

Article

Thermally Enhanced Bioremediation of NAPL Polluted Soil-Water Resources

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Abstract: The use of conventional techniques for physico-chemical remediation of hydrocarbon such non-aqueous phase liquids (NAPL)-polluted sites may disturb the natural biotic settings of the (sub)-surface. However, natural attenuation has been reported very slow and sometime results as incomplete removal under prevailing site conditions. In particular, microbial growth is quite slow in cold regions, which reduces the applicability of bioremediation in treating NAPL-polluted soil-water. Thus, this study aims to evaluate the thermally enhanced bioremediation techniques to treat NAPL-polluted soil-water using practical experiments. A one-dimensional large column setup was designed and fabricated for this purpose. The column was integrated with automatic temperature controlling baths to maintain different soil-water temperatures (4 °C, 20 °C, 28 °C, and 36 °C), which was circulated through the porous media filled in the column setup. Results show a high dissolution rate of toluene, the selected light NAPL, at an elevated temperature of 28–36 °C. The biodegradation rates of the NAPL were found to be 0.002 mg L/h, 0.008 mg L/h, 0.012 mg L/h, and 0.015 mg L/h at soil-water temperature levels of 4 °C, 20 °C, 28 °C, and 36 °C, respectively. It was found that at high soil-water temperature (28 °C and 36 °C), a significant increment in microbial actions accelerates the biodegradation rate of NAPL in the subsurface system. The outcomes of this study may help in treating NAPL-polluted sites using solar or geo-thermal based heating systems for thermally enhanced bioremediation.

Keywords: hydrocarbon; groundwater; mineral soils; temperature; microbes; biodegradations



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1. Introduction

Sandy aquifer regions are at high risk of hydrocarbon contamination in and around areas of oil-handling activities. Hydrocarbons such as nonaqueous phase liquids (NAPLs) are of particular concern under varying environmental conditions because of their high sensitivity to subsurface variability, which enables them to spread widely in the subsurface [1,2]. NAPLs are not freely miscible in water, but a small quantity of NAPLs dissolved with water is sufficient to seriously degrade its quality. Based on their density, NAPLs are classified as light and dense NAPLs, known as LNAPLs and DNAPLs, respectively [3]. Leakage of LNAPLs presents a major risk to ecosystems as they can result in widespread pollution from small spillages, while DNAPL can persist in subsurface for a long time.

The LNAPL pool retained around the capillary fringe and groundwater table dissolves with groundwater and subsequently moves to surrounding locations due to advection, diffusion, and dispersion mechanisms of mass transport [1–3]. Groundwater flow regimes play a significant role in the dissolution and spread of pure-phase light NAPLs and in the movement of its dissolved form to the surrounding locations. The dynamic nature of the groundwater table causes spreading of NAPLs in the smear zone, which considerably increases the NAPL–water interface area, resulting in accelerated dissolution of the pure-phase LNAPL in surrounding groundwater resources. The dissolved NAPL moves along

with groundwater, which forms a plume feature with varying concentration of NAPL compounds. Thus, it is important to thoroughly understand the fate and transport of NAPLs under varying subsurface conditions in order to frame a suitable engineered remediation strategy.

Natural attenuation of these pollutants has been reported very slow in soil-water resources [4,5], especially in cold regions [6]. On the other hand, ex situ/physico-chemical treatments require high costs and create a great deal of disturbance in natural settings [7]. Thus, enhanced bioremediation is gaining attention to remediate NAPL-polluted sites [5,8]. Therefore, in order to enhance the degradation rate, engineered bioremediation is practiced using additives to the polluted site and/or customizing subsurface parameters. Since subsurface parameters are difficult to control directly in field conditions, they can be modified indirectly using injection and extraction wells that aid in the overall bioremediation process. To maintain favorable conditions in enhanced bioremediation, injection wells are used to supply both aerobic and anaerobic electron acceptors, along with the nutrients and potential microbes in the contaminated zone, while extraction wells are used for controlling plume spreading in downgradient locations. Hot water supply to improve microbial actions is an emerging technique. Thus, thermally enhanced bioremediation was adopted in many NAPL-polluted sites and reported encouraging response of native microbes, which results in a high removal rate of these pollutants [9–13]. Although thermally enhanced bioremediation has been studied for different soils, it has not been performed at varying temperature levels in continuous flow systems. Mostly batch experiments were performed for completely mixed setups to observe the impact of temperature [4]. However, in large-scale engineered bioremediation, microbial populations and their surrounding environmental conditions are important for hastening the process of biodegradation. In particular, it is recommended to achieve enhanced removal of NAPL by maintaining favorable environmental conditions, addition of nutrients, and/or bacterial strain to the polluted site. Out of several environmental factors, temperature variations play a crucial role in maintaining the biodegradation rate of LNAPL compounds in subsurface. Thus, the main objective of this study is to investigate thermally enhanced bioremediation of NAPL by hot water flooding in a one-dimensional column setup for maintaining the soil-water temperature conditions favorable for microorganisms.

2. Materials and Methods

A one-dimensional glass column setup was fabricated and filled uniformly for conducting the experiments. Indian standard clean sand of 650 grade-II having particle size less than 1 mm and greater than 0.5 mm was used as the experimental porous media. The column was integrated with automatic cold and hot baths to maintain groundwater temperatures of 4 °C, 20 °C, 28 °C, and 36 °C. Figure 1 shows the schematic diagram of the column setup with an inner diameter of 14.2 cm and a height of 140 cm. Sand was packed up to 70 cm to create a sandy subsurface formation, and the remaining top 70 cm was kept as headspace to maintain aerobic condition throughout the experiment. The water-filled column was left overnight to saturate the sand and then allowed to drain. Filtration screens were fixed around the inlet and outlet valves to prevent the entrance of the sand particles into the connecting viton tubes. Piezometers were attached to the column to measure the positions of the groundwater table. Similarly, an automatically controlling water bath was connected to the same peristaltic pump to provide varying temperature water. A thermometer and a temperature sensor were attached to cold and hot baths, respectively. A flow-controlling valve was attached to cold/hot baths before the peristaltic pump to allow the required cold/hot water to flow in the system. Sampling ports (P1–P4) were installed in the saturated zone at a uniform space of 10 cm to each other. One head-space sampling port was also used to collect the vapor phase samples.

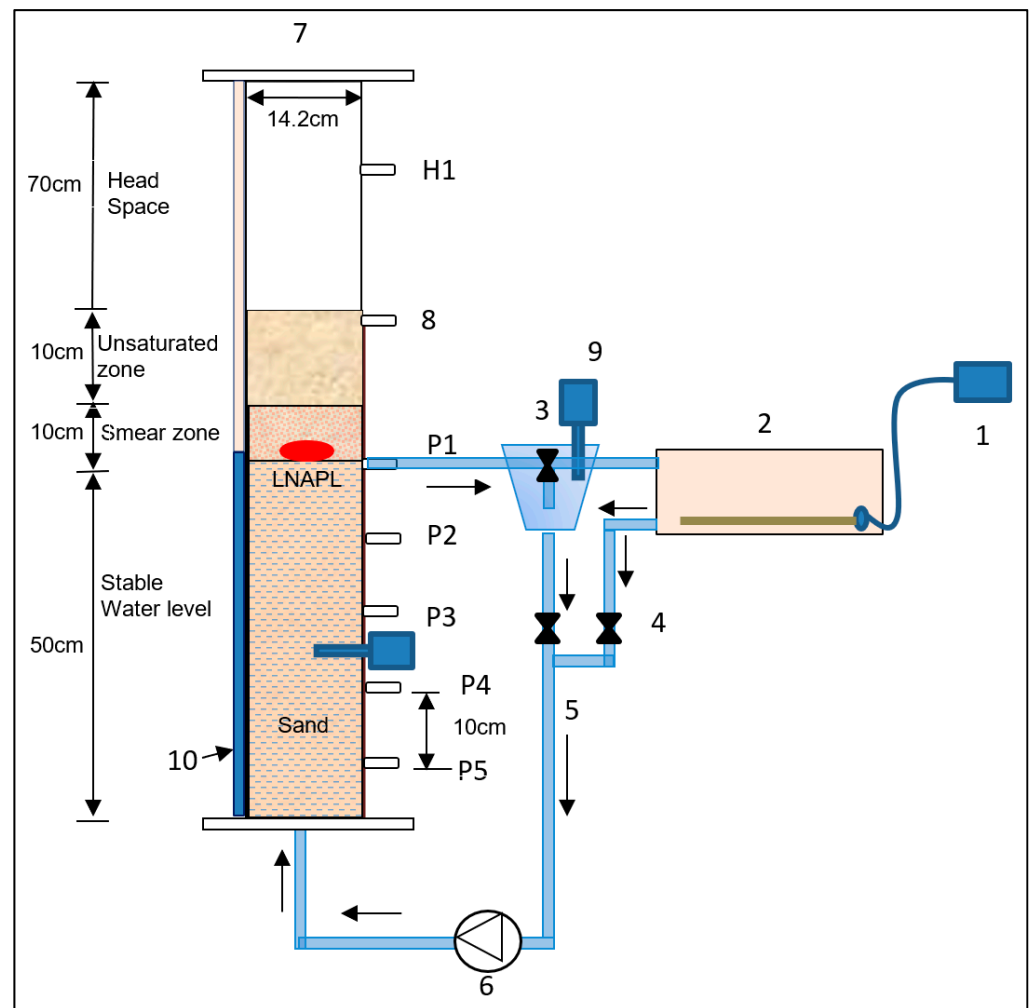


Figure 1. Schematic diagram of the laboratory 1D column setup used to investigate the thermally enhanced bioremediation of NAPL in soil-water. 1: Electric board; 2: hot water bath; 3: cold water bath; 4: flow controlling valve; 5: viton tube cover with cotton; 6: peristaltic pump; 7: column setup; 8: injection port; 9: thermometer; 10: piezometer; P1-P4 are sampling ports.

A tracer transport experiment was performed first to estimate vertical dispersivity. A solution of tap water and sodium chloride with an initial concentration of 1000 mg/L was injected continuously to the column from the bottom to maintain the uniform flow against gravity. The water samples were collected from port 1 for 6 h with an interval of 0.5 h and analyzed using portable conductivity meter. Thereafter, experiments regarding NAPLs' fate and transport were started by recirculating cold water at a rate of 1.2 m/day to mimic low temperature at, e.g., 4 °C inside the column. Once the background column temperature reached at 4 °C, 5 mL of Toluene (a representative light NAPL) was spiked from the injection port as marked in Figure 2. Subsequently, three other sets of experiments were performed at soil-water temperatures of 20 °C, 28 °C, and 36 °C by recirculating heated water with same flow velocity through the column setup. Soil-water temperature was continuously recorded using a thermometer attached to a column and cold bath. Soil water samples were collected routinely from sampling ports to analyze dissolved toluene concentrations and microbial populations. Simulation of toluene transport was performed by solving the advection–dispersion equation numerically as described in Gupta et al. [1].

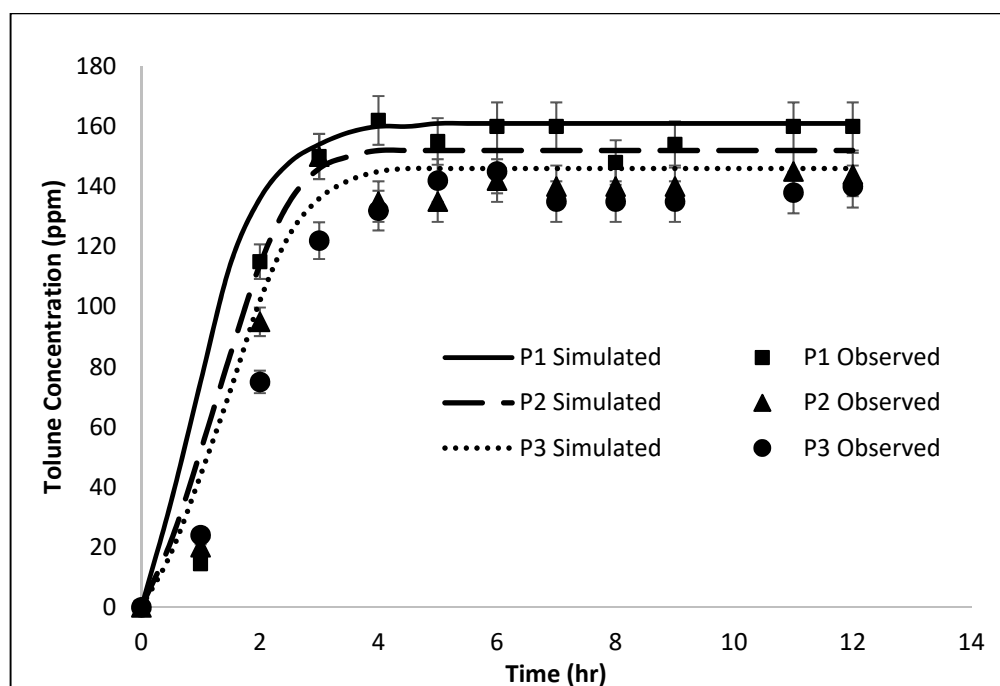


Figure 2. Concentration of NAPL at sampling port P1 (10 cm depth), P2 (20 cm depth), and P3 (30 cm depth) of column setup observed at soil-water temperature of 36 °C.

Agilent GC-MS (Model No. 5977) equipped with a fast electronics model was used to measure the selected NAPL concentration. Standard solutions of 2.5–100 mg/L concentration of toluene were prepared using stock solution to calibrate the peak area–concentration response. A Chrompack capillary column (30 m × 0.25 mm, Silicone coating of 0.25 μm) was used for toluene analysis. Helium (99.9% pure gas from Sigma Gas, New Delhi, India) was employed as the carrier gas at a flow rate of 25 mL/min. Air and nitrogen with a flow rate of 20 mL/min were used throughout the experiments. The temperature of injection port, oven, and detector port was kept at 150 °C, 120 °C, and 150 °C, respectively, during the measurements.

Microbial population was counted using heterotrophic standard plate count method (No. 9215C) [14]. In this method, colony-forming units (CFU) for live heterotrophic bacteria was estimated for collected soil-water samples during laboratory experiments. Immediately, all collected samples were diluted with a factor of 10¹ to 10⁵ and before shaking them for 15 s. Growth media were prepared using a combination of 20 g protease peptone; 1.5 g of K₂HPO₄, 1.5 g of MgSO₄, 7 g H₂O, and 20 g of Agar. Final pH of media was adjusted to 7.2 by adding 1 N NaOH before autoclaving at 121 °C for 15 min. The laminar air flow setup was wiped with 70% ethanol and UV light for the 15 min. Well-marked plates were poured with 30 mL prepared growth media and were kept still for a few minutes to solidify agar surface. Thereafter, diluted samples were inoculated with the help of spreader on agar surface of respective plates. Successively, these plates were kept for 48 h at 36 ± 1 °C for incubation before the colonies were counted using the quadrat method.

3. Results

Four series of laboratory experiments were performed to investigate the role of different soil-water temperature levels on toluene biodegradation using a continuous flow system of the vertical column setup. BTCs of the NAPL are presented in Figures 2–5 for the 36 °C, 28 °C, 20 °C, and 4 °C cases, respectively. Figures 2–5 show a higher equilibrium concentration at upper port (P1) than at the lower ports (P2 and P3) in all experimental cases. Furthermore, the equilibrium concentration (140–160 mg/L) at port 1 (P1) was higher in the case of a column having 36 ± 2 °C soil-water temperature (Figure 2). Further, the equilibrium concentrations at 28 °C, 20 °C, and 4 °C ranged from 112–120, 80–100, and

40–55 mg/L, respectively, at the same port. This clearly indicates that the high temperature accelerates the dissolution of NAPLs, which significantly contributes towards the high concentration of dissolved NAPLs in the soil water plume. A concentration range of 100–150 mg/L of toluene was observed to be most effective for bioavailability [2], which can also accelerate the degradation rate significantly. The biodegradation rates of NAPL were found to be 0.002, 0.008, 0.012, and 0.015 mg-L/h at soil-water temperatures of 4 °C, 20 °C, 28 °C, and 36 °C, respectively. This indicates that higher temperature ranges, particularly 28–36 °C, can considerably enhance the biodegradation rates of NAPL-polluted sites.

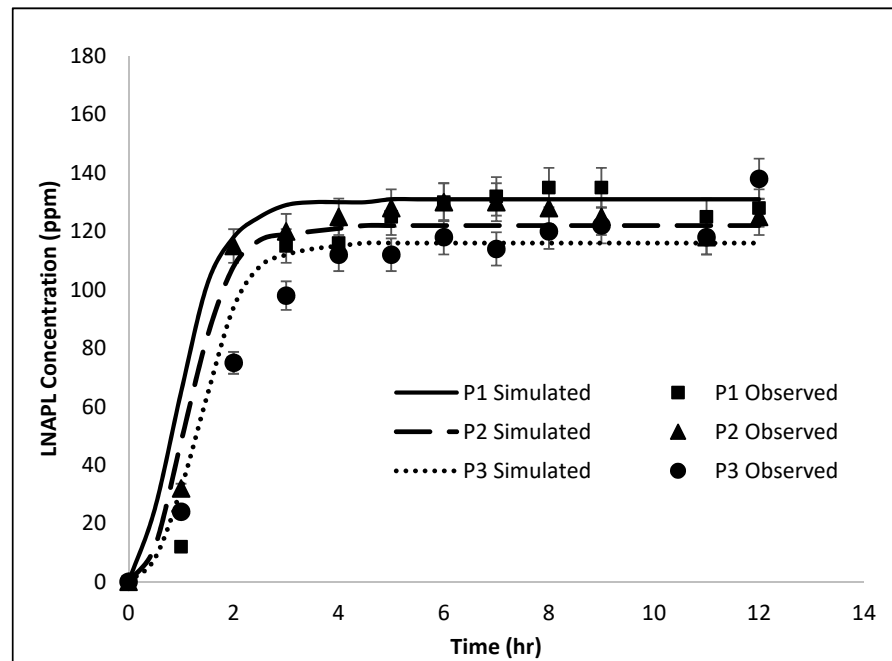


Figure 3. Concentration of NAPL at sampling ports P1, P2, and P3 of column setup at soil-water temperature of 28 °C.

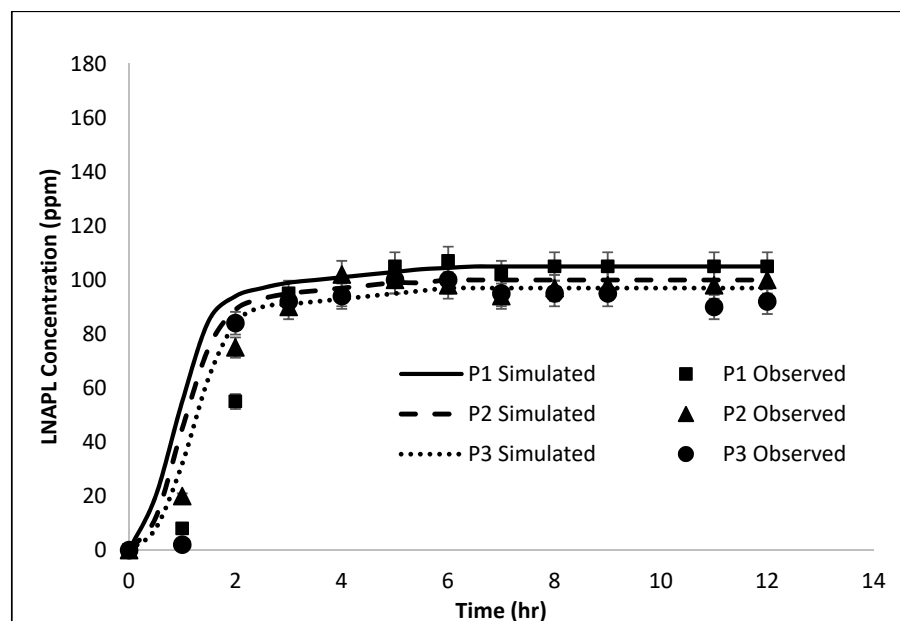


Figure 4. Concentration of NAPL at sampling ports P1, P2, and P3 of column setup at soil-water temperature of 20 °C.

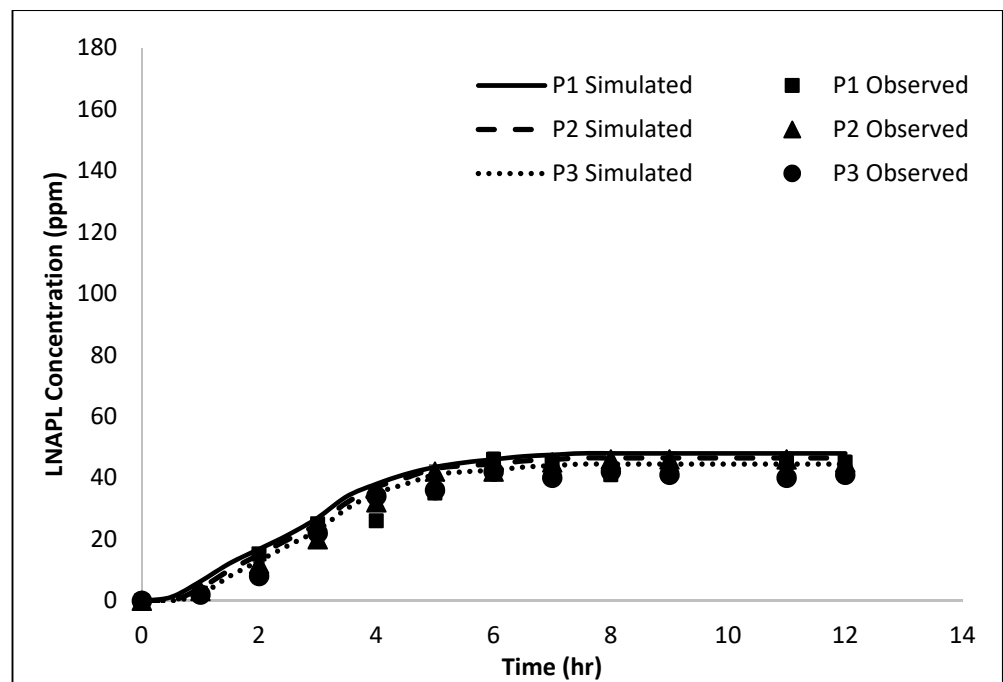


Figure 5. Concentration of the NAPL at sampling ports P1, P2, and P3 of column setup at soil-water temperature of 4 °C.

Estimated CFU per ml of pore water shows an increasing microbial number with high soil-water temperature. Initial microbial population sizes of the samples collected from the P1 port were 28×10^4 CFU/mL, 3.2×10^4 CFU/mL, 2.4×10^4 CFU/mL, and 1.2×10^4 CFU/mL at 36 °C, 28 °C, 20 °C, and 4 °C, respectively. Furthermore, initial microbial populations of the samples collected from P3 port were 2.3×10^4 CFU/mL, 2.6×10^4 CFU/mL, 0.6×10^4 CFU/mL, and 0.6×10^4 CFU/mL, respectively at these temperature levels. Population density increases to 120×10^4 CFU/mL, 264×10^4 CFU/mL, 85×10^4 CFU/mL, and 8.6×10^4 CFU/mL in samples collected from P1 after experiment at 36 °C, 28 °C, 20 °C, and 4 °C, respectively. Furthermore population density was comparatively low, i.e., 116×10^4 CFU/mL, 142×10^4 CFU/mL, and 72×10^4 CFU/mL, 1.4×10^4 CFU/mL in samples collected from P2 after experiment at these temperature levels. Higher initial and final microbial density in the upper port (P1) than the lower port (P2) indicates the aerobic nature of the microbes. The bloom of microbial population accelerates the degradation of the NAPL at high temperatures (Figure 6). Microbial number was high in regions of optimal dissolved concentration, i.e., 140–150 mg/L at temperatures of 28 °C and 36 °C. Suarez and Rifai [15] reported that a temperature of 10–36 °C is optimum for NAPL degraders. A relatively low range of temperatures seems to be due to the different NAPLs selected by the authors.

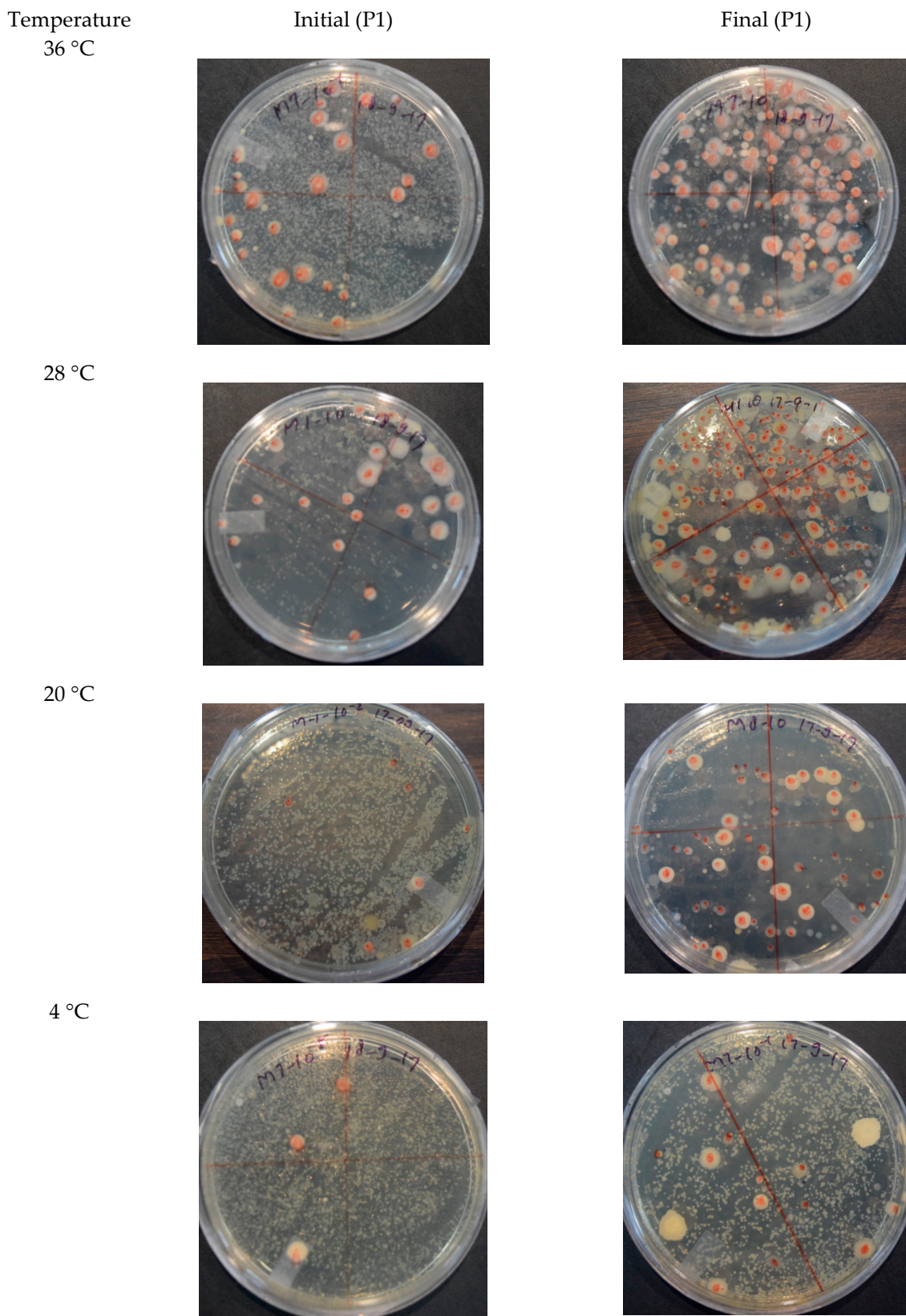


Figure 6. Pictures of Petri plates presenting microbial colony at different temperature levels.

4. Discussion

The release of NAPLs from pipelines and storage tanks increases the risk of environmental hazards during their transport and refining activities and such releases are spotted

frequently on the ground. The spilled NAPLs infiltrate and redistribute in the soil and can also reach the groundwater table. The transport behavior of NAPLs in soils has been extensively studied [16]. Gupta et al. [1] and Gupta and Yadav [2] performed multiscale laboratory experiments to enhance the understanding of the transport behavior of NAPLs in soils under varying subsurface conditions. Batch experiments performed by Gupta and Yadav [2] indicate that the biodegradation of light NAPL increases by increasing its dissolved concentration and remains at a maximum till 100 mg/L before it starts decreasing with the increment in substrate concentration. However, Gupta et al. [1] observed a high biodegradation of NAPL in concentration ranges from 140–160 mg/L in 2D sand tank experiments under fluctuating water table conditions. This indicates that the microbes can survive and participate in degradation of NAPL compounds under their concentration range of 100–160 mg/L in large domains under varying environmental conditions. On the basis of findings of Gupta et al. [1] and Gupta and Yadav [2], the biodegradation rates were calculated for dissolved NAPLs in this study at different groundwater temperature conditions (Figure 7). The figure clearly indicates that the high temperature accelerates the dissolution of NAPL from the free phase, which significantly contributes to raising its dissolved concentration in soil water plume [17,18]. The water-soluble compounds from NAPL pools enhances the bioavailability, which is used as a carbon source by the native microbes to improve biodegradation of hydrocarbons.

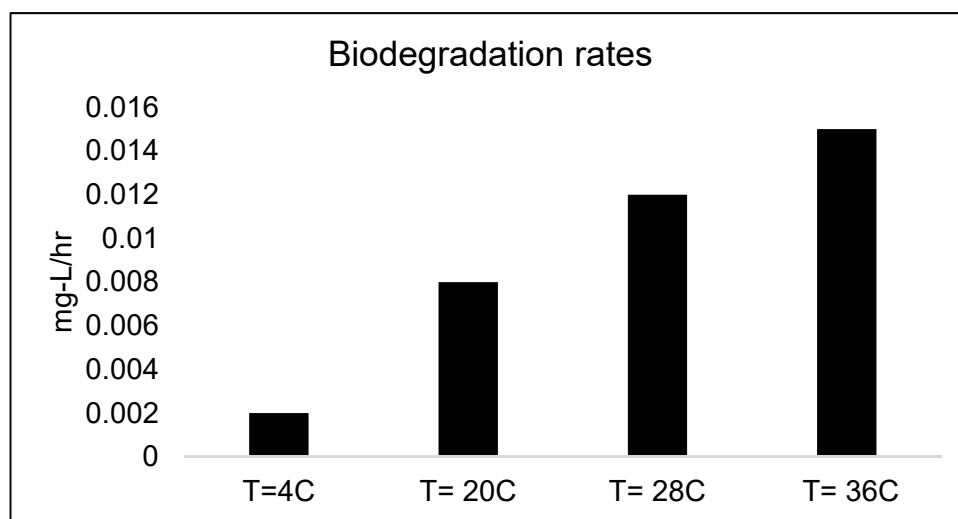


Figure 7. Estimated biodegradation rate of NAPL in column setup for port P1 at different soil-water temperature levels. P1 is sampling top sampling port.

Encouraging roles of elevated temperature on biodegradation of hydrocarbons were reported in the past for different geographical regions at constant temperatures [12]. However, the use of the impact of varying temperatures to evaluate their relative performance polluted soil-water systems was not investigated. Moreover, most earlier studies were performed using completely mixed batches rather than continuous flow systems. A 90% removal of the hexadecane from soil after 40 days, accompanied with an increase in bacterial numbers, was observed by Perfumo et al. [12]. Microbial bloom was also observed in all high-temperature cases by the authors. Thus, the dissolved NAPL concentration acts as an easily available carbon source to native microbes and thus increases microbial colonies in the affected resources. This rare-to-dominant microbial shift also illustrates the potential role in degradation of NAPL compounds under hot-water flooding conditions. Likewise, Yadav and Hassanizadeh [4] highlighted that the lower temperature reduces the microbial activity due to inadequate bioavailability of nutrients. Previous studies indicate that proteobacteria and actinobacteria are major NAPL degraders in soils [19–21], and it was demonstrated that the microbial bloom takes place during hot water flooding. The

outcomes of this study will particularly help in implementing geothermally or solar based heating to treat NAPL-polluted sites.

5. Conclusions

Thermally enhanced biodegradation by hot-water flooding has encouraged practitioners to enhance the removal of hydrocarbon pollutants quickly. We found that hot-water (28–36 °C) flooding accelerates the removal rate of toluene, the selected NAPL, and thus may act as excellent tool for remediation of hydrocarbon polluted sites in cold regions/seasons. At severely polluted sites, soil-water temperature can be raised by several means to hasten microbial activities for bioremediating the polluted resources in limited time. This approach can help to further reduce bioremediation cost by applying hot water from nearby geothermal, solar based heating systems or by return flow taking place from industries.

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