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Monitoring the Hydrocarbon-Utilizing Bacteria in Crude Oil Remediation Using Biostimulant of Mint Leaf (*Mentha piperita*)

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Abstract

The conceptual monitoring of hydrocarbon-utilizing bacteria (HUB) during crude oil bioremediation using a mint leaf (Mentha piperita) biostimulant was investigated. The nutrient composition of the biostimulant was evaluated by determining the available nitrogen, phosphorus, potassium (NPK), and the presence of HUB in both room-dried and sun-dried samples. Mint leaves (Mentha piperita) were processed into a fine powder (nano-sized particles) to prepare the biostimulant. Microorganisms isolated and identified from crude oil-contaminated loamy soil were classified as HUB and fungi. Microbial performance was evaluated using different bioreactors designated A1, A2, B1, B2, and C. Bioreactors A1 and A2 contained room-dried biostimulant at dosages of 50 g and 100 g, respectively, whereas B1 and B2 contained sun-dried biostimulant at the same dosages. Bioreactor C served as the control. Microbial activity was monitored for 35 days, during which the concentration of HUB was determined. Higher microbial growth was observed in the bioreactors containing the room-dried biostimulant than in those containing the sun-dried biostimulant. This variation was attributed to differences in the physicochemical properties and NPK nutrient composition of the processed mint leaf (Mentha piperita) powder. The conceptual growth behavior of the HUB was monitored from Day 7 to Day 35 and was characterized by distinct growth phases. The observed pattern included the absence of a lag phase, followed by acceleration, progressive, stationary, and decline (death) phases. The greatest increase in bacterial population occurred during the acceleration and progressive phases. This rapid microbial growth enhanced the efficiency of crude oil bioremediation, and microbial activity was not adversely affected by environmental factors. Overall, the study demonstrated that the nutrient content of mint leaf (Mentha piperita) effectively enhanced the bioremediation of crude oil-contaminated soil by stimulating the growth and activity of hydrocarbon-utilizing microorganisms.

Keywords: Biostimulant, crude oil, HUB, *Mentha piperita*, mint leaf, monitoring

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INTRODUCTION

Various studies have investigated the remediation of crude oil-contaminated soil, water, and air using different remediation strategies. A wide range of remediants has been applied for the treatment of contaminated sites through either in situ or ex situ approaches [1, 2]. Several researchers have employed organic materials, inorganic materials, and combinations of both for crude oil remediation, demonstrating varying degrees of effectiveness [3]. Further studies have developed bioremediation models using powdered Moringa oleifera seed shells, yeast, and NPK fertilizer to remediate crude oil-contaminated swampy soil [4, 5]. These investigations focused on biokinetic modeling to determine key functional parameters, including the specific rate of total petroleum hydrocarbon (TPH) degradation, the maximum specific TPH degradation rate, and the equilibrium constant for TPH degradation [6–8].

Other researchers have investigated the use of various plant-derived materials to enhance the remediation of crude oil-contaminated soils. Their findings demonstrated that plant extracts can serve as effective and environmentally friendly raw materials for bioremediation applications [9–13]. Numerous studies have also evaluated the use of plant extracts, animal wastes, and other biomaterials, including mushrooms, as nutrient-supplying agents to stimulate microbial activity during the bioremediation of crude oil-contaminated soils [14–19].

However, limited information is available regarding the use of mint leaves (*Mentha piperita*) as a biostimulant for the remediation of crude oil-contaminated soils. Therefore, this study investigated the effectiveness and potential of powdered mint leaves (*Mentha piperita*) as a nutrient source for enhancing the bioremediation of crude oil-contaminated loamy soil.

MATERIALS AND METHODS

Materials Used in Setting Up the Experiment

The following materials were used for the bioremediation of crude oil-contaminated soil: loamy soil, crude oil, plastic containers, and a biostimulant.

Experimental Procedures

One kilogram of loamy soil was placed into plastic containers labeled A1, A2, B1, B2, and C. Each reactor was contaminated with 100 mL of crude oil. Reactors A1 and B1 each received 50 g of the biostimulant, whereas reactors A2 and B2 each received 100 g. The biostimulant used in reactors A1 and A2 was prepared from room-dried mint leaves, whereas that used in reactors B1 and B2 was prepared from sun-dried mint leaves. Reactor C served as the control and did not receive any biostimulant. Soil samples were collected at 7-day intervals for microbial analysis to isolate and identify the hydrocarbon-utilizing microorganisms.

Microbial Analysis

Samples were collected from the different bioreactor setups, and the microorganisms were isolated and identified using the methods described by Buchanan et al. [15] and Gerhardt et al. [16].

RESULTS AND DISCUSSION

The results obtained from this investigation are presented in the following figures. The observed trends demonstrate distinct microbial growth phases during the bioremediation process, indicating that changes in remediation efficiency were closely associated with microbial activity.

Figure 1 compares the hydrocarbon-utilizing bacterial (HUB) counts in bioreactors treated with 50 g and 100 g of the biostimulant, along with the untreated control. The results showed that microbial growth increased in reactors A1 and A2, whereas only slight growth was observed in the control reactor (C). The enhanced bacterial growth in the treated reactors indicates that the biostimulant provided nutrients that promoted microbial proliferation. The order of bacterial growth was HUB A2 (100 g) > HUB A1 (50 g) > HUB C (control).

Figure 2 compares the hydrocarbon-utilizing fungi (HUF) isolated from the different bioreactors and their ability to degrade TPH. The results demonstrated that fungal growth increased with increasing biostimulant dosage, following the order HUF A2 (100 g) > HUF A1 (50 g) > HUF C (control). In the treated reactors (A1 and A2), no lag phase was observed; instead, the microbial growth progressed through the progressive, stationary, and decline (death) phases. In the control reactor, no lag phase was observed, and only a slight increase occurred during the progressive phase before the stationary and decline phases.

Figure 3 compares the growth characteristics of hydrocarbon-utilizing bacteria (HUB) in response to nutrients supplied by the mint leaf (*Mentha piperita*) biostimulant. The comparison includes bioreactors treated with 100 g and 50 g of the biostimulant, as well as the untreated control. The results showed that bacterial growth followed the order HUB B2 (100 g) > HUB B1 (50 g) > HUB C (control). Similar to the previous observations, no lag phase was detected. Instead, the microbial population progressed through the progressive, stationary, and decline (death) phases, demonstrating the effectiveness of the biostimulant in supplying nutrients that enhanced the growth and activity of HUB.

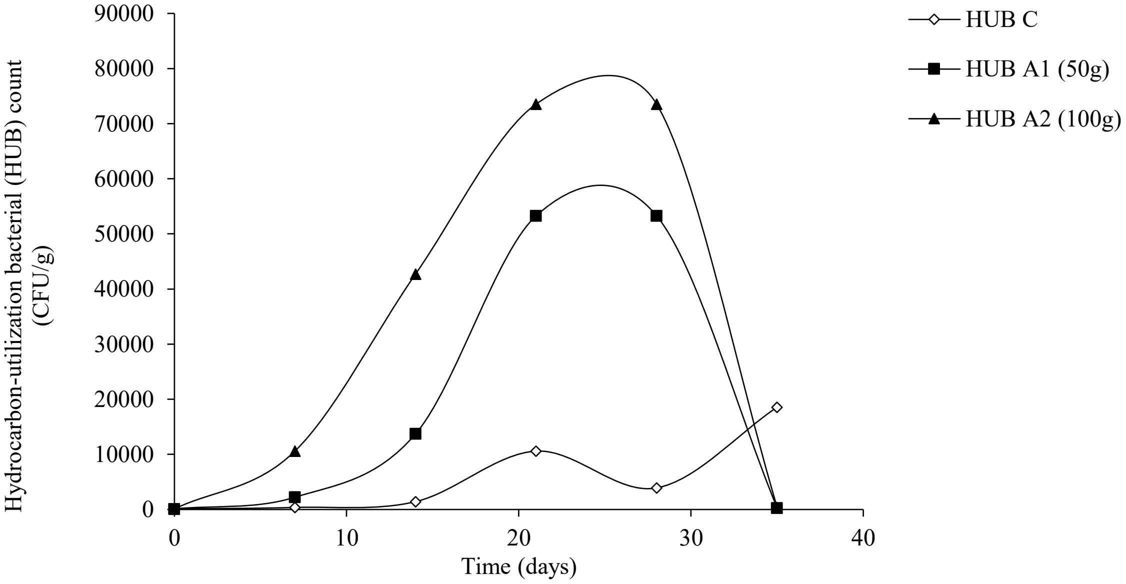

Figure 1. Comparison of hydrocarbon-utilizing bacterial (HUB) counts versus time in sun-dried *Mentha piperita* biostimulant treatments at 50 g and 100 g dosages, with the control.

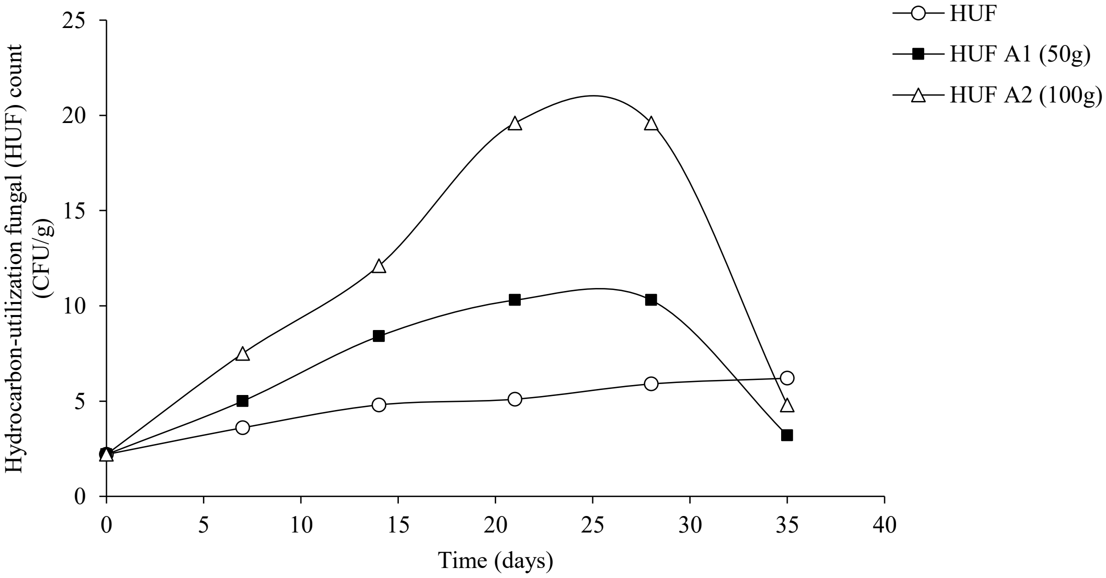

Figure 2. Comparison of hydrocarbon-utilizing fungal (HUF) counts versus time in sun-dried *Mentha piperita* biostimulant treatments at 50 g and 100 g dosages, compared with the control.

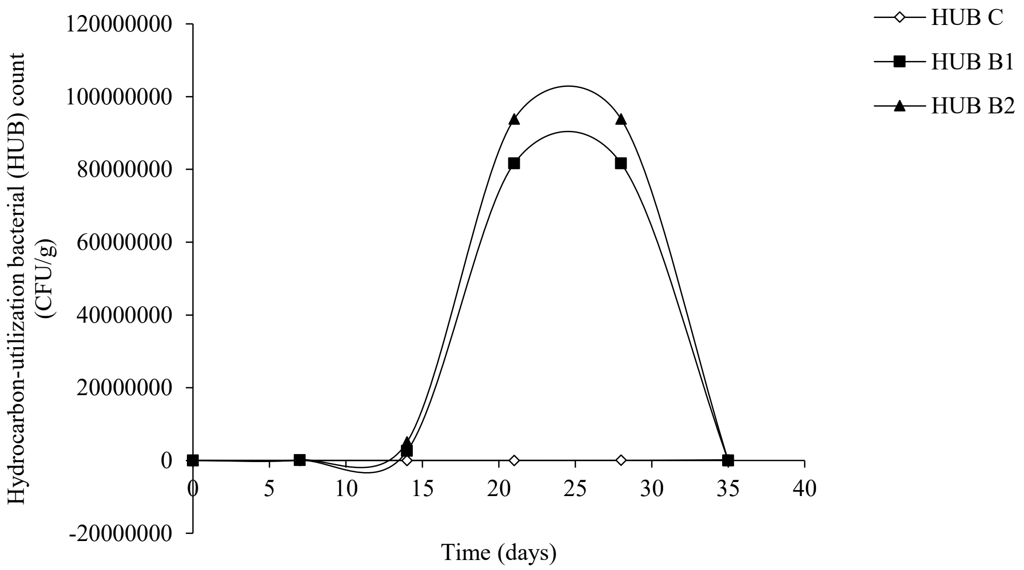

Figure 3. Comparison of hydrocarbon-utilizing bacterial (HUB) counts versus time in room-dried *Mentha piperita* biostimulant treatments at 50 g and 100 g dosages, compared with the control.

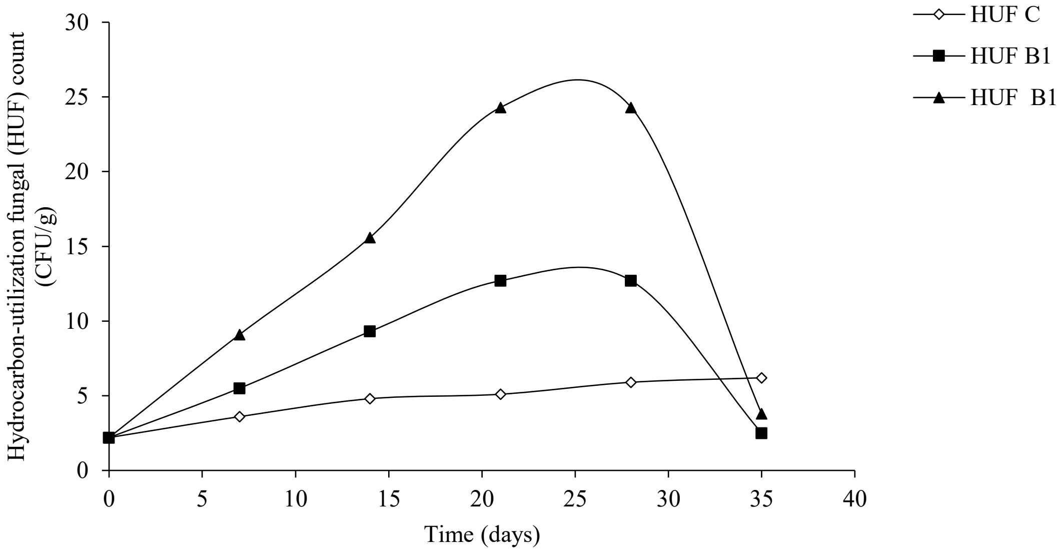

Figure 4. Comparison of hydrocarbon-utilizing fungal (HUF) counts versus time in room-dried *Mentha piperita* biostimulant treatments at 50 g and 100 g dosages, compared with the control.

Figure 4 shows the trend in hydrocarbon-utilizing fungal (HUF) growth in bioreactors B1, B2, and the control (C) over a 35-day incubation period. The results revealed an increase in microbial counts from the beginning of the experiment to Day 21, with no observable lag phase. The progressive growth phase occurred from Day 7 to Day 21, followed by a stationary phase between Days 21 and 28 and a decline (death) phase from Day 28 to Day 35. The effect of biostimulant dosage was also evident, with fungal growth following the order HUF B2 (100 g) > HUF B1 (50 g) > HUF C (control).

CONCLUSION

Room-dried and sun-dried mint leaves (*Mentha piperita*), investigated in the present study, were found to support a consortium of hydrocarbon-degrading microorganisms, including heterotrophic bacteria and fungi. In addition to providing a suitable habitat for these microorganisms, the mint leaf biostimulant supplies essential macro- and micronutrients that stimulate indigenous hydrocarbon-degrading microorganisms and enhance the overall microbial community involved in bioremediation. Furthermore, the observed treatment efficiency may be partially attributed to the introduction of beneficial, non-genetically modified microorganisms associated with the mint leaves, which complement the degradative activities of the indigenous microbial population in the contaminated soil. Based on these findings, the application of *Mentha piperita* leaves as an organic biostimulant for the remediation of crude oil-contaminated soil is recommended during the dry season. During the wet season, increased soil moisture may promote anaerobic conditions that can reduce the activity of aerobic hydrocarbon-degrading bacteria, thereby decreasing the efficiency of the bioremediation process.

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