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Research Article

Bioremediation of Petroleum-Contaminated Soil Using *Saccharomyces cerevisiae*: A Cost-Effective Nature-Based Approach for Environmental Remediation

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Abstract

The increasing global demand for petroleum as an energy source and industrial raw material has led to intensified activities in its extraction, transportation, and refining, resulting in significant environmental contamination, particularly soil pollution. Petroleum hydrocarbons are persistent in the environment due to their hydrophobic nature and low biodegradability, allowing them to accumulate in soil ecosystems for extended periods. Many petroleum constituents, especially polycyclic aromatic hydrocarbons (PAHs), are toxic and have been associated with carcinogenic, mutagenic, and ecotoxic effects, posing serious risks to human health, terrestrial organisms, and aquatic ecosystems. This study evaluated the potential of *Saccharomyces cerevisiae* for the bioremediation of petroleum-contaminated soil under controlled laboratory conditions over a 28-day period. Soil samples were artificially contaminated and treated with different concentrations of yeast (1% and 2%), while a control flask received no microbial treatment. Total petroleum hydrocarbons (TPH) were quantified using EPA Method 418.1 and spectrophotometric analysis at 320 nm. The results demonstrated a significant reduction in hydrocarbon concentration in yeast-treated samples compared to the control. The highest degradation was observed in the flask treated with 2% *S. cerevisiae*, indicating that increased microbial concentration enhances biodegradation efficiency. These findings suggest that *S. cerevisiae* has strong potential as a cost-effective and environmentally friendly agent for the bioremediation of petroleum-contaminated soils.

1. Introduction

Soil contamination caused by oil spills is a major global environmental challenge in the contemporary world. Petroleum-contaminated soils pose serious risks to human health, contribute to groundwater pollution that limits its usability, reduce agricultural productivity, and lead to significant economic losses. Exposure to petroleum hydrocarbons may occur through direct contact with contaminated soil, inhalation of volatile compounds, or ingestion of contaminated water derived from polluted subsurface sources. The toxic effects of petroleum hydrocarbons on microorganisms, plants, animals, and humans are well documented [1, 2]. Petroleum contamination primarily results from

activities such as oil extraction, refining, transportation, storage, and accidental spills. Because petroleum products are often transported over long distances through pipelines and maritime tankers, they are highly susceptible to leakage and accidents. This risk is further intensified by the geographical mismatch between oil-producing regions and major consumer markets, necessitating extensive global transportation networks.

It has long been established that certain microorganisms possess the ability to degrade petroleum hydrocarbons and utilize them as their sole source of carbon and energy [3]. As a result, various remediation strategies have been developed for petroleum-contaminated soils, including physical, chemical, and biological methods. Among these, biological approaches are generally considered more cost-effective, environmentally friendly, and sustainable [3, 4].

Bioremediation techniques aim to enhance the natural degradation of hydrocarbons in soil through microbial activity. However, the effectiveness of microbial degradation is often limited by the low solubility, high molecular weight, and strong adsorption of certain hydrocarbon fractions, which reduce their bioavailability to microorganisms. Microbial degradation involves the production of specific enzymes that catalyze the breakdown of hydrocarbon compounds through complex metabolic pathways. Nevertheless, the absence or insufficiency of key enzymes may slow down or inhibit complete degradation of petroleum hydrocarbons [5, 6].

Bioremediation is defined as the process by which living organisms, particularly microorganisms, are used to detoxify and degrade environmental pollutants into less harmful forms. Microorganisms play a crucial role in ecosystem nutrient cycling by utilizing contaminants as sources of carbon and energy for growth and metabolism. In this study, *Saccharomyces cerevisiae* (yeast) is proposed as a biological agent for the degradation of petroleum hydrocarbons due to its metabolic adaptability and potential biotechnological applications [7, 8].

2. Methods

2.1. Preparation of Soil Samples

Soil samples were collected from the IUKL campus at a depth of 20–40 cm below the soil surface to minimize surface contamination. The collected samples were air-dried for 48 hours at room temperature and subsequently homogenized by passing through a 2 mm sieve to remove debris and ensure uniform particle size. The dried soil was then transferred into clean glass beakers and examined for the possible presence of hydrocarbons. This was performed by mixing the soil with distilled water and allowing it to settle for 24-48 hours. The supernatant was visually inspected for the presence of hydrocarbons, which is indicated by the formation of a thin oily layer or iridescent (rainbow-like) film on the water surface. Following preliminary assessment, the soil samples were sterilized at 65°C for 24 hours to eliminate indigenous microbial activity prior to experimental contamination.

2.2. Soil Contamination with Petroleum

The sterilized soil samples were artificially contaminated with petroleum to simulate polluted conditions. A total of 200 g of soil was thoroughly mixed with 60 mL of petroleum in sterile containers. The mixture was homogenized to ensure even distribution of hydrocarbons throughout the soil matrix.

2.3. Preparation of *Saccharomyces cerevisiae*

Saccharomyces cerevisiae was obtained from a glycerol stock stored at -80°C. The culture was revived by inoculating into YPD (Yeast Extract Peptone Dextrose) broth and incubated at 37°C for 24 hours. After incubation, colonies were streaked onto YPD agar plates to obtain pure cultures. Morphological identification was confirmed by microscopic observation of single colonies to ensure culture purity before further use.

2.4. Viable Cell Count

The viable cell concentration of *S. cerevisiae* was determined using serial dilution techniques. A 1:100 dilution was prepared by adding 1 mL of yeast culture to 99 mL of sterile diluent. The diluted suspension was then loaded into a hemocytometer (counting chamber), and viable cells were counted under a microscope. The cell concentration was calculated and used to standardize inoculum size for biodegradation experiments.

2.5. Biodegradation Experiment Setup

Biodegradation studies were conducted using three conical flasks, each containing 100 mL of Mineral Salt Medium (MSM) and 20 g of petroleum-contaminated soil.

The experimental setup was as follows:

- Flask A (Control): MSM + contaminated soil (no yeast)
- Flask B: MSM + contaminated soil + 1% (v/v) *S. cerevisiae*
- Flask C: MSM + contaminated soil + 2% (v/v) *S. cerevisiae*

All flasks were incubated at 30°C for 28 days under controlled laboratory conditions. The experiment was performed following the method described by [9].

2.6. Determination of Petroleum Hydrocarbon Degradation (EPA Method 418.1)

Residual petroleum hydrocarbons were quantified using EPA Method 418.1, which is based on spectrophotometric determination of Total Petroleum Hydrocarbons (TPH). Samples were collected at 7-day intervals (Day 0, 7, 14, 21, and 28). Each sample was treated with sodium

sulfate and silica gel to remove moisture and polar impurities. The samples were then centrifuged for 10–20 minutes, followed by filtration into clean glass cuvettes. Absorbance was measured using a spectrophotometer, and TPH concentrations were calculated based on standard calibration curves. The percentage degradation of petroleum hydrocarbons was determined and plotted over the 28-day incubation period to compare treatment efficiency.

3. Result

Table 1: Samples for the degradation part

Flask A	Flask B	Flask C
MSM media + 20g of contaminated soil	MSM media + 1% of yeast + 20g of soil contaminated petroleum hydrocarbons	MSM Media + 2% of yeast + 20g of soil contaminated petroleum hydrocarbons

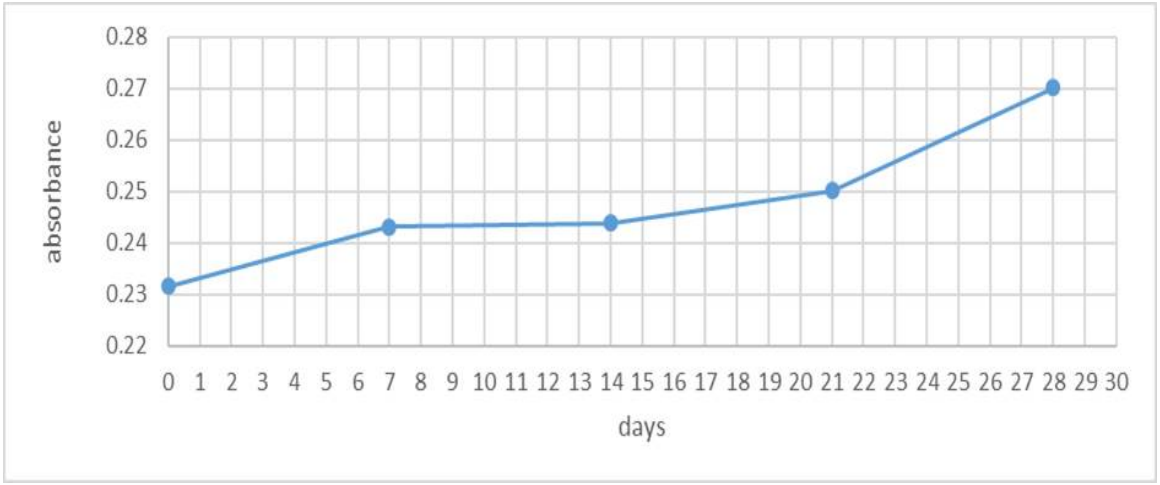

Figure 1: This graph shows the absorbance at 320 nm and the changes according to number of the days. The graph describes about flask A which contains the soil contaminated with petroleum hydrocarbons and the MSM media

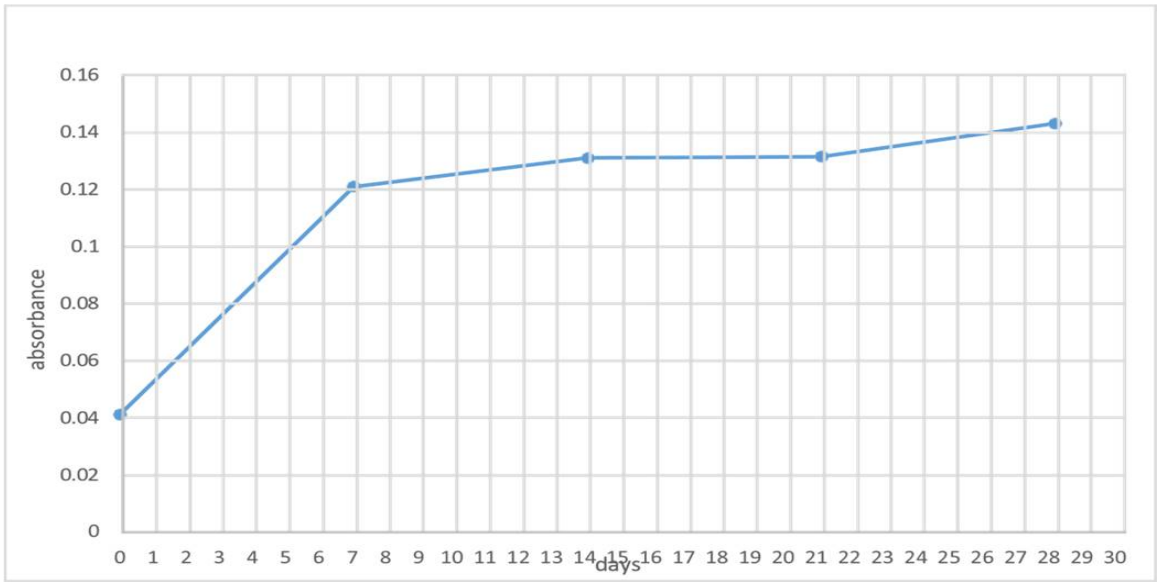

Figure 2: This graph shows the absorbance at 320 nm and the changes according to number of the days. The graph describes flask B which contains which contains the soil contaminated with petroleum hydrocarbons and the MSM media and 1% of yeast

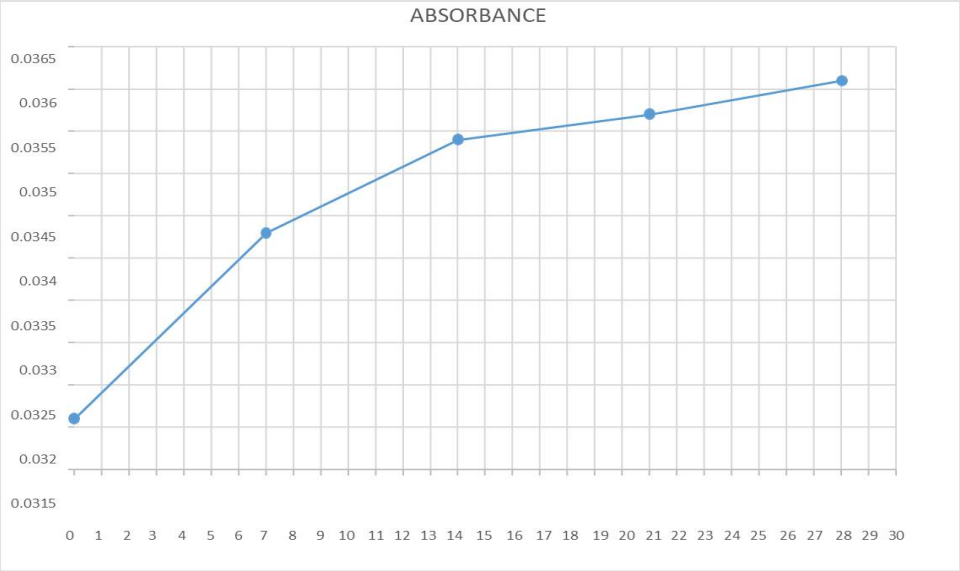

Figure 3: This graph shows the absorbance at 320 nm and the changes according to number of the days. The graph describes flask C which contains the soil contaminated with petroleum hydrocarbons and the MSM media and 2% of yeast

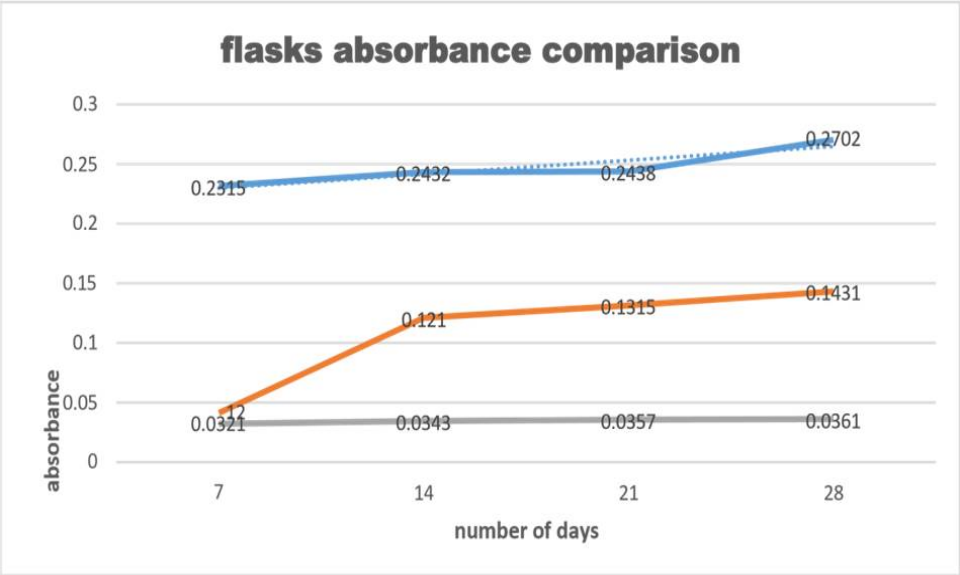

Figure 4: This graph shows a combination of the three graphs, it is comparing the activity of the degradation of the petroleum hydrocarbons in flask B and C have quite different while A is totally different which means the yeast degraded the hydrocarbons [8]

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Total hydrocarbons degraded

The amount of petroleum hydrocarbons obtained using the EPA method was calculated in mg/L. The initial amount in each flask was 20 mL. After 28 days, flask B contained 11 mL and flask C 9 mL. Flask A was the control, and the petroleum amount did not change.

So
Calculating the petroleum hydrocarbons in the sample using the formula of EPA mg/L
Where

$$\frac{R \times D}{V}$$

R = mg of Petroleum Hydrocarbons as obtained after the degradation
D = extract dilution factor
V = volume of sample, in liters
Flask B
11 ml to mg is 110 mg

$$\frac{110\ \text{mg}}{0.12\ \text{L}} \times 916.67\ \text{mg/L}$$

Flask C
Calculating the petroleum hydrocarbons in the sample using the formula of EPA mg/L
Where

$$\frac{R \times D}{V}$$

R = mg of Petroleum Hydrocarbons as obtained after the degradation
D = extract dilution factor
V = volume of sample, in liters

$$\frac{900}{0.12}$$

Flask c is containing 750mg/L

After 28 days of incubation, the TPH in flask B is 916.67 mg/L and in flask C is 750 mg/L, indicating that the TPH in the sample has decreased. Flask B and flask C differ in the percentage of yeast, which is why flask C is lower than flask B. The yeast (*Saccharomyces cerevisiae*) uses a sole carbon source. This might be due to efficient hydrocarbon uptake via specific receptor sites for binding hydrocarbons, a unique feature that assists in the emulsification and transport of hydrocarbons into the cell, and the presence of enzymes that introduce molecular oxygen into the carbon and generate intermediates that subsequently enter the common energy-yielding catabolic pathway [5, 10, 11].

4. Discussion

4.1. Preparation of Soil Samples

Soil samples collected from the campus were air-dried and homogenized for 48 hours to ensure uniformity. The dried samples were then subjected to a preliminary hydrocarbon screening test. The soil was mixed with distilled water in a beaker and allowed to settle for 24–48 hours.

Visual inspection of the water surface revealed no iridescent film or oily layer, indicating that the collected soil was initially free from detectable hydrocarbon contamination prior to experimental treatment. This confirms that any subsequent hydrocarbon presence was due to artificial contamination introduced during the experiment.

4.2. Viable Cell Count of *Saccharomyces cerevisiae*

The viable and non-viable cells of *Saccharomyces cerevisiae* were determined using a haemocytometer. A total of 257 cells/mL were counted across all chambers, of which 189 cells/mL were viable and 68 cells/mL were non-viable.

The calculated cell viability percentage was 73.54%, indicating that a substantial proportion of the yeast culture was metabolically active and suitable for use in the biodegradation experiment. This level of viability is adequate for effective microbial activity in hydrocarbon degradation processes.

4.3. Degradation of Petroleum Hydrocarbons

Table 1 summarizes the experimental setup, where:

- Flask A (Control): Mineral Salt Medium (MSM) + 20 g contaminated soil (no yeast)
- Flask B: MSM + 20 g contaminated soil + 1% *S. cerevisiae*
- Flask C: MSM + 20 g contaminated soil + 2% *S. cerevisiae*

This setup was used to evaluate the effect of different yeast concentrations on petroleum hydrocarbon degradation.

Spectrophotometric Analysis

Spectrophotometric measurements were carried out at a wavelength of 320 nm to determine the concentration of Total Petroleum Hydrocarbons (TPH) in the samples over the 28-day incubation period. The control flask (Flask A) showed consistently high absorbance values ($\sim$0.27) throughout the experiment. This indicates that no significant degradation of hydrocarbons occurred in the absence of microbial activity. In contrast, Flask B (1% yeast treatment) showed a gradual reduction in absorbance over time, reaching approximately 0.15 after 28 days, indicating partial biodegradation of petroleum hydrocarbons due to microbial activity. Flask C (2% yeast treatment) exhibited the lowest absorbance value ($\sim$0.036), demonstrating the highest level of hydrocarbon degradation among all treatments. This suggests that increasing the concentration of *S. cerevisiae* enhances the biodegradation efficiency. Overall, the results indicate a clear trend of decreasing absorbance with increasing yeast concentration, confirming that *S. cerevisiae* contributes to the biodegradation of petroleum hydrocarbons in contaminated soil. These findings are consistent with previous studies reporting that microbial activity significantly reduces hydrocarbon concentrations through enzymatic breakdown processes.

5. Conclusion

This study demonstrates that *Saccharomyces cerevisiae* is an effective biological agent for the degradation of petroleum hydrocarbons in contaminated soil under laboratory conditions. The results revealed a clear reduction in Total Petroleum Hydrocarbons (TPH) in yeast-treated samples compared to the control, with the highest degradation efficiency observed at 2% yeast concentration. The findings confirm

that petroleum biodegradation is influenced by microbial concentration, with higher yeast density enhancing hydrocarbon breakdown. The study further establishes that *S. cerevisiae* can contribute to the detoxification of petroleum-contaminated soils through enzymatic degradation and improved hydrocarbon bioavailability. In conclusion, *Saccharomyces cerevisiae* presents a promising, cost-effective, and environmentally friendly alternative for petroleum bioremediation. Future research should focus on field-scale applications, optimization of environmental conditions, and evaluation of microbial consortia to improve degradation efficiency and practical applicability in real contaminated environments.

Article Information

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Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

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