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Evaluation on Bioremediation Kinetics of Petroleum-Contaminated Soils Using Plant-Based Amendments

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Abstract

The effectiveness of bioremediation processes is strongly influenced by environmental conditions, reactor design parameters, microbial characteristics, and pollutant properties. This study investigates the combined effects of environmental-related factors, reactor design considerations, organism-related characteristics, and pollutant properties on the degradation of total petroleum hydrocarbons (TPH) in swampy and clay soils amended. Laboratory-scale remediation experiments were conducted over 84 days using amendment dosages ranging from 20 to 100 g. The impacts of temperature, pH, nutrient availability, moisture content, electron acceptors, and mass transfer limitations on microbial activity and hydrocarbon degradation were evaluated. Results showed that optimized environmental conditions significantly enhanced microbial metabolism, leading to substantial TPH removal efficiencies. Maximum TPH degradation values of 97.78%, 97.38%, 98.50%, and 97.69% were achieved in mango leaf-treated swampy soil and mango leaf-treated clay soil, respectively, after 42 days. Degradation trends indicated faster TPH removal in clay soils during the first 3 weeks, while swampy soils exhibited superior performance in the later remediation stages. Kinetic analysis using first-order, second-order, and Michaelis–Menten models revealed that the first-order kinetic model provided the most reliable prediction of TPH degradation over time. The findings demonstrate that bioremediation efficiency is governed by the interplay of environmental control, reactor design optimization, microbial adaptability, and pollutant characteristics. The study confirms the viability of plant-based amendments as sustainable, low-cost alternatives for the remediation of petroleum-contaminated soils.

Keywords: Bioremediation, environmental factors, kinetic modeling, plant-based amendments, total petroleum hydrocarbons

INTRODUCTION

Petroleum hydrocarbon contamination of soil environments remains a significant environmental concern due to increased industrial activities associated with oil exploration, transportation, storage, and refining. Accidental spills and chronic leakages have resulted in the widespread contamination of terrestrial and wetland ecosystems, particularly in developing regions. Hydrocarbon-contaminated soils pose serious ecological and public health risks owing to the persistence, toxicity, and bioaccumulative nature of petroleum compounds. Consequently, the development of effective and environmentally sustainable remediation strategies has become a major focus of environmental engineering research [1].

Bioremediation has emerged as a preferred remediation technique owing to its cost-effectiveness, minimal environmental disturbance, and ability to achieve the complete mineralization of pollutants. This process relies on the metabolic

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activity of microorganisms to degrade complex organic contaminants into less harmful products. However, the efficiency of bioremediation is not solely dependent on microbial presence but is strongly influenced by environmental conditions, reactor design parameters, microbial population dynamics, and pollutant characteristics [2–4].

Environmental factors, such as temperature, pH, nutrient availability, moisture content, oxygen supply, and salinity, directly affect microbial growth, enzyme production, and pollutant bioavailability. Temperature influences enzymatic reaction rates, pollutant solubility, and microbial metabolism. Within optimal ranges, increased temperatures enhance biodegradation rates, whereas extreme temperatures inhibit microbial activity. Similarly, pH also affects microbial cell membrane integrity, enzyme stability, and pollutant chemistry. Most hydrocarbon-degrading bacteria thrive under near-neutral pH conditions, although certain fungi and specialized bacteria can operate under acidic or alkaline environments [5–7].

Nutrient availability is another critical factor that governs the efficiency of bioremediation. Hydrocarbon-contaminated soils are often deficient in nitrogen and phosphorus, which are essential for microbial biomass synthesis and energy metabolism. Biostimulation using nutrient-rich organic amendments has been widely reported to significantly enhance biodegradation rates. Moisture content also plays a crucial role, as microbial activity occurs in aqueous environments and water availability influences substrate diffusion and osmotic balance [8].

In addition to environmental conditions, reactor design-related factors determine the extent to which optimal conditions can be maintained during bioremediation. The reactor configuration, mixing efficiency, hydraulic retention time, and mass transfer characteristics influence microbial–substrate contact and process stability. Poor reactor design may result in inadequate aeration, thermal stratification, and microbial washout, thereby reducing remediation efficiency [9].

Organism-related factors, including microbial population density, diversity, and metabolic capabilities, further influence biodegradation outcomes. Indigenous microorganisms that are adapted to contaminated environments often exhibit superior degradation capabilities. Mixed microbial consortia are generally more effective than single strains owing to their synergistic metabolic interactions. Pollutant-related factors such as molecular structure, solubility, concentration, and toxicity also determine biodegradability, with complex and high-molecular-weight hydrocarbons exhibiting greater resistance to microbial attack [10].

Plant-based amendments, such as mango leaves, have gained increasing attention owing to their nutrient content, biodegradability, and local availability. These materials enhance soil fertility, improve microbial growth, and stimulate hydrocarbon degradation. Despite extensive research on bioremediation, few studies have comprehensively evaluated the combined effects of environmental, reactor, organism, and pollutant-related factors on remediation kinetics using plant-based amendments across different soil types [11].

Therefore, this study aimed to evaluate the influence of these factors on TPH degradation in swampy and clayey soils amended with mango leaves. The study further applied kinetic modeling to predict the degradation behavior and identify the most suitable model for describing the TPH removal dynamics.

METHODOLOGY

Materials Preparation

Mango leaves were collected from the Rivers State University Agricultural Farm, whereas swampy and clay soil samples were obtained from the Eagle Island mangrove area in Port Harcourt. Approximately 20 liters of crude oil were sourced from a certified crude oil production company in

the State of the Rivers. All samples were transported to the laboratory for subsequent preparation and analysis.

The mango leaves used as the treatment material were thoroughly washed with distilled water to remove any adhering impurities. The washed leaves were air-dried under sunlight for approximately three days and subsequently oven-dried to remove residual moisture. The dried samples were crushed and sieved to obtain a uniform particle size of 2 mm. The sieved mango leaf biomass was divided into five portions weighing 20, 40, 60, 80, and 100 g for use in the remediation experiments.

Soil pH

The pH values of the soil samples before and during the treatment period were determined under laboratory conditions. For each sample, a 10% (w/v) suspension of air-dried soil was prepared using deionized water and allowed to equilibrate for approximately one hour. The suspension was subsequently filtered through Whatman filter paper, and the pH of the resulting filtrate was measured using a calibrated Hanna HI 2211 pH/ORP meter. All pH measurements were conducted in accordance with ASTM D4972.

Electrical Conductivity

The moisture contents of the soil samples were evaluated using a standard gravimetric technique. For each determination, 10 g of soil was placed in a clean crucible and dried in a laboratory oven at 105°C for 24 h to remove inherent moisture. After drying, the samples were transferred to a desiccator and allowed to cool for approximately 30 minutes. The cooled samples were then re-weighed until a constant mass was achieved. Moisture content was subsequently calculated as a percentage using Equation (1).

$$MC(\%) = \frac{w_1 - w_2}{w_1} \times 100\% \tag{1}$$

Where, MC = moisture content (%), w_1 = initial weight of the soil sample (g), and w_2 = weight of the dried soil sample (g).

Total Organic Content

The total organic carbon (TOC) content was determined using a wet oxidation trimetric procedure. Approximately 1.0 g of finely ground, representative soil samples were weighed in duplicate into separate 250 mL beakers. A potassium dichromate solution (10 mL) was added to each beaker and gently swirled to ensure complete wetting of the soil. Subsequently, 20 mL of concentrated sulfuric acid was carefully introduced using an automatic pipette to direct the acid stream into the soil suspension.

The mixture was gently agitated to promote uniform contact, and then vigorously swirled for approximately one minute to enhance the oxidation efficiency. Beakers were allowed to stand undisturbed for 30 min on an insulated surface. Thereafter, 100 mL of distilled water was added, followed by the introduction of 3–4 drops of the diphenylamine indicator. The resulting solution was titrated with 0.5 N ferrous sulfate until the endpoint was reached. Blank determination, conducted using distilled water instead of soil, was performed under identical conditions to standardize the dichromate solution. The total organic carbon content was calculated using the formula given in Equation (2).

$$TOC = Blank - \frac{volume\,of\ soil\ sample\ titre \times 0.195}{weight\ of\ soil\ sample} \times 100\% \tag{2}$$

Nitrogen Content

The nitrogen content of the soil samples was determined using the Kjeldahl digestion and distillation method in accordance with APHA (1998), Method 4500-NO_3-B. Approximately 10 g of air-dried soil was ground to pass through a 0.5 mm sieve and containing an estimated 10 mg of nitrogen was weighed into a clean, dry 500 mL Macro-Kjeldahl flask. Twenty milliliters of distilled water were added, and the mixture was gently swirled for approximately two minutes before being allowed to stand for up to 30 min.

Following equilibration, a 1 g catalyst tablet consisting of a K_2SO_3–H_2O mixture and an additional 10 g of potassium sulfate was introduced into the flask. This was followed by the careful addition of 30 mL of concentrated sulfuric acid using an automatic pipette. The flask was placed on a digestion stand and gradually heated. As moisture evaporated and frothing subsided, the heating intensity was increased until a clear digest was obtained, ensuring that sulfuric acid reached approximately halfway up the neck of the flask [12].

After digestion, the flask was allowed to cool, and 100 mL of distilled water was slowly added. The digest was then quantitatively transferred into a clean 750 mL Macro-Kjeldahl flask. To prevent bumping during distillation, residual sand particles were retained in the original flask; these residues were rinsed four times with 50 mL of distilled water, and the washings were combined with the main digest.

For ammonia recovery, 50 mL of boric acid indicator solution was placed in a 500 mL Erlenmeyer flask positioned beneath the condenser of the distillation apparatus. The digestion flask was connected to the distillation unit, and approximately 150 mL of 10 N sodium hydroxide was added to the funnel to render the solution alkaline. Distillation continued until 150 mL of the distillate was collected. The ammonium-nitrogen in the distillate was determined by titration with 0.01 N standard sulfuric acid using a burette graduated at 0.1 mL intervals. The endpoint is indicated by a color change from green to pink. The percentage nitrogen content of the soil was calculated using Equation (3):

$$N(\%) = \frac{(T - B) \times N \times 1400}{S} \times 100\% \quad (3)$$

Where, T = Sample Titration (ml), B = Blank Titration (ml), N = Normality of H_2SO_4 and.
S = Sample weight (mg).

Phosphorous Content

The phosphorus content of the soil samples was determined using the American Public Health Association (APHA) standard method (APHA, 1999). Approximately 1.0 g of a representative soil sample was weighed into a clean extraction flask, followed by the addition of 10 mL of Bray P-1 extraction solution composed of 0.025 N hydrochloric acid and 0.03 N ammonium fluoride. The mixture was shaken vigorously for one minute to ensure effective extraction and was subsequently filtered.

An aliquot of 5 mL of the filtrate was transferred into a 25 mL volumetric flask and diluted with distilled water to approximately 20 mL. Thereafter, 4 mL of ascorbic acid reagent, prepared by dissolving 1.056 g of ascorbic acid in 200 mL of molybdate–tartrate solution, was added, and the solution was further diluted to volume. The resulting mixture was allowed to stand for at least 30 min to allow for full color development. The phosphorus concentration was determined after the development of a stable and clear coloration [13].

LITERATURE REVIEW

pH

Similar to temperature, pH plays a critical role in microbial growth and metabolic activity by influencing the ionic properties of microbial cells. Microorganisms exhibit minimum, optimum, and

maximum pH ranges for growth; most bacteria grow optimally between pH 6 and 7.5, although some species, such as acidophiles, prefer acidic conditions, whereas alkaliphiles thrive under alkaline conditions. Fungi generally tolerate lower pH values than bacteria do. In bioreactors, the operating pH must be carefully controlled to provide conditions that maximize microbial growth and enzymatic activity.

The pH also affects pollutant behavior, which in turn influences bioremediation efficiency. For example, the solubility and redox state of metals are pH-dependent, and different metal forms and valences can affect the microbial activity differently. Metal solubility generally increases under acidic conditions, whereas alkaline conditions favor metal precipitation. In some cases, lower pH values facilitate the attachment of metal ions to microbial cell surfaces. Additionally, acid-producing microorganisms can enhance the solubility of metal ions, further influencing the biodegradation processes. To maintain the optimal pH for microbial activity, buffers are often included in the growth medium, and acids or bases can be added during bioreactor operation to adjust the pH as needed [14].

Nutrients

Microorganisms require nutrients for growth and metabolic activity, as these elements are essential for biosynthesis and energy production. Carbon is the primary element required in the largest quantity, forming the basis for all cellular components. Other essential macro- and micronutrients include hydrogen, oxygen, nitrogen, sulfur, phosphorus, iron, calcium, and magnesium, which collectively maintain a balanced nutritional environment in the bioreactors. These nutrients can either be supplied through the reactor media or, in some cases, microorganisms can utilize the pollutants as a primary or supplementary energy source through co-metabolism.

Moisture

Water is a critical factor for microbial growth and enzymatic activity, as most cellular biochemical reactions occur under aqueous conditions. Appropriate water content ensures the maintenance of osmotic balance, which is vital for microbial survival and metabolic efficiency. The availability of water for microbial growth was quantified as water activity. Most microorganisms thrive at water activities ≥ 0.98 or higher, although osmotolerant species can survive under lower water activity conditions [15].

Reactor Design Factors

Bioreactor design directly affects microbial performance and pollutant degradation efficiency. The reactor size, configuration, and mode of operation must ensure the optimal physical, chemical, and biological conditions for effective bioremediation. This includes proper transport of gases, liquids, and solids to maintain uniform conditions for microbial activity and metabolite distribution. Inadequate reactor design, particularly poor mixing, can reduce process stability, promote thermal stratification, and limit the contact between microbes and substrates. Therefore, adequate mixing is critical for maintaining uniform conditions, enhancing mass transfer, and improving microbial process efficiency. Hydraulic retention time (HRT) must also be optimized; excessively long HRTs reduce substrate loading and microbial density, whereas very short HRTs may prevent effective pollutant degradation or lead to washout of microorganisms [16].

Organism-Related Factors

Microbial community composition, population density, and interspecific or intraspecific interactions significantly influence bioremediation outcomes. Microorganisms possess diverse metabolic pathways that enable their adaptation to various ecological conditions, including exposure to xenobiotics. Certain bacteria and fungi have demonstrated superior pollutant-degrading capabilities, and bioremediation may involve single or mixed cultures, depending on the pollutant and environmental context. Bioaugmentation requires that introduced microorganisms coexist with indigenous populations. Screening and selection of strains with high degradation potential, including those that avoid the accumulation of toxic intermediates, are crucial for maximizing bioremediation efficiency.

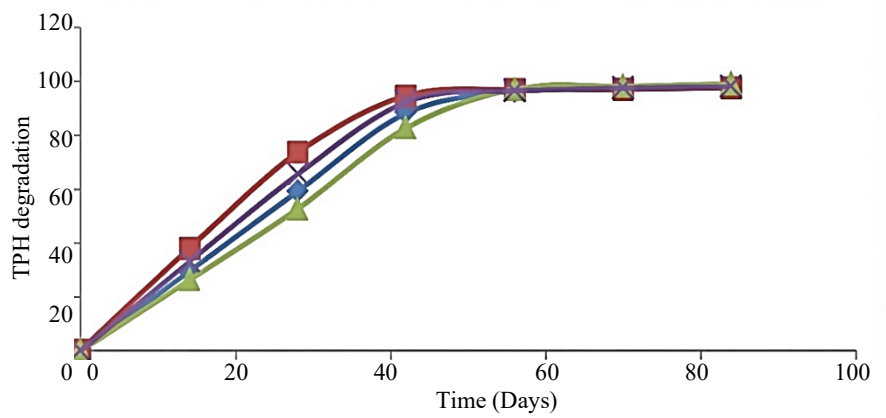

Figure 1. Performance of mango leaf samples in swampy and clay soils after 42 days.

Pollutant-Related Factors

Physicochemical properties of pollutants, such as solubility, volatility, molecular structure, concentration, and toxicity, strongly influence biodegradation. Bioremediation efficiency is affected by pollutant complexity, presence of mixed contaminants, and bioavailability. For example, highly recalcitrant compounds degrade more slowly even under optimal microbial and environmental conditions. Understanding these pollutant characteristics is essential for designing effective bioremediation strategies and selecting appropriate microbial consortia [17].

RESULTS AND DISCUSSION

Figure 1 shows the percentage degradation of TPH in swampy and clay soils obtained at 100 g of the weight of mango leaf treatments over time. The percentage of TPH removed from the soil increased with time from 0% to 97.78%, 97.38%, 98.50%, and 97.69%, respectively.

The results showed that the degradation of TPH in mango leaves was faster in clay soil for the first three weeks, but in the last three weeks, the treatment performance was dominant in swampy soil.

CONCLUSION

This study demonstrated that bioremediation efficiency is governed by a complex interaction of environmental conditions, reactor design parameters, microbial characteristics, and pollutant properties. Optimized temperature, pH, moisture, nutrient availability, and oxygen supply significantly enhanced microbial metabolism and TPH degradation in both the swampy and clay soils. The application of mango leaves effectively stimulated microbial activity, resulting in TPH removal efficiencies exceeding 97% after 42 days.

Soil type influenced the degradation behavior, with clay soils showing faster early-stage degradation and swampy soils dominating in later stages. Kinetic modeling confirmed that first-order kinetics best described the TPH degradation trends, providing a reliable predictive framework for remediation planning.

Overall, these findings confirm the viability of plant-based amendments as sustainable and cost-effective bioremediation agents. This study provides valuable insights into the optimization of bioreactor design and environmental control strategies for petroleum-contaminated soil.

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