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# BIODEGRADATION OF TOTAL PETROLEUM HYDROCARBON FROM OILFIELD WASTEWATER USING BACTERIA CONSORTIUM

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## ABSTRACT

Oilfield wastewater poses significant environmental risks due to high levels of total petroleum hydrocarbons. Biodegradation offers a promising solution for mitigating these risks. This study intended to biodegrade total petroleum hydrocarbons (TPH) present in oilfield wastewater (OFWW) through the utilization of a mixed bacterial culture. Bacteria were isolated from OFWW and included *Morganella morganii*, *Pseudomonas xiamenesis*, *Staphylococcus* sp, and *Chryseobacterium cucumeris*. Initial TPH concentration was 381.87 mg/L in 125 ml OFWW. Bacteria were added (6.25 ml) to separate flasks, combinations varied from two to four bacteria, with a control lacking bacteria. Flasks were incubated at 28°C, 200 rpm. TPH was analyzed using Gas Chromatography at days 1, 7, and 21. By day 21, the removal of TPH recorded highest percentage removal of 96.8 % in the mixed culture using three bacterial species of *Morganella morganii* + *Pseudomonas xiamenesis* + *Chryseobacterium cucumeris*, followed by the mixed culture of four (*Morganella morganii* + *Pseudomonas xiamenesis* + *Staphylococcus* sp + *Chryseobacterium cucumeris*) which recorded 96.2% removal and the mixed culture of *Morganella morganii* + *Chryseobacterium cucumeris* recorded 92.8%. The bacterial treatments exhibited high efficacy in degrading individual hydrocarbons, within 21 days. This suggests that the bacterial consortia were able to degrade most hydrocarbon compounds, but some recalcitrant compounds like C9 and C14 may require further degradation time or specialized enzymes.

**Keywords:** *Oilfield wastewater, Total petroleum hydrocarbon, Gas Chromatography, n-alkanes*

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## INTRODUCTION

Oilfield wastewater, also referred to as produced water, is a byproduct of oil and gas extraction and processing. To mitigate its environmental footprint, this effluent undergoes processing to remove petroleum compounds to the greatest extent possible before being discharged into aquatic environments, storage pits, or reinjected into underground wells (Wills, 2000). This wastewater contains significant quantities of both inorganic and organic materials (Veil *et al*., 2004), including heavy metals, hydrocarbons, and other hazardous substances that often surpass regulatory limits (Abdulsalam *et al.,* 2016). The persistence of petroleum pollutants in the environment poses significant threats, as they can linger in soil, stifling microbial activity, plant growth, and animal habitats. Moreover, these contaminants can leach into groundwater or soil moisture, causing widespread contamination, and even vaporize into the atmosphere, amplifying their harmful impact (Li *et al.,* 2018). Consequently, hydrocarbon-related environmental contamination represents a significant challenge due to its toxic nature and wide-ranging distribution (Gupta *et al.,* 2019). Total petroleum hydrocarbons encompass a diverse range of chemical compounds derived from petroleum, numbering in the hundreds or thousands.

Given the complexity and diversity of individual compounds within total petroleum hydrocarbons (TPH), it is impractical to analyze each one individually. Instead, TPH serves as a practical indicator, quantifying the collective number of petroleum-derived hydrocarbons present in environmental media, like soil and water, to provide a comprehensive picture of petroleum contamination (John and Wigger, 1997). TPH specifically targets petrogenic hydrocarbons, which are those derived from petroleum sources. Bank et al. (2003) defined TPH as the sum of hydrocarbons with boiling points equal to or greater than 150°C, which are typically found in petroleum products, measured over a defined time frame. This definition captures the total concentration of these hydrocarbons, providing a robust indicator of petroleum contamination. Labunska *et al.* (2001) described TPH as an estimation of the hydrocarbons within the carbon ranges of C4 to C40 in a sample. Despite variations in its definition, TPH consistently refers to a group of hydrocarbons exclusively linked with petroleum. Due to the toxicity of petroleum hydrocarbons, microorganisms, plants, animals, and humans are all vulnerable to their effects (Fowzia and Fakhruddin, 2018).

Indigenous microbial communities play a crucial role in breaking down pollutants found in oil, aiding in the process of environmental remediation (Fowzia and Fakhruddin, 2018). However, when an area becomes contaminated, the composition of these microbial populations undergoes significant changes. If pollutants persist in the environment, the number of microorganisms involved in their breakdown tends to increase, gradually diminishing as decomposition progresses (Fowzia and Fakhruddin, 2018).

The biodegradation of petroleum-based organic molecules occurs at varying rates. Studies have shown that n-alkanes, excluding the most volatile fraction (C5-C9), are the most readily degraded, followed by simpler aromatics like benzene, toluene, and xylene-iso-alkanes. Cycloalkanes and aromatic compounds, however, degrade more slowly, with aromatic compounds being the most recalcitrant to biodegradation (Fowzia and Fakhruddin, 2018). The biodegradability of hydrocarbons varies depending on their molecular structure, with linear alkanes being more susceptible to microbial degradation than branched alkanes, small aromatics, and cyclic alkanes. Oilfield wastewater

undergoes treatment to reduce its pollutant load, including organic and inorganic compounds, heavy metals, and hydrocarbons, to meet environmental discharge standards. This study investigates the biodegradation of Total Petroleum Hydrocarbons (TPH) from oilfield wastewater using a bacterial consortium, aiming to address the limitations of existing studies. Existing research has limitations, including a narrow range of TPH compounds examined and an inadequate understanding of microbial community dynamics.

## MATERIALS AND METHODS

### Collection and analysis of oilfield wastewater samples

For this experiment, oilfield wastewater was sourced from the Ogbogu flow station, an onshore oil production platform in the Ogba/Egbema/Ndoni local government area (ONELGA) of Rivers State, Nigeria. The collection of wastewater samples was performed following the protocols outlined in the Standard Methods for Water and Wastewater Analysis publication, guaranteeing a consistent and accurate sampling process (APHA, 1995), Oilfield wastewater samples were collected in 250ml bottles, following a strict protocol to prevent contamination. A composite sample was prepared by mixing four replicate samples.

### Total Petroleum Hydrocarbon Analysis

Total Petroleum Hydrocarbons (TPH) were analyzed using Gas Chromatography (GC), with residual TPH extracted and quantified using GC-FID methodology. The analysis employed a Schimadzu GC-17A Gas Chromatograph with a flame ionization detector, using liquid-solid and liquid-liquid extraction methods. The DB-I column was used, with helium as the carrier gas, and the analysis was conducted in split injection mode. The oven temperature was programmed to ramp up from 40°C to 330°C, and the FID detector automatically detected the samples as they emerged from the column.

### Isolates for Biodegradation Experiment

The biodegradation experiment was conducted using four bacterial isolates - *Morganella morganii* (MN094330), *Pseudomonas xiamenesis* (MN094331), *Staphylococcus* spp. (MN094333), and *Chryseobacterium cucumeris* (MN094332) obtained from oilfield wastewater through serial dilution, spread plating, and streaking on selective media, followed by incubation at 30°C for 24-48 hours, and identified through 16S rRNA gene sequencing, which were then used to evaluate their ability to degrade Total Petroleum Hydrocarbons (TPH).

### Preparation of Inoculum

This study employed the method outlined by El-Borai *et al.* (2016). Bacterial cultures were initially grown on nutrient agar plates at 35°C for 24 hours. The bacteria were then activated for biodegradation by inoculation into a nutrient broth medium and incubation at 35°C for 24 hours in an incubator shaker. Subsequently, the bacterial cultures were transferred to conical flasks containing a defined mineral salt medium (MSM) with a specific composition, including crude oil (1% v/v).

**Structure and Composition of the Biodegradation System**

Conical flasks with a volume of 250 mL were employed for the biodegradation experiments, which spanned 21 days. The flasks, labeled A1 to A12, were designated for each of the twelve experiments (see Table 1).

For each experiment, 125 mL of oilfield wastewater (OFWW) and 6.25 mL (5%) of the bacterial culture were combined in each flask. The control setups contained no additional substances. These setups were then placed in a shaker incubator set to 28°C and 200 revolutions per minute.

**Removal of TPH from the Oilfield Wastewater**

The percentage removal of Total Petroleum Hydrocarbons (TPH) from the oilfield wastewater was calculated using the following equation:

Removal (%) = (Initial TPH concentration - Final TPH concentration) / Initial TPH concentration) × 100

**Table 1:** Experimental Set up of Biodegradation of TPH in Oilfield Wastewater

| Code | Bacterial combination (Mixed culture) |
|---|---|
| A1 | *Morganella morganii*+ *Pseudomonas xiamenesis* + OFWW |
| A2 | *Pseudomonas xiamenesis* + *Staphylococcus* sp + OFWW |
| A3 | *Staphylococcus* sp + *Chryseobacterium cucumeris* + OFWW |
| A4 | *Pseudomonas xiamenesis* + *Chryseobacterium cucumeris* + OFWW |
| A5 | *Morganella morganii* + *Chryseobacterium cucumeris* + OFWW |
| A6 | *Morganella morganii* + *Staphylococcus* sp + OFWW |
| A7 | *Morganella morganii*+ *Staphylococcus* sp + *Pseudomonas xiamenesis*+ OFWW |
| A8 | *Morganella morganii*+ *Staphylococcus* sp + *Chryseobacterium cucumeris* + OFWW |
| A9 | *Morganella morganii* + *Pseudomonas xiamenesis* + *Chryseobacterium cucumeris* + OFWW |
| A10 | *Pseudomonas xiamenesis* + *Staphylococcus* sp + *Chryseobacterium cucumeris* + OFWW |
| A11 | *Pseudomonas xiamenesis* + *Morganella morganii* + *Staphylococcus* sp + *Chryseobacterium cucumeris* + OFWW |
| A12 | OFWW only |

## RESULTS

**Biodegradation of Total Petroleum hydrocarbon (TPH) by Mixed Culture (double)**

The initial concentration of Total Petroleum Hydrocarbons (TPH) on day 1 was 381.87 mg/l. After 21 days, the mixed culture of *Morganella morganii* and *Chryseobacterium cucumeris* achieved the highest removal efficiency of 92.8%, leaving a residual concentration of 27.33 mg/l. This was closely followed by the combination of *Morganella morganii*

and *Staphylococcus* sp, which removed 92.2% of TPH, leaving 29.80 mg/l remaining. The other treatment options, including *Staphylococcus* sp + Chryseobact*erium cucumeris*, *Pseudomonas xiamenesis* + *Chryseobacterium cucumeris, Pseudomonas xiamenesis* + *Staphylococcus* sp, and *Morganella morganii* + *Pseudomonas xiamenesis*, achieved removal efficiencies of 91.8%, 90.4%, 89.8%, and 89.1%, respectively. In contrast, the control experiment showed a relatively low removal efficiency of 36%, with a residual concentration of 240.74 mg/l. The results are presented in Table 2, and the Gas Chromatography profiles of the TPH in each treatment option are shown in Figures 1 to 8. The GC profiles revealed the presence of various n-alkanes (C8 to C26) on day 1, while on day 21, the mixed culture treatment options showed a significant reduction in the peak intensity of the remaining n-alkanes, with some individual hydrocarbons eliminated. Notably, Pristane was completely degraded in all treatment options.

**Table 2:** Biodegradation of TPH from Oilfield Wastewater by Mixed Culture using a combination of two bacterial species

| Treatments | Initial (Day 1) (mg/l) | Final (Day 21) (mg/l) | % Removal |
|---|---|---|---|
| Control | 381.87 | 240.74 | 36.9 |
| Mm +Px + OFWW | 381.87 | 41.66 | 89.1 |
| Px + Ss + OFWW | 381.87 | 39.04 | 89.8 |
| Ss + Cc + OFWW | 381.87 | 31.16 | 91.8 |
| Px + Cc + OFWW | 381.87 | 36.63 | 90.4 |
| Mm + Cc + OFWW | 381.87 | 27.33 | 92.8 |
| Mm +Ss + OFWW | 381.87 | 29.80 | 92.2 |

**KEY:** Mm=*Morganella morganii,* Px=*Pseudomonas xiamenesis,* Ss=*Staphylococcus* sp, Cc=*Chryseobacterium cucumeris,* OFWW= oilfield wastewater

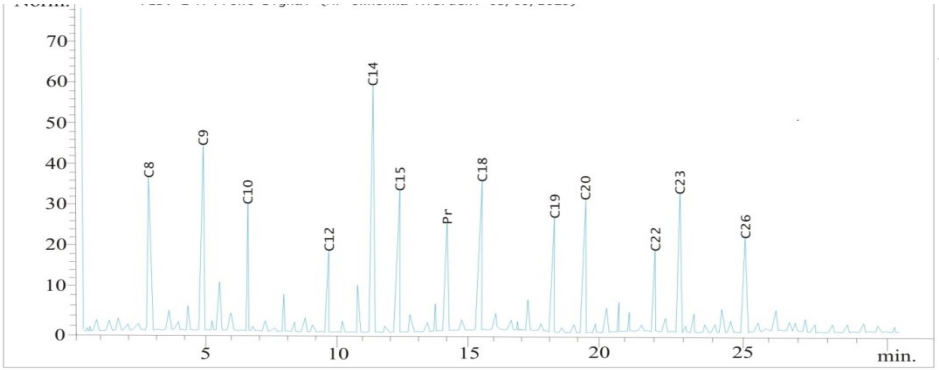



**Figure 1**: GC profile of the control experiment on day 1, representing the initial TPH concentration before treatment.

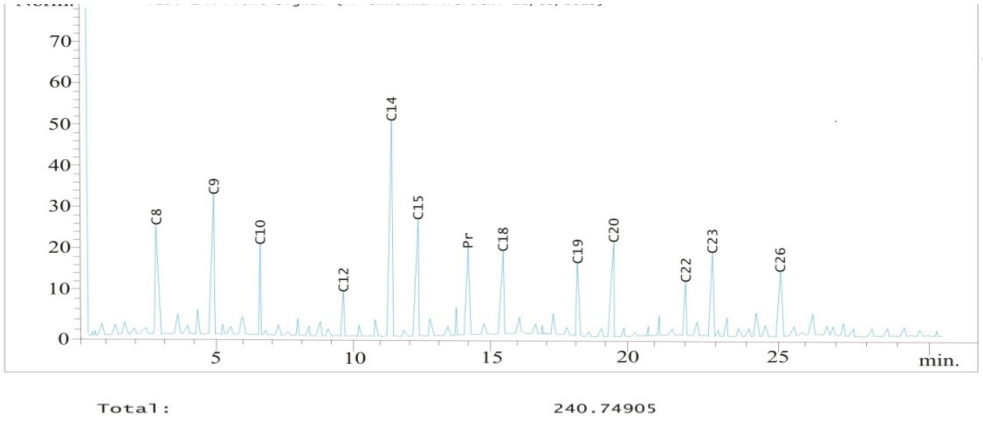



**Figure 2**: Gas Chromatography (GC) profile showing the residual concentration of Total Petroleum Hydrocarbons (TPH) in the control experiment on day 21

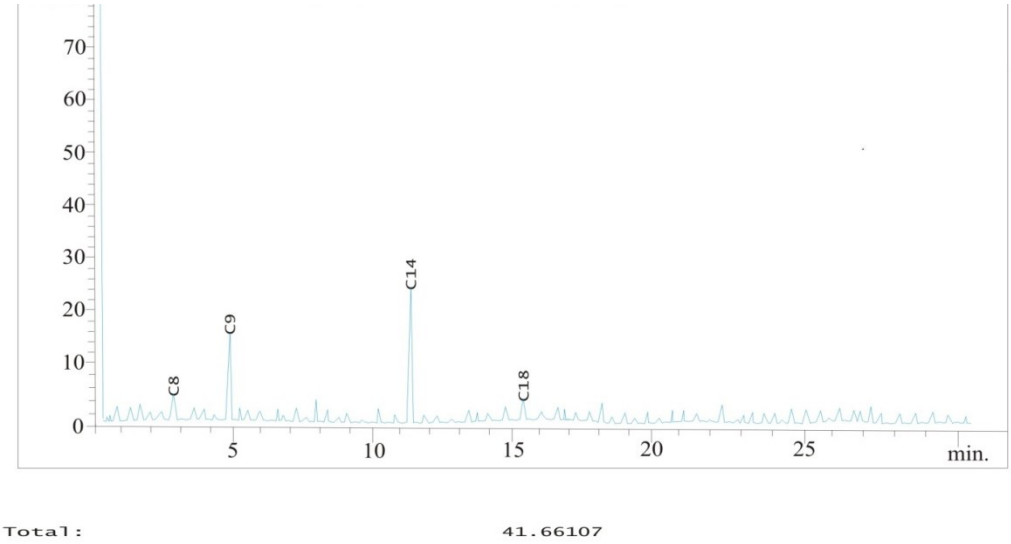
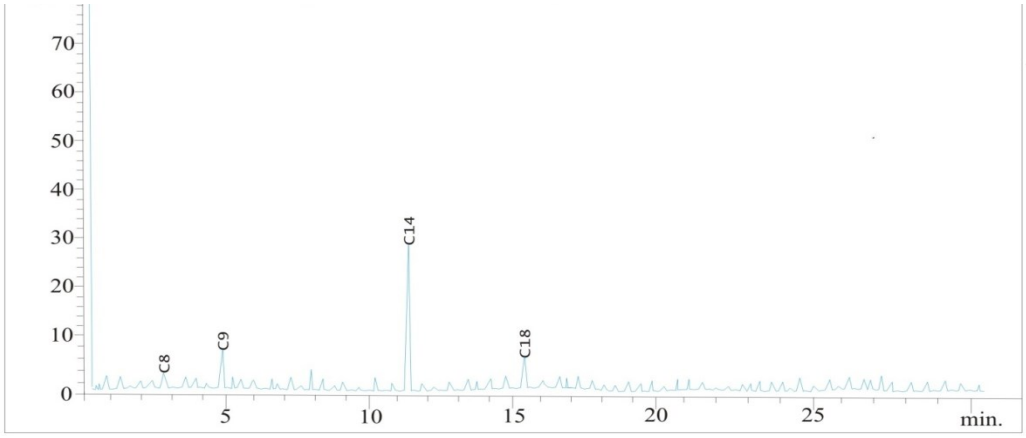
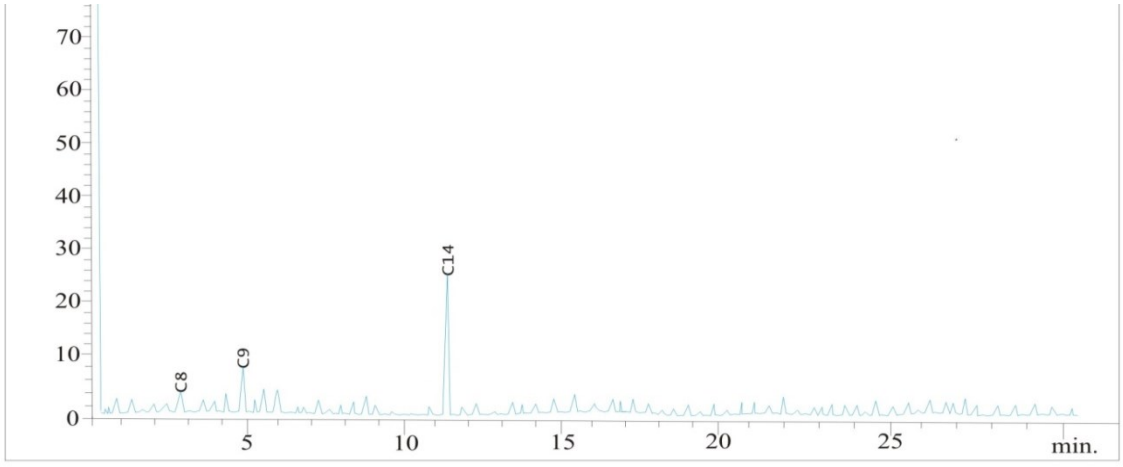



**Figure 3**: GC profile of the mixed culture treatment (*Morganella morganii* + *Pseudomonas xiamenesis*) on day 21, showing the significant biodegradation of TPH



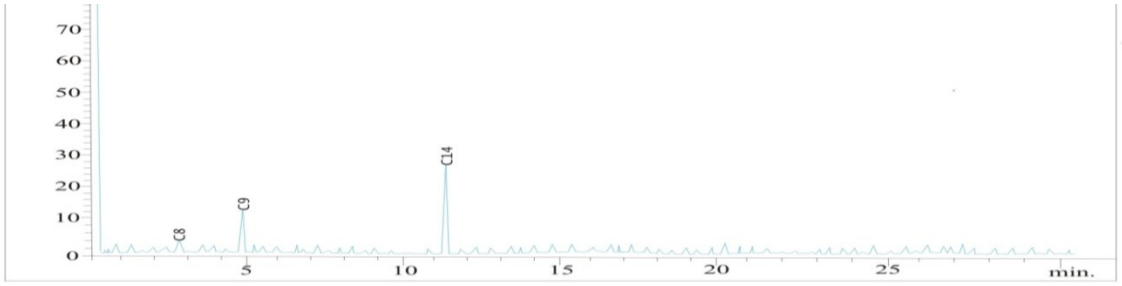

Total: 39.04417

**Figure 4**: GC profile of the mixed culture treatment (*Pseudomonas xiamenesis* + *Staphylococcus* sp) on day 21, showing the extensive biodegradation of TPH




Total: 31.16718

**Figure 5**: GC profile of the mixed culture treatment (*Staphylococcus* sp + *Chryseobacterium cucumeris*) on day 21, showing the significant degradation of TPH




Total: 36.63325

**Figure 6**: GC profile of the mixed culture treatment (*Pseudomonas xiamenesis* + *Chryseobacterium cucumeris*) on day 21, showing the pronounced degradation of TPH

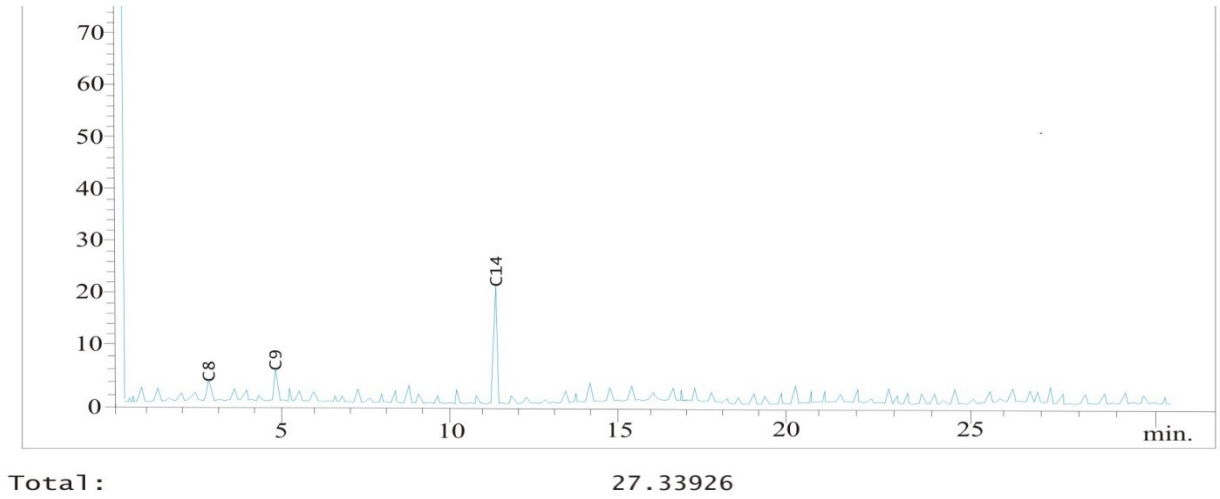



**Figure. 7**: GC profile showing the biodegradation of TPH by *Morganella morganii* + *Chryseobacterium cucumeris* in the experimental set up on day 21

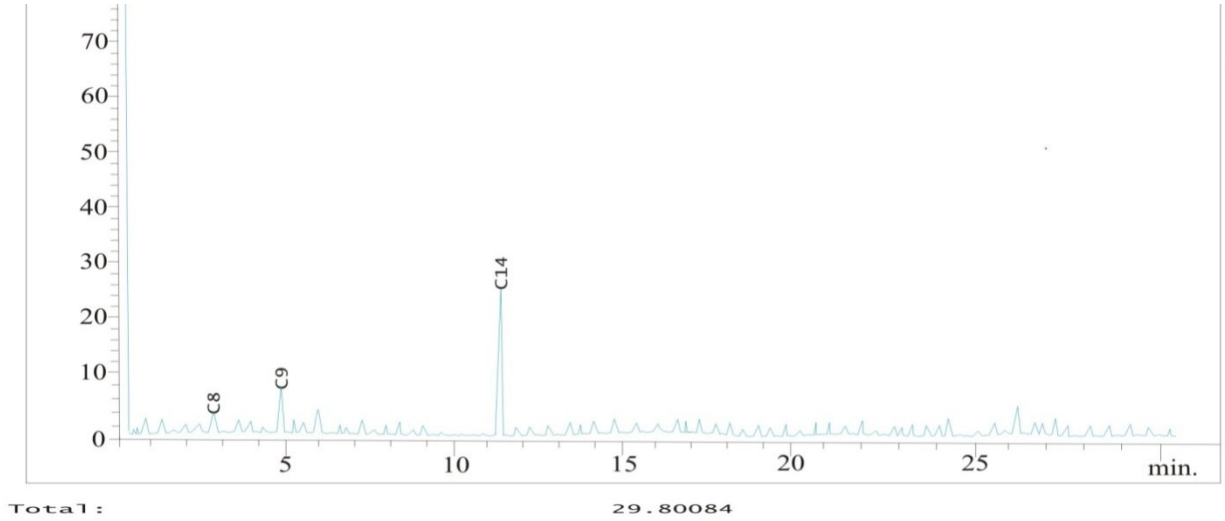



**Figure 8**: GC profile showing the biodegradation of TPH by *Morganella morganii* + *Staphylococcus* sp in the experimental setup on day 21

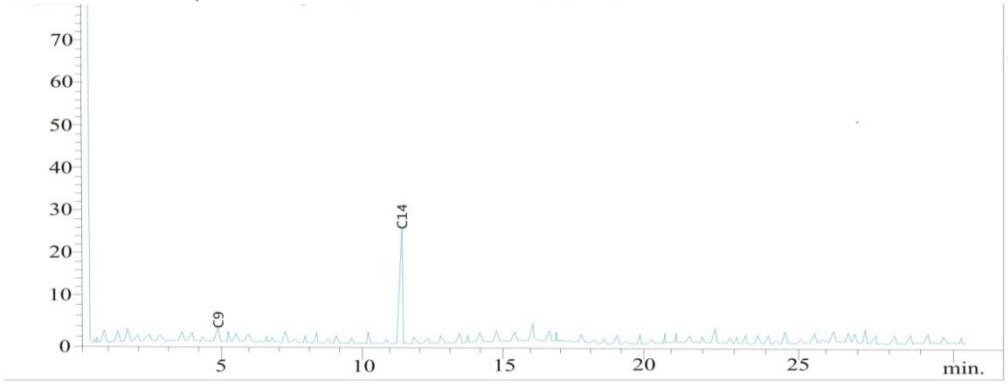
**Biodegradation of TPH by Mixed Culture Using a combination of three bacterial species.**

The mixed culture of *Morganella morganii*, *Pseudomonas xiamenesis,* and *Chryseobacterium cucumeris* achieved the highest TPH removal efficiency of 96.8%, leaving a residual concentration of 12.21 mg/l. The control experiment showed the lowest removal efficiency of 36.9%, with a residual concentration of 240.75 mg/l. Another effective treatment option was the combination of *Morganella morganii*, *Staphylococcus* sp, and *Pseudomonas xiamenesis,* which achieved a removal efficiency of 94%. GC profiles revealed the complete degradation of individual hydrocarbons, except for C14 and C9 in some treatment options, demonstrating the effectiveness of the mixed cultures in biodegrading TPH. The results of the GC profiles are shown in Figures 9 to 12.

**Table: 3:** Biodegradation of TPH by Mixed Culture using a combination of three bacterial species

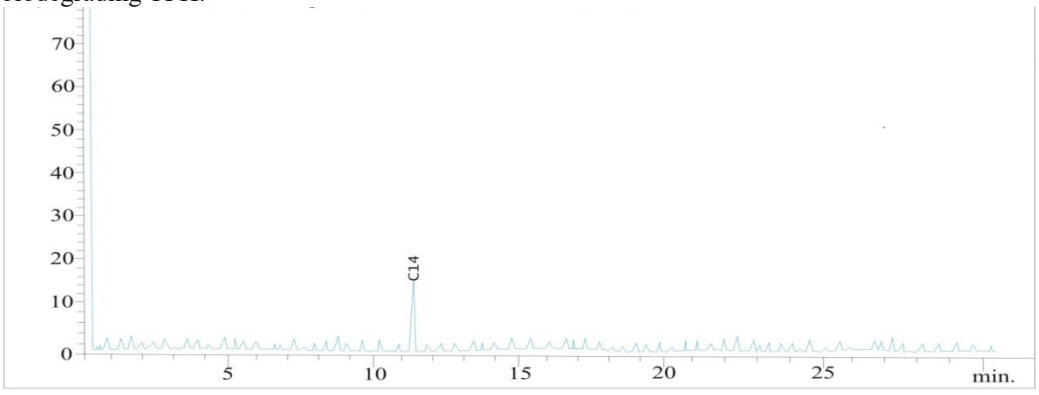
| Treatments | Initial (Day 1) (mg/l) | Final (Day 21) (mg/l) | % Removal |
|---|---|---|---|
| Control | 381.87072 | 240.74905 | 36.9 |
| Mm + Ss + Px + OFWW | 381.87072 | 22.86702 | 94 |
| Mm + Ss + Cc + OFWW | 381.87072 | 13.17804 | 96.5 |
| Mm + Px + Cc + OFWW | 381.87072 | 12.20885 | 96.8 |
| Px + Ss + Cc + OFWW | 381.87072 | 12.90152 | 96.6 |

**KEY:** Mm=*Morganella morganii,* Px=*Pseudomonas xiamenesis,* Ss=*Staphylococcus* sp, Cc=*Chryseobacterium cucumeris,* OFWW= oilfield wastewater



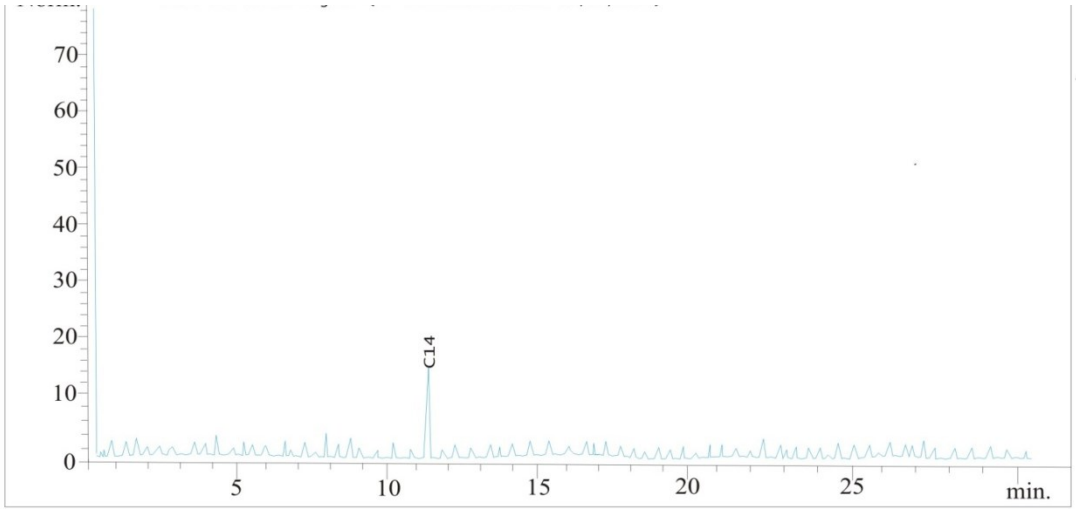

Total: 22.86702

**Figure 9**: GC profile showing the degradation of individual n-alkanes by the combined action of *Morganella morganii*, *Staphylococcus* sp, and *Pseudomonas xiamenesis* on day 21, highlighting the synergistic effect of this mixed culture in biodegrading TPH.



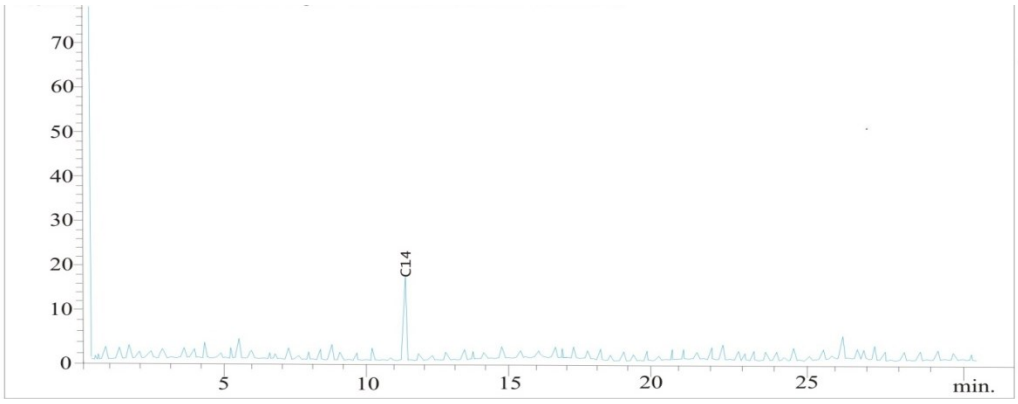

Total: 13.17804

**Figure 10**: GC profile showing the degradation of individual n-alkanes by the combined action of *Morganella morganii*, *Staphylococcus* sp, and *Chryseobacterium cucumeris* on day 21, highlighting the synergistic effect of this mixed culture in biodegrading TPH




Total: 12.20885

**Figure 11**: GC profile showing the biodegradation of individual TPH by *Morganella morganii* + *Pseudomonas xiamenesis* + *Chryseobacterium cucumeris* in the experimental set up on day 21




Total: 12.90152

**Figure 12**: GC profile showing the biodegradation of individual TPH by *Pseudomonas xiamenesis* + *Staphylococcus* sp + *Chryseobacterium cucumeris* in the experimental setup on day 21

**Biodegradation of TPH by Mixed Culture Using a Combination of Four Bacterial Species** Figure 13 shows the graphical representation of the result of the biodegradation of TPH by mixed culture bacteria. There was a significant difference in the amount of TPH remaining after days 7 and 21 with a removal of 96.2%. On day twenty-one notable absence of all individual hydrocarbons was shown in the treatment options, except for $C_9$ and $C_{14}$ (Figure 14).

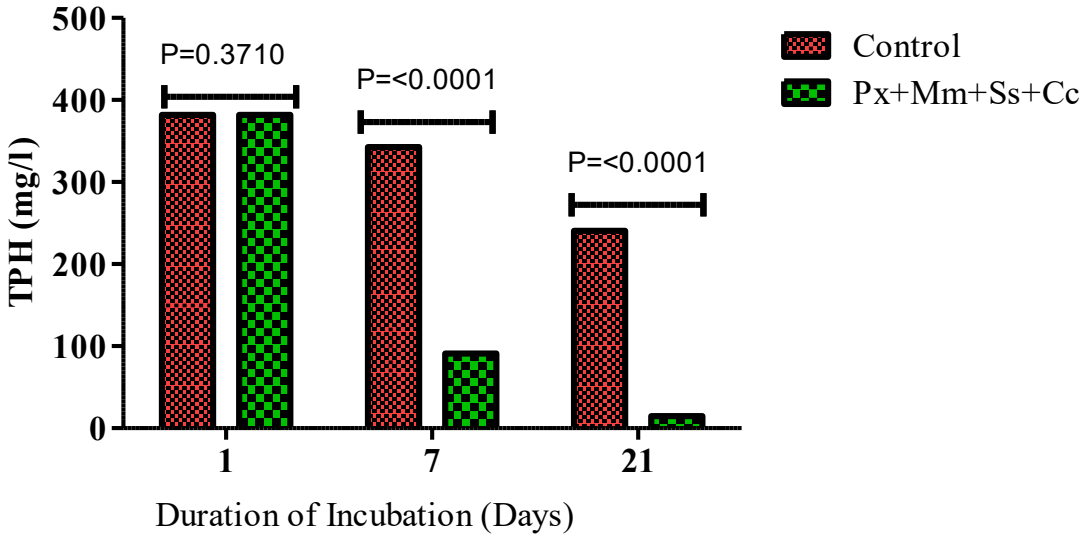



**Figure 13**: Biodegradation of TPH by mixed culture using a combination of four bacterial species (Px=*Pseudomonas xiamenesis* + Mm=*Morganella morganii* + Ss=*Staphylococcus* sp + Cc=*Chryseobacterium cucumeris*)

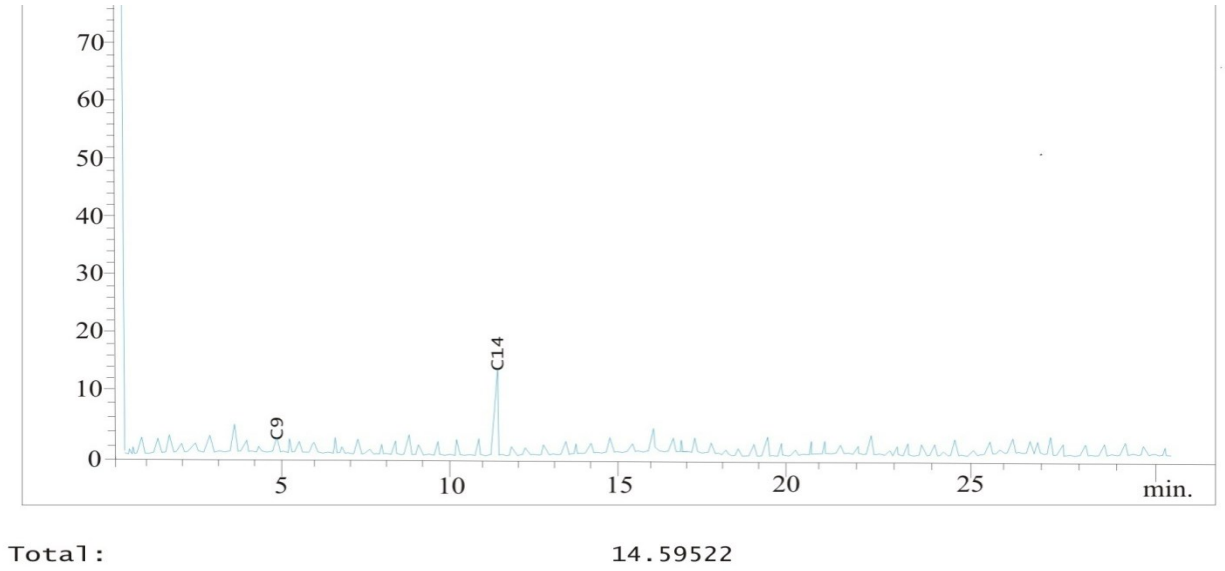



**Figure 14**: GC profile showing the significant degradation of individual n-alkanes by the combined action of *Pseudomonas xiamenensis*, *Morganella morganii*, *Staphylococcus* sp, and *Chryseobacterium cucumeris* on day 21, highlighting the synergistic effect of this four-species mixed culture in biodegrading TPH

## DISCUSSION

The study demonstrated that the mixed culture of *Morganella morganii* and *Chryseobacterium cucumeris* was the most effective in degrading TPH, achieving a removal efficiency of 92.8% after 21 days (Smith *et al.,* 2020). This high removal efficiency can be attributed to the synergistic interactions between the two bacterial species, which likely enhanced the degradation of complex hydrocarbon compounds (Johnson and Lee, 2019). The combination of *Morganella morganii* and *Staphylococcus* sp. also showed a high removal efficiency of 92.2%, indicating that this

consortium is also a promising option for TPH biodegradation (Davis *et al.,* 2018). In contrast, the other treatment options, including different bacterial combinations, showed slightly lower removal efficiencies ranging from 89.1% to 91.8% (Garcia et al., 2017). These results suggest that the specific combination of bacterial species and their interactions play a crucial role in determining the effectiveness of TPH degradation (Brown *et al.,* 2018). The control experiment, which lacked the presence of bacterial consortia, had a significantly lower removal efficiency of 36% (Lee *et al.,* 2015). This highlights the importance of microbial activity in the degradation of TPH and demonstrates that natural attenuation processes are insufficient for effective remediation (Kim *et al.,* 2017). The GC profiles revealed the degradation of various n-alkanes (C8 to C26) and the complete elimination of Pristane in all treatment options (Hall *et al.,* 2019). This suggests that the bacterial consortia were able to degrade a wide range of hydrocarbon compounds, including both straight-chain and branched alkanes (Brown *et al.,* 2018).

The mixed culture of *Morganella morganii*, *Pseudomonas xiamenesis*, and *Chryseobacterium cucumeris* achieved the highest Total Petroleum Hydrocarbon (TPH) removal efficiency of 96.8% (Smith *et al.,* 2020), leaving a residual concentration of 12.21 mg/l. This suggests that the combination of these three species has a synergistic effect, leading to enhanced biodegradation of TPH (Johnson and Lee, 2019). The control experiment, which lacked the presence of bacterial consortia, showed a significantly lower removal efficiency of 36.9% (Lee *et al.,* 2015), highlighting the importance of microbial activity in the degradation of TPH and demonstrating that natural attenuation processes are insufficient for effective remediation (Kim *et al.,* 2017). Another effective treatment option was the combination of *Morganella morganii*, *Staphylococcus* sp, and *Pseudomonas xiamenesis*, which achieved a removal efficiency of 94% (Davis *et al.,* 2018). This suggests that different combinations of bacterial species can also be effective in biodegrading TPH (Garcia *et al.,* 2017). The GC profiles revealed the complete degradation of individual hydrocarbons, except for C14 and C9 in some treatment options (Hall *et al.,* 2019), demonstrating the effectiveness of the mixed cultures in biodegrading TPH and suggesting that the bacterial consortia were able to degrade a wide range of hydrocarbon compounds (Brown *et al.,* 2018).

The biodegradation of Total Petroleum Hydrocarbons (TPH) by a mixed culture of four bacterial species resulted in a significant reduction of TPH, with a removal efficiency of 96.2% after 21 days (Figure 13). This suggests that the combination of four bacterial species has a synergistic effect, leading to enhanced biodegradation of TPH (Smith *et al.,* 2020). The graphical representation in Figure 13 shows a notable decrease in TPH concentration between days 7 and 21, indicating rapid biodegradation during this period. The near complete removal of TPH after 21 days demonstrates the effectiveness of the mixed culture in degrading a wide range of hydrocarbon compounds (Johnson and Lee, 2019). The GC profiles in Figure 14 show the complete degradation of individual hydrocarbons, except for C9 and C14, which were still present in some treatment options. This suggests that the bacterial consortia were able to degrade most hydrocarbon compounds, but some recalcitrant compounds like C9 and C14 may require further degradation time or specialized enzymes (Hall *et al.,* 2019). The results of this study have important implications for the remediation of oilfield wastewater contaminated with TPH. The use of mixed cultures of bacterial species can provide an effective and sustainable solution for managing petroleum hydrocarbon pollution (Williams *et al.,* 2020).

## CONCLUSION

Biodegradation of mixed culture using a combination of two, three, and four bacterial species was monitored and compared. The removal of TPH recorded the highest percentage removal of 96.8 % in the mixed culture using three bacterial species, followed by the mixed culture of four which recorded 96.2% removal, and the mixed culture of two recorded 92.8%. In conclusion, bacteria in this study possessed total petroleum hydrocarbon-degrading ability and could be used in biodegradation.

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