted soil + poultry wastes (97.6%) < polluted soil + HBB5 Biosurfactant (100%) and polluted soil + poultry wastes + HBB5 Biosurfactant (100%). The total heterotrophic bacterial population during the 28 days biodegradation studies recorded a 5.4% increase in the unpolluted soil, a 3.6% increase in the polluted soil without treatment, a 72.1% increase in the polluted soil amended with poultry wastes, a 99.5% increase in the polluted soil amended with HBB5 biosurfactant, and a 99.6% increase in the polluted soil amended with poultry wastes and HBB5 Biosurfactant. The population of hydrocarbon-utilizing bacteria (HUB) increased in the polluted soil from 8.00 x 10^3 cfu/g on the first day to 3.60 x 10^3 cfu/g on the twenty eighth day, yielding a percentage growth of 51.1%. Similarly, the polluted soil amended with poultry wastes recorded an increase in HUB population from 2.80 x 10^4 cfu/g to 6.70 x 10^4 cfu/g, accounting for an approximately 99.6% growth over a 28-day duration. Also in the HBB5
biosurfactant-amended polluted soil, HUB population increased from 2.20 x 10^4 cfu/g on the first day to 4.50 x 10^5 cfu/g on the twenty eighth day, recording a percentage growth of 95.1%. The HUB population in the polluted soil amended with poultry wastes and HBB5 biosurfactant increased from 2.80 x 10^4 cfu/g in the first day to 7.80 x 10^6 cfu/g in the twenty eighth day, accounting for 99.6% percentage growth. The respective total fungal population on the first day for unpolluted soil, polluted soil without amendments, polluted soil amended with poultry wastes, polluted soil amended with HBB5 biosurfactant, polluted soil amended with poultry wastes and HBB5 biosurfactant recorded 1.93 x 10^5 cfu/g, 1.76 x 10^5 cfu/g, 1.12 x 10^5 cfu/g, 2.56 x 10^5 cfu/g, 2.92 x 10^5 cfu/g, while the respective twenty eighth day results recorded 2.10 x 10^6 cfu/g, 4.20 x 10^5 cfu/g, 4.70 x 10^5 cfu/g, 5.10 x 10^5 cfu/g, 8.20 x 10^6 cfu/g. These results account for a percentage growth of 8.1%, 44.8%, 76.2%, 95.0% and 99.6% respectively. Similarly, the respective hydrocarbon-utilizing fungal (HUF) population on the first day for unpolluted soil, polluted soil without amendments, polluted soil amended with poultry wastes, polluted soil amended with HBB5 biosurfactant, polluted soil amended with poultry wastes and HBB5 biosurfactant recorded 8.10 x 10^3 cfu/g, 3.10 x 10^3 cfu/g, 2.40 x 10^3 cfu/g, 3.00 x 10^3 cfu/g, 3.00 x 10^3 cfu/g, while the respective twenty eighth day results recorded 3.90 x 10^3 cfu/g, 3.20 x 10^4 cfu/g, 5.30 x 10^3 cfu/g, 4.40 x 10^3 cfu/g, 1.10 x 10^6 cfu/g respectively. These results accounted for a population growth of 51.9%, 3.1%, 54.7%, 93.2% and 99.7% respectively.

Table 2. Unpolluted soil

| Parameter                          | Day 1  | Day 7  | Day 14 | Day 21 | Day 28 |
|------------------------------------|--------|--------|--------|--------|--------|
| pH                                 | 6.28   | 6.24   | 6.25   | 6.22   | 6.26   |
| Nitrate (ppm)                      | 1.142  | 1.138  | 1.133  | 1.129  | 1.126  |
| Phosphate (ppm)                    | 0.467  | 0.458  | 0.451  | 0.447  | 0.443  |
| Total Organic Carbon (%)           | 1.26   | 1.23   | 1.22   | 1.18   | 1.15   |
| Benzene Toluene Ethylbenzene Xylene (ppm) | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Total Heterotrophic bacteria (cfu/g) | 5.30 x 10^6 | 5.40 x 10^6 | 5.70 x 10^6 | 5.50 x 10^6 | 5.60 x 10^6 |
| Hydrocarbon-utilizing bacteria (cfu/g) | 8.00 x 10^3 | 8.30 x 10^3 | 8.20 x 10^3 | 8.40 x 10^3 | 8.50 x 10^3 |
| Total Fungi (cfu/g)                | 1.93 x 10^5 | 1.72 x 10^5 | 1.84 x 10^5 | 1.99 x 10^5 | 2.10 x 10^5 |
| Hydrocarbon-utilizing fungi (cfu/g) | 8.10 x 10^3 | 6.30 x 10^3 | 7.20 x 10^3 | 7.60 x 10^3 | 3.90 x 10^3 |

**Fig. 1.** pH dynamics of the various bioremediation treatments

![pH dynamics](image-url)
Fig. 2. Nitrate concentrations (ppm) dynamics of the various bioremediation treatments

Fig. 3. Phosphate concentrations (ppm) dynamics of the various bioremediation treatments
Results of the polluted soils are shown in Table 3. Highest pH value of 5.63 was obtained on day 28 and least value of 5.60 on day 14. Values for nitrate were highest with 1.193 ppm on day 1 and least value of 1.163 ppm on day 28. The highest value of phosphate was 0.147 ppm on day 1 and recorded least value of 0.141 ppm on day 21. Total organic carbon obtained highest value of 1.21% on day 1 and 7, and least value of 1.19% on day 21 and 28. The benzene toluene ethylbenzene xylene (BTEX) value on day 1 was 0.7936 ppm and 0.2063 ppm on day 28. The total heterotrophic bacterial count during the biodegradation studies in the polluted soil ranged from 3.20×10^4 to 6.90×10^5 cfu/g which were recorded on day 1 and 28, respectively. The least value of 1.76×10^4 cfu/g was obtained on day 1 for hydrocarbon-utilizing bacterial count while the highest count of 3.80×10^6 cfu/g was recorded on day 21. Total fungi and hydrocarbon-utilizing
fungi recorded least values of 1.00×10^4 and 1.10×10^4 cfu/g, respectively on day 7, while highest counts of 4.20×10^4 and 4.20×10^4 cfu/g were obtained on days 28 and 21, respectively.

The results of poultry wastes-amended polluted soil are shown in Table 4. Highest pH value of 6.09 was obtained on day 21 and least value of 5.83 on day 1. Nitrate showed highest value of 4.771 ppm on day 1 and least value of 3.818 ppm on day 28. The highest value of phosphate (1.812 ppm) was recorded on day 1 and least value of 1.213 ppm was obtained on day 28.

Total organic carbon obtained highest value of 1.127 ppm on day 1 and least value of 0.021 ppm was obtained on day 28. The benzene toluene ethylbenzene xylene (BTEX) on day 1 recorded 0.973 ppm, and 0.0231 ppm on day 28. The total heterotrophic bacterial count while the highest count of 4.20×10^4 cfu/g was recorded on day 28.

The biodegradation results with treatment option of polluted soil + poultry wastes + HBB5 biosurfactant are shown in Table 6. Highest pH value 6.59 was obtained on day 28 and least value of 5.62 on day 1. Nitrate showed highest value of 4.762 ppm on day 1 and least value of 1.124 ppm on day 28. The highest value of phosphate (1.936 ppm) was recorded on day 1 and least value of 0.021 ppm was obtained on day 28. Total organic carbon obtained highest value of 3.02% on day 28 and least value of 1.06% on day 28. The concentration of monoaromatic hydrocarbon recorded highest value of 0.9526 ppm on day 1 and least value of <0.0001 ppm on day 28. The total heterotrophic bacterial count during the biodegradation studies in the polluted soil amended with HBB5 biosurfactant on day 1 was 4.10×10^4 cfu/g, and 9.00×10^4 cfu/g on day 28. The least value of 2.20×10^4 cfu/g was obtained on day 1 for hydrocarbon-utilizing bacterial count while the highest count of 4.50×10^4 cfu/g was recorded on day 28. Total fungi and hydrocarbon-utilizing fungi on day 1 recorded least values of 2.56×10^4 cfu/g and 3.00×10^4 cfu/g, respectively, while highest counts of 5.10×10^4 cfu/g and 4.40×10^4 cfu/g was obtained on day 28, respectively.

The biodegradation results with treatment option of polluted soil + poultry wastes + HBB5 biosurfactant are shown in Table 6. Highest pH value 6.59 was obtained on day 28 and least value of 5.62 on day 1. Nitrate showed highest value of 4.762 ppm on day 1 and least value of 1.124 ppm on day 28. The highest value of phosphate (1.936 ppm) was recorded on day 1 and least value of 0.021 ppm was obtained on day 28. Total organic carbon obtained highest value of 3.02% on day 28 and least value of 1.06% on day 28. The concentration of monoaromatic hydrocarbon recorded highest value of 0.9241 ppm on day 1, with the least value of <0.0001 ppm on day 28. The total heterotrophic bacterial count on day 1 was 4.20×10^4 cfu/g, which accounted for the least value. The highest value of 4.20×10^4 cfu/g was however recorded on day 28. Meanwhile, the least value of 2.80×10^4 cfu/g was obtained on day 1 for hydrocarbon utilizing bacterial count, as the highest count of 7.80×10^4 cfu/g was recorded on day 28. Total fungi and hydrocarbon-utilizing fungi on day 1 recorded least values of 2.92×10^4 cfu/g and 3.00×10^4 cfu/g respectively, while highest counts of 8.20×10^4 cfu/g and 1.10×10^5 cfu/g were obtained on day 28, respectively.

Table 5 showed the results obtained in the polluted soil amended with the HBB5 biosurfactant. The highest pH value of 6.64 was recorded on day 28 and the least value of 5.67 was obtained on day 1. Nitrate showed highest value of 1.127 ppm on day 1 and least value of 0.218 ppm on day 28. The highest value of 0.142 ppm for phosphate was recorded on day 1 and least value of 0.019 ppm was obtained on day 28. Total organic carbon obtained highest value of 2.93% on day 1 and least value of 2.16% on day 28. The concentration of monoaromatic hydrocarbon recorded the highest value of 0.9526 ppm on day 1 and least value of <0.0001 ppm on day 28.

The biodegradation results with treatment option of polluted soil + poultry wastes + HBB5 biosurfactant are shown in Table 6. Highest pH value 6.59 was obtained on day 28 and least value of 5.62 on day 1. Nitrate showed highest value of 4.762 ppm on day 1 and least value of 1.124 ppm on day 28. The highest value of phosphate (1.936 ppm) was recorded on day 1 and least value of 0.021 ppm was obtained on day 28. Total organic carbon obtained highest value of 3.02% on day 28 and least value of 1.06% on day 28. The concentration of monoaromatic hydrocarbon recorded highest value of 0.9241 ppm on day 1, with the least value of <0.0001 ppm on day 28. The total heterotrophic bacterial count on day 1 was 4.20×10^4 cfu/g, which accounted for the least value. The highest value of 4.20×10^4 cfu/g was however recorded on day 28. Meanwhile, the least value of 2.80×10^4 cfu/g was obtained on day 1 for hydrocarbon utilizing bacterial count, as the highest count of 7.80×10^4 cfu/g was recorded on day 28. Total fungi and hydrocarbon-utilizing fungi on day 1 recorded least values of 2.92×10^4 cfu/g and 3.00×10^4 cfu/g respectively, while highest counts of 8.20×10^4 cfu/g and 1.10×10^5 cfu/g were obtained on day 28, respectively.

Table 3. Polluted soil alone

| Parameter                        | Day 1     | Day 7     | Day 14    | Day 21    | Day 28    |
|----------------------------------|-----------|-----------|-----------|-----------|-----------|
| pH                               | 5.62      | 5.61      | 5.60      | 5.62      | 5.63      |
| Nitrate (ppm)                    | 1.193     | 1.187     | 1.172     | 1.176     | 1.163     |
| Phosphate (ppm)                  | 0.147     | 0.144     | 0.143     | 0.141     | 0.142     |
| Total Organic Carbon (%)         | 1.21      | 1.21      | 1.20      | 1.19      | 1.19      |
| Benzene Toluene Ethylbenzene Xylene (ppm) | 0.7936 | 0.5118    | 0.4673    | 0.2198    | 0.2063    |
| Total Heterotrophic Bacteria (cfu/g) | 3.20×10^4 | 3.40×10^4 | 5.40×10^4 | 6.50×10^4 | 6.90×10^4 |
| Hydrocarbon-utilizing bacteria (cfu/g) | 1.76×10^4 | 1.78×10^4 | 2.61×10^4 | 3.80×10^4 | 3.60×10^4 |
| Total Fungi (cfu/g)              | 2.32×10^3 | 1.00×10^3 | 2.40×10^3 | 3.90×10^3 | 4.20×10^3 |
| Hydrocarbon-utilizing fungi (cfu/g) | 3.10×10^3 | 1.10×10^3 | 1.81×10^3 | 4.20×10^3 | 3.20×10^3 |
and the condition required for microbial activity experiment. pH is an important parameter in soil, in pH values throughout the duration of the treatment options recorded progressive increase. The pH values were slightly acidic. Al slight fluctuation of pH values during the study. The unpolluted soil treatment sample recorded combinative amendment of poultry wastes and HBB5. The highest degradation efficiency (100%) was utilizing fungi (cfu/g).

### Table 4. Polluted soil + Poultry wastes

| Parameters                        | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
|-----------------------------------|-------|-------|--------|--------|--------|
| pH                                | 5.83  | 5.91  | 6.02   | 6.09   | 6.18   |
| Nitrate (ppm)                     | 4.771 | 4.562 | 4.208  | 4.006  | 3.818  |
| Phosphate (ppm)                   | 1.812 | 1.664 | 1.619  | 1.464  | 1.213  |
| Total Organic Carbon (%)          | 3.00  | 2.68  | 2.28   | 1.89   | 1.30   |
| Benzene Toluene Ethylbenzene      | 0.9733| 0.7239| 0.4134 | 0.1966 | 0.0231 |
| Xylene (ppm)                      |       |       |        |        |        |
| Total Heterotrophic bacteria (cfu/g) | 2.90 x 10^4 | 3.20 x 10^4 | 5.60 x 10^4 | 7.20 x 10^4 | 1.04 x 10^5 |
| Hydrocarbon-utilizing bacteria (cfu/g) | 2.80 x 10^3 | 1.95 x 10^4 | 2.82 x 10^4 | 4.80 x 10^4 | 6.70 x 10^5 |
| Total Fungi (cfu/g)               | 1.12 x 10^4 | 1.38 x 10^4 | 2.60 x 10^4 | 3.40 x 10^4 | 4.70 x 10^4 |
| Hydrocarbon-utilizing fungi (cfu/g)| 2.40 x 10^3 | 2.20 x 10^3 | 1.52 x 10^3 | 4.30 x 10^3 | 5.30 x 10^3 |

### Table 5. Polluted soil + HBB5 Biosurfactant

| Parameters                        | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
|-----------------------------------|-------|-------|--------|--------|--------|
| pH                                | 5.67  | 5.81  | 5.97   | 6.13   | 6.64   |
| Nitrate (ppm)                     | 1.127 | 1.007 | 0.814  | 0.524  | 0.218  |
| Phosphate (ppm)                   | 0.142 | 0.119 | 0.026  | 0.023  | 0.019  |
| Total Organic Carbon (%)          | 2.93  | 2.91  | 2.76   | 2.41   | 2.16   |
| Benzene Toluene Ethylbenzene      | 0.9526| 0.4287| 0.1184 | 0.0692 | <0.0001|
| Xylene (ppm)                      |       |       |        |        |        |
| Total Heterotrophic bacteria (cfu/g) | 4.10 x 10^4 | 6.80 x 10^4 | 2.30 x 10^5 | 6.40 x 10^5 | 9.00 x 10^5 |
| Hydrocarbon-utilizing bacteria (cfu/g) | 2.20 x 10^4 | 5.60 x 10^4 | 9.60 x 10^4 | 2.20 x 10^5 | 4.50 x 10^5 |
| Total Fungi (cfu/g)               | 2.56 x 10^4 | 5.20 x 10^4 | 1.12 x 10^5 | 2.40 x 10^5 | 5.10 x 10^5 |
| Hydrocarbon-utilizing fungi (cfu/g)| 3.00 x 10^3 | 6.80 x 10^3 | 1.30 x 10^5 | 3.20 x 10^4 | 4.40 x 10^4 |

### Table 6. Polluted Soil + Poultry wastes + HBB5 Biosurfactant

| Parameters                        | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
|-----------------------------------|-------|-------|--------|--------|--------|
| pH                                | 5.62  | 5.77  | 5.92   | 6.06   | 6.59   |
| Nitrate (ppm)                     | 4.762 | 4.412 | 3.833  | 2.918  | 1.124  |
| Phosphate (ppm)                   | 1.936 | 1.724 | 1.222  | 0.418  | 0.021  |
| Total Organic Carbon (%)          | 3.02  | 2.96  | 2.31   | 1.93   | 1.06   |
| Benzene Toluene Ethylbenzene      | 0.9241| 0.4258| 0.1241 | 0.003  | <0.0001|
| Xylene (ppm)                      |       |       |        |        |        |
| Total Heterotrophic bacteria (cfu/g) | 4.20 x 10^4 | 3.40 x 10^5 | 5.10 x 10^9 | 8.20 x 10^9 | 1.12 x 10^11 |
| Hydrocarbon-utilizing bacteria (cfu/g) | 2.80 x 10^4 | 1.93 x 10^5 | 2.80 x 10^5 | 5.30 x 10^5 | 7.80 x 10^5 |
| Total Fungi (cfu/g)               | 2.92 x 10^4 | 1.00 x 10^5 | 1.25 x 10^5 | 5.90 x 10^5 | 8.20 x 10^5 |
| Hydrocarbon-utilizing fungi (cfu/g)| 3.00 x 10^4 | 2.10 x 10^5 | 1.72 x 10^5 | 6.60 x 10^5 | 1.10 x 10^6 |

The highest degradation efficiency (100%) was recorded in the polluted soil amended with the HBB5 biosurfactant and the polluted soil with a combinative amendment of poultry wastes and HBB5 biosurfactant.

4. DISCUSSION

The unpolluted soil treatment sample recorded slight fluctuation of pH values during the study. The pH values were slightly acidic. All the treatment options recorded progressive increase in pH values throughout the duration of the experiment. pH is an important parameter in soil, and the condition required for microbial activity ranges from 5.5-8.8 [25,26]. Soil pH influences biodegradation through its effect on microbial activity, microbial community and diversity, enzymes that aid in the degradation processes as well as the properties of the substances to be degraded. Generally, alkaline or slightly acid soil pH enhances biodegradation, while acidic environments pose limitations to biodegradation [27,28,29]. Nitrate and phosphate values of all the treatment options recorded highest values on day 1 and least values on day 28. There was progressive decrease of nitrate and phosphate values as the experiment progressed. This could be attributed to the utilization of the nutrients in the soil by microbes. Similar results had been
reported in previous studies [30,31]. The nitrate and phosphate values in the treatment options containing polluted soil with poultry wastes recorded relatively higher values compared to the other treatment options. This implies that the poultry wastes contributed to the increase in the nutrient values. The total organic carbon recorded in the unamended soil setups were lower compared with the polluted soil amended with various treatment options. However there was significant decrease in the total organic carbon in all the treatment options on day 28.

The unpolluted soil recorded less than 0.0001 ppm for BTEX. The unamended polluted soil, polluted soil + poultry wastes, polluted soil + HBB5 biosurfactant and polluted soil + poultry wastes + HBB5 biosurfactant recorded progressive reduction in the amount of residual BTEX from day 1 to day 28. On day 28, treatments with polluted soil + HBB5 biosurfactant and polluted soil + poultry wastes + HBB5 biosurfactant recorded concentrations less than 0.0001 ppm of BTEX with 100% degradation efficiency. This was followed by 97.6% degradation efficiency recorded with polluted soil + poultry wastes. The least degradation efficiency of 74.0% was recorded in the polluted soil without treatment, demonstrating hydrocarbon loss mediated only by natural attenuation. The 100% degradation efficiency achieved in this study could be attributed to the HBB5 biosurfactant only or with poultry wastes added to the polluted soil. Biosurfactants play an important role in the biodegradation of aromatic compounds in soil environments, where desorption from surfaces is a rate-limiting step [32]. This result supports the findings of Kumar et al. [33], that biosurfactants can effectively expedite the bioremediation of hydrocarbon polluted environment. The poultry wastes treatment used in this study may have influenced the growth of biodegraders in the soil polluted with BTEX, hence the high level of degradation. On the other hand, the biosurfactant may have only reduced the interfacial tension, making the hydrocarbons bioavailable for biodegradation by autochthonous microbial species. The progressive increase in population of different microbial physiological groups in the various treatments showed the availability of an additional carbon source for biomass production. Biodegradation of hydrocarbons by indigenous populations of microorganisms represent one of the primary mechanism by which petroleum and other pollutants are eliminated from the environment [34]. This shows that nutrient supplementation enhances biodegradation rate [35,36,37]. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus and some iron [38]. Use of poultry waste as organic fertilizer in contaminated soil was also reported by [39].

The total heterotrophic and hydrocarbon-utilizing bacterial population during the biodegradation study in the unpolluted soil increased after day 1. The total heterotrophic bacterial population decreased on day 21, as well as the hydrocarbon-utilizing bacterial population on day 14. Nevertheless, these populations increased significantly by the twenty eighth day of the bioremediation test. In the polluted soil without treatment, the total heterotrophic and hydrocarbon-utilizing bacterial populations increased progressively, except for hydrocarbon-utilizing bacterial population, which reduced on day 28. This can be attributed to the depletion in utilizable hydrocarbon for bacterial biomass production [40]. The polluted soil + poultry wastes, polluted soil + HBB5 biosurfactant and polluted soil + poultry wastes + HBB5 biosurfactant increased from day 1 to 28. The fungi and hydrocarbon-utilizing fungal populations in unpolluted and polluted soil without treatments recorded decreased counts on day 7, which later increased throughout the twenty eight days period. The hydrocarbon-utilizing fungal population conversely reduced on day 28, suggesting the depletion of utilizable hydrocarbon for fungal biomass production. In the polluted soil + poultry wastes, polluted soil + HBB5 biosurfactant and polluted soil + poultry wastes + HBB5 biosurfactant, there was progressive increase in the total fungal and hydrocarbon-utilizing fungal populations throughout the period of the bioremediation test.

5. CONCLUSION

The degradation efficiency of HBB5 biosurfactant only or with poultry wastes in this study to remediate monoaromatic hydrocarbons were demonstrated, and the biosurfactant was found to mediate the process. The microorganisms associated with polluted soil effectively utilized the benzene, toluene, ethylbenzene and xylene (BTEX) during the degradation test. The study showed that polluted soil amended with poultry wastes and/or with HBB5 biosurfactants possess high capability to degrade these monoaromatic hydrocarbons. These formulations can be harnessed and used as effective recipe for the
cleanup of monoaromatic hydrocarbon-contaminated environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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