https://doi.org/10.17221/66/2023-SWR

# Application of biostimulation and bioventing system as bioremediation strategy for the treatment of crude oil contaminated soils

*Ifeanyi Michael Smarte Anekwe, Yusuf Makarfi Isa**

*School of Chemical and Metallurgical Engineering, University of the Witwatersrand, Johannesburg, South Africa*

**Corresponding author: yusuf.isa@waits.ac.za*

**Citation:** Anekwe I.M.S., Isa Y.M. (2024): Application of biostimulation and bioventing system as bioremediation strategy for the treatment of crude oil contaminated soils. Soil & Water Res., 19: 100–110.

**Abstract:** The purpose of this study was to evaluate the application of biostimulation and bioventing for the treatment of crude oil-contaminated soils. The research needed to check how various industrial biostimulants will perform in the treatment of contaminated soils and whether or not there is a synergetic effect that has to do with the stimulant composition. Soil samples used for this study were collected from South Africa. The soil samples were air-dried for 24 h and subsequently passed through a standard sieve of 2 mm screen. The soil consists of 79.32% sand (2.00–0.02 mm), 14.71% silt (0.02–0.002 mm) and 5.97% clay (< 0.002 mm). A microcosm system containing 1 kg of soil contaminated with crude oil (5% w/w) for biostimulation (BSTc) treatment was amended with varying ratios of municipal wastewater (MWW) and brewery wastewater (BWW) to investigate the possible synergy. The bioventing (BVTc) treatment involves the supply of atmospheric air to the bioreactors through the vadose zone for 30mins flow duration every 48 h intervals at ambient condition for 28 days. The BSTc and BVTc treatments recorded 48–58% and 54–75% total petroleum hydrocarbon (TPH) removal efficiencies, respectively, as the BWW amendment noted appreciable removal compared to MWW, while the control treatment recorded 35%. The result showed that the attempt to boost the TPH removal efficiency using the bioventing with the wastewater amendment was effective, as the presence of enough oxygen in the system resuscitated the activities of the microbial community for enhanced TPH biodegradation. This study inferred that combined bioventing and biostimulation techniques proved to be an effective bioremediation strategy for the treatment of crude oil contaminated soils and could serve as a vital tool towards the mitigation of pollution aftermath faced by communities involved in oil production and/or processing activities.

**Keywords:** biostimulation; bioventing; brewery wastewater; crude oil; municipal wastewater

Environmental pollution has been identified as a major challenge to the survival of both terrestrial and aquatic habitats, which has contributed to the degradation and contamination of soil and water bodies. This can be attributed to industrialization, which includes mining and exploration of mineral resources. Crude oil spillage and acid mine drainage are the major water and soil contaminants attributed to industrial and/or human activities; these pollution sources reduce the availability of potable water and arable lands for agricultural purposes. Remediation of crude oil-contaminated soils using physical, chemical and thermal methods has been widely criticized due to its expensive, non-eco-friendly nature and inability to meet remediation purposes. Recently, bioremediation has been considered as an alternative soil treatment strategy (Shahsavari et al. 2017) over physiochemical methods due to the former's ability to provide a clean, green, effective, affordable and environmentally friendly approach for the treatment of contaminated soils while the latter has been criticized for its hazardous, expensive nature and possible recontamination of site. Biostimulation is a bioremediation technique that employs the use of organic and/or inorganic nutrient amendments for the stimulation of the indigenous microbial community to enhance the biodegradation of degradable contaminants. The success of biostimulation application on contaminated soils depends on several factors such as temperature, aeration, pH (Varjani & Upasani 2017), nutrients availability and bioavailability of contaminants to degrading microbes (Benyahia & Embaby 2016; Varjani & Upasani 2017). The application of the organic nutrient has been viable in the treatment of crude oil-contaminated soils. Nutrients present in organic substrate vary according to source, and this contributes to its efficacy for the bioremediation of contaminated soils.

The application of organic substrates such as wastewater (Aburto-Medina et al. 2012; Agarry & Latinwo 2015), agricultural waste (Chijioke-Osuji et al. 2014; Wang et al. 2016), sewage sludge (Ling & Isa 2006; Chorom et al. 2010), animal waste (Agarry et al. 2010; Chijioke-Osuji et al. 2014; Adekunle et al. 2017,), municipal refuse (Adekunle 2011; Liu et al. 2018) have been studied for petroleum contaminated soils and results showed that these amendments can improve the bioavailability of crude oil contaminant in the soil, revive the activities of microbial population, and enhance hydrogenase for effective degradation of hydrocarbons. Meanwhile, excessive nutrient concentration in the treatment system can hinder biodegradation efficiency, as Oudot et al. (1998) and Chaillan et al. (2006) reported the negative effect of high nutrient concentration (NPK) on hydrocarbon removal efficiency. The stimulation of the natural degradative ability of the native microbial community may be challenging, especially when selecting the appropriate organic or inorganic amendments or biostimulants required for a large-scale application. However, when exploring feasible approaches to carry out the remediation exercise effectively, it is crucial to understand that the contaminated environment is made up of a diverse microbial population with different degradation potentials (Alexander 1999), and these indigenous microorganisms are typically present in relatively limited numbers. To circumvent these drawbacks, changes in physicochemical parameters (pH, temperature, electron donors or acceptors, etc.), as well as a "niche adjustment" by inoculating competent microbes into these systems, are possible strategies that may be adopted for effective bioremediation. Hence, hydrocarbon remediation can be activated or improved by introducing nutrients and oxygen into the polluted soil (Agarry & Latinwo 2015) or through the inoculation of genetically modified organisms into the soil (Barathi & Vasudevan 2003).

In bioventing, the contaminated environment is stimulated by a suitable air supply or aeration to provide sufficient oxygen for microbial activities (Møller et al. 1996; Thomé et al. 2014; Hinchee 2017) for an effective bioremediation process. The bioventing treatment of polluted soil requires the controlled delivery of air or oxygen directly or indirectly into the subsurface unsaturated zone of the polluted site to resuscitate aerobic processes. Bioventing of polluted soil has been studied; the early work focuses on the use of water (as a source of oxygen) to provide oxygen for sub-surface area ventilation, which is less efficient as compared to gas ($O_2$) penetration rate. This also contributes to the disadvantage of using hydrogen peroxide for the bioventing process since the low power of penetration restricts the availability of oxygen for microbial activities in the vadose region (Hinchee et al. 1991). Similarly, investigations by Huling et al. (1990) to determine the efficacy of $H_2O_2$ for bioventing revealed that the high concentration of $H_2O_2$ up to 100 mg/L of soil solution may have an inhibitory effect on the biodegradation rate of the contaminated sample and that the toxicity and stability of $H_2O_2$ depend on the type of contaminants, sites and other environmental conditions, hence, air supply becomes more effective and economical (Hinchee & Arthur 1991; Lee & Swindoll 1993).

The application of organic or inorganic nutrients and air injection to enhance the direct oxidation of contaminants while providing sufficient nutrients for the degrading microbes and increasing aerobic biodegradation has received much interest recently (Agarry & Ogunleye 2012; Zhang et al. 2021). The modification of a polluted environment with organic or inorganic nutrients and air-injection contributes to the reinforcement of soil-based microorganisms (Lee & Swindoll 1993; Agarry & Latinwo 2015) which results in a substantial decrease in total petroleum hydrocarbon (TPH) concentration. These nutrients appear to release alkalinity into the environment, which increases the pH and promotes crude oil remediation (Adekunle 2011; Al-Kindi & Abed 2016; Liu et al. 2018). However, in developing countries, these inorganic chemical fertilizers are expensive and insufficient for agricultural purposes, not to mention their application for oil spill clean-up. As a result, it is necessary to look for less expensive and eco-friendly ways to improve petroleum hydrocarbon breakdown. One of these possibilities is the utilization of organic waste effluents as bulking agents and microbial biomass sources.

Nonetheless, research on devising cost-effective and environmentally friendly methods of removing petroleum hydrocarbons from polluted soil needs further exploration. To the best of our knowledge, there is a paucity of literature on the combined effect of different wastewater effluent applications together with oxygen supply for stimulating indigenous microorganisms in petroleum hydrocarbon-contaminated soils. Therefore, the objective of this study is to investigate the potentials of the brewery and municipal wastewaters and the mixture of these two alone or in combination with bioventing for the treatment of crude oil-contaminated soils.

## MATERIAL AND METHODS

### Material

Soil samples used for this study were collected from Durban, South Africa, at a depth of 2–3 cm (humic horizon, non-agricultural soil). The soil samples were air-dried for 24 h and subsequently passed through a standard sieve of 2 mm screen. The soil consists of 79.32% sand (2.00–0.02 mm); 14.71% silt (0.02 to 0.002 mm), and 5.97% clay (< 0.002 mm). Soil samples for the bioremediation treatment were preserved in a polyethylene bag for future use. The results of the characterization show that the soil has a light texture, mainly due to the high sand content. According to the United State Department of Agriculture soil taxonomy, these soil samples, characterized by their high sand content (sandy soils), most likely belong to the Entisol and Spodosol soil orders (Deckers et al. 2003). Crude oil was collected from a local oil refinery, while brewery wastewater (BWW) and municipal wastewater (MWW) were obtained from South African Brewery (SAB) and South African wastewater treatment facility, respectively. Wastewaters were stored in high density polyethylene containers kept in the refrigerator and mixed thoroughly before use so that there is consistent composition across the volume.

### Methodological approach

**Preparation of crude oil contaminated soil.** One kg of soil sample was spiked with 50 g of crude oil (5% w/w*)* and agitated in a mechanical shaker to achieve a homogenous mixture of the two components. A concentration of 5 % (w/w) was adopted to obtain severe contamination of soil samples, as a concentration above 3% has been reported to be increasingly detrimental to the soil structure (Osuji et al. 2005). The contaminated soil was kept for 2 days to allow for ageing and to mimic a real contaminated soil scenario, after which different bioremediation treatments were applied.

**Biostimulation and bioventing studies for crude oil-contaminated soil.** Biostimulation (BSTc) and bioventing (BVTc) studies for crude oil-contaminated soil treatment consist of six and four bioreactors, respectively, containing 1 kg of contaminated soil each. Five BSTc and three BVTc bioreactors were amended with wastewaters at varying ratios, while one BVTc bioreactor received only atmospheric air (without wastewater amendment) (Table 1). The remaining bioreactor designated for bioattenuation (BATc), which was neither supplemented with wastewater nor ventilated, served as the control treatment. All BVTc treatments were ventilated, and atmospheric air was supplied to the bioreactors through the vadose unsaturated zone using the air compressor pump to allow for adequate air circulation around the bioreactors. Air was supplied at 3 L/min for 30 min every 48 h since bioventing will be more economical if the lowest flow rate and highest flow interval are considered (Thomé et al. 2014). Bioventing treatments for crude oil-contaminated soils were performed at ambient tem-

Table 1. Composition of biostimulation, bioventing and bioattenuation treatment systems for crude oil contaminated soils (soil bulk density = 1 650 kg/m$^3$)

| Bioreactors | BWW | MWW | Atmospheric air ($O_2$) | Loading ratio (BWW : MWW) |
|---|---|---|---|---|
| | (mg/kg soil) | | | |
| BSTc-1 | 100 | 0 | – | 4 : 0 |
| BSTc-2 | 75 | 25 | – | 3 : 1 |
| BSTc-3 | 50 | 50 | – | 1 : 1 |
| BSTc-4 | 25 | 75 | – | 1 : 3 |
| BSTc-5 | 0 | 100 | – | 0 : 4 |
| BVTc-1 | 0 | 0 | √ | 0 : 0 |
| BVTc-2 | 100 | 0 | √ | 4 : 0 |
| BVTc-3 | 0 | 100 | √ | 0 : 4 |
| BVTc-4 | 50 | 50 | √ | 1 : 1 |
| BATc | 0 | 0 | – | 0 : 0 |

BSTc – biostimulation; BVTc – bioventing; BATc – bioattenuation; BWW – brewery wastewater; MWW – municipal wastewater perature for 28 days study period. Each 1 L bioreactor was used in static mode during the bioremediation process. Samples (~ 2 g) were collected every week from all bioreactors for the determination of total petroleum hydrocarbon residue.

### Analytical procedure and instruments

**Mechanical extraction and determination of total petroleum hydrocarbon.** The soil samples were dried in an oven at a low temperature (35 °C), pulverized using mortar and pestle and sieved using a standard sieve size of 63 μm to ensure grain size homogeneity. The mechanical extraction of crude oil from the sample was performed using 5 g of homogenized soil mixed with dichloromethane (DCM) and acetone in a ratio of 2 : 1 (4 : 2 mL) in a 250 mL glass jar. The glass jar was covered with aluminium foil to prevent solvent loss and shaken vigorously at 200 rpm on a mechanical shaker for 90 min to allow for effective residue TPH extraction. To recover the residue (filtrate), the solution was filtered using Whatman filter paper and subsequently with a syringe filter and the filtrate (extract) was transferred to a volumetric flask of 50 mL and made up to a known volume. Due to their strength, reliability and ability not to conflict with hydrocarbon fractions such as benzene, toluene, and xylene (BTX), and $C_5$-$C_9$ (Okop & Ekpo 2012), DCM and acetone are the most suitable solvent for oil extraction from soil samples. Gas chromatography-mass spectrometry (GC-MS QP 2010, Shimadzu, Japan) equipped with a Rxi-5ms column was used to determine total petroleum hydrocarbon. The chemical oxygen demand (COD) was determined through a standard method using a COD reactor (Hach, USA) and spectrophotometry (DR 3900, Hach, USA) (Talinli & Anderson 1992). Total nitrogen was determined by the semi-micro Kjeldahl method (Bremner & Mulvaney 1982), and the available phosphorus was determined by Brays No. 1 method (Olsen & Sommers 1982) while the total degrading bacteria were determined by the vapour phase transfer method according to Amanchukwu et al. (1989).

Table 2 shows that brewery waste effluents contain more nitrogen, microbial count and COD content than MWW, which implies nutrient deficiency in MWW when compared to BWW. According to these findings, brewery waste effluents can provide more nutrients to boost microbial density and activity and serve as an effective stimulant for the biodegradation of hydrocarbon-contaminated soil. Furthermore, the organic component of brewery wastewater (reported as COD) is usually readily biodegradable, as it primarily consists of sugar, soluble starch, ethanol, and volatile fatty acids (Bassey & Inyang 2012).

## RESULTS AND DISCUSSION

**Biostimulation of crude oil contaminated soils.** Biostimulation results showed that the amendment of contaminated soil with wastewaters (BWW and MWW) facilitated the biodegradation of crude oil, as shown in Figure 1. It is evident from the result that TPH removal was relatively fast within the first 2 weeks in all treatments except BSTc-5, which recorded 56.62% overall removal efficiency in week 4 at 1 011.07 mg/day average removal rate. The TPH removal efficiency for BSTc-1 increased (after week 1) by 7.11% and 10.17% in week 2 and week 3, respectively, to attain 58.39% in week 4 at a 1 042 mg/day average removal rate. However, BSTc-2, 3, and 4 showed a similar trend in relation to other treatments but recorded removal efficiency of less than 50% in week 4. The average removal efficiency of > 15% was observed for these treatments (BSTc-2, 3, and 4) in week 1, but as BSTc-2 and BSTc-3 increased by > 8% (9.58% and 10.16%, respectively), BSTc-4 only recorded a 2.03% increase in removal efficiency in week 2. Also, the TPH removal efficiencies of treatment BSTc-2, 3 and 4 plateaued at 48.67%, 49.67%, and 48.81% with an average removal rate of 869.11, 886.96 and 871.61 mg/day, respectively, attributed to > 5% increase in removal efficiencies of treatments BSTc-2 and BSTc-4 in week 4, while BSTc-3 recorded 2.87% increase which is a decline from 10.16% increased ef-

Table 2. Physicochemical and microbiological characterization of soil, brewery and municipal wastewaters

| Wastewater/ Composition | pH | COD (mg/L) | Total nitrogen | Available phosphorus | Microbial count |
|---|---|---|---|---|---|
| BWW | 8.2 | 750 | 52.68 ± 0.07 mg/L | 9.63 ± 0.04 mg/L | $1.7 \times 10^6$ CFU/mL |
| MWW | 7.9 | 704 | 45.53 ± 8.7 mg/L | 15.5 ± 0.5 mg/L | $1.1 \times 10^6$ CFU/mL |
| Soil | 7.2 | – | 2.80 ± 0.1 g/kg soil | 0.37 ± 0.01 g/kg soil | $3.0 \times 10^6$ CFU/g |

BWW – brewery wastewater; MWW – municipal wastewater; COD – chemical oxygen demand

https://doi.org/10.17221/66/2023-SWR

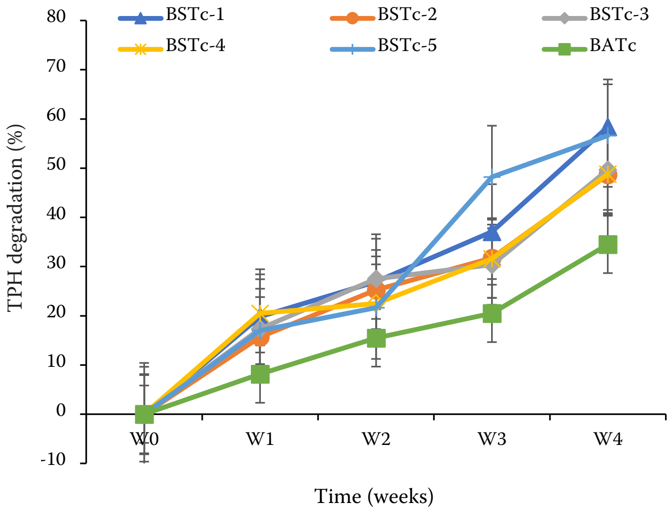

Figure 1. Time course for the biodegradation of total petroleum hydrocarbon (TPH) under bioattenuation, municipal wastewater, brewery wastewater and combined wastewater effluents amendment

BSTc – biostimulation; BATc – bioattenuation; error bars show the standard deviation of duplicate samples from treatment systems

ficiency in the previous week. The control treatment, BATc, recorded the lowest TPH removal efficiency of 34% at 616.07 mg/day average TPH removal rate. The reduction of TPH concentration can be attributed to the wastewater amendment.

**Bioventing of crude oil contaminated soils.** Crude oil removal from the contaminated soil was evident in all the bioreactors, both the vented and vented + nutrient amendment treatments. Figure 2 showed that the introduction of atmospheric air into the treatment reactors improved the bioremediation process in the first week of treatment, which recorded (in week 1) > 15% removal efficiencies in all treatments that received nutrient amendment + air (BSTc-2, BVTc-3, and BVTc-4). The treatment that was only ventilated (BVTc-1) recorded 11.25%, while the control treatment (that was neither amended with nutrients nor ventilated (BATc)) recorded < 10% removal efficiency in the first week. BVTc-1 (air) and BVTc-4 (wastewaters + air) maintained > 9% average increase in TPH weekly removal efficiency from week 1 till the end of the treatment with 54.93% and 61.47% at 980.89 and 1 097.68 mg/day average TPH removal rate, respectively. However, BVTc-3 (MWW + air) TPH removal efficiency increased by 3.44% in week 2, with an average increase of > 19% from week 2 to week 4 at a 1 071.79 mg/day average removal rate. BSTc-2, which was amended with BWW and ventilated, recorded the highest removal efficiency of 74.75% at an average removal rate of 1 334.82 mg per day. In comparison to other treatments, the control treatment BATc recorded the least removal efficiency (34.5%). The removal efficiencies of the BVTc treatment follow the trend BVTc-2 > BVTc-4 > BVTc-3 > BVTc-1 > BATc. The weekly TPH reduction efficiencies in all treatments amended with nutrients and/or ventilated were observed to show an appreciable progressive increase from week 1 to week 4.

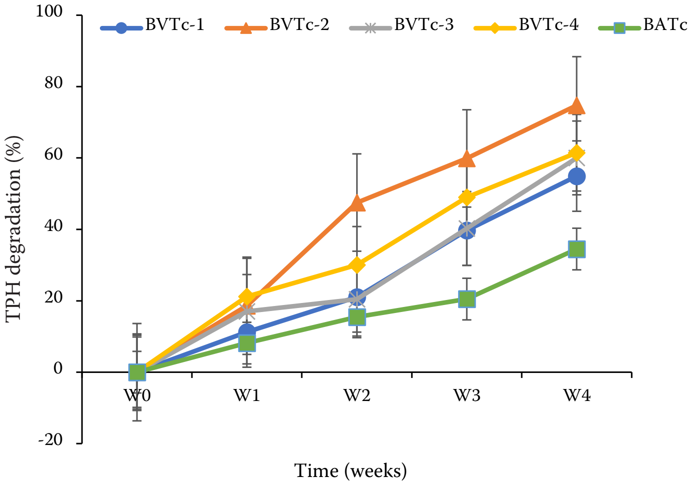

Figure 2. Time course for the biodegradation of total petroleum hydrocarbon (TPH) under bioattenuation, bioventing alone and combined wastewaters (brewery wastewater and municipal wastewater) effluents amendment with bioventing

BVTc – bioventing; BATc – bioattenuation; error bars show the standard deviation of duplicate samples

The first week of BSTc treatment observed the modification of environmental conditions for microbial activities by the organic substrate (BWW and MWW), accompanied by the acclimatization of oil-degrading microbes in the treatment system similar to the findings of Mbah and Obahiagbon (2018), which resulted in appreciable TPH weekly removal efficiency with respect to time, within the first 14 days and beyond (Mbah & Obahiagbon 2018). The increase in the TPH weekly reduction efficiency recorded from week 1 to week 4 with the BSTc treatments suggests a possible correlation with an increase in microbial load as the introduction of nutrients provides a favourable condition that invigorates the native microorganisms in the soil for effective degradation which has been validated in different studies (Chijioke-Osuji et al. 2014; Agarry & Latinwo 2015) as the microbial growth and metabolism is a factor of hydrocarbon reduction (Chen et al. 2019), hence, microbial population growth is proportional to the rate of TPH removal (Agarry & Latinwo 2015; Mbah & Obahiagbon 2018). Organic substrates like MWW and BWW simultaneously served as a bulking agent, pH buffering agent, organic substitute, fertilizer, and soil conditioner during the treatment of TPH contaminated soil to enhance biomass growth and metabolism, which was also found to be a proactive natural surfactant that improves the solubilization of petroleum pollutants to increase the bioavailability of hydrocarbons to degrading microbes and degradation of hydrocarbon fractions (Adams et al. 2017). Amenorfenyo et al. (2019) reported that wastewater contains organic nutrients (N and P), which also act as a carrier for immobilizing oil-degrading strains (Adekunle 2011) as microbes grow well in wastewater by consuming organic nutrients and turning them into usable biomass for hydrogenase (Adekunle 2011). Also, wastewater was reported to contain a range of microbes that were highly resistant to toxic contaminants and had exceptional organic contaminant degradation capability (Agarry & Latinwo 2015). The biodegradation efficiency depends on microbial viability in the environmental natural system, which is a limiting factor in bioremediation (Joo et al. 2008).

The treatments amended with BWW only (BSTc-1) showed slightly more appreciable removal efficiency (58.39%) than MWW (BSTc-5), which recorded 56.62%, while the use of mixed substrates (BWW + MWW) at varying ratios showed possible synergy between the wastewaters with an average removal efficiency of 49.05%, which is slightly below that of single substrate (57.50% average). The improved performance of pure wastewater sources (brewery or municipal wastewater) compared to their mixtures can be attributed to several factors. For example, treatment with BWW-only enriched effluent had the highest removal efficiency due to the high nutrient (nitrogen) content responsible for a significant microbial load in BWW, which is essential for increased biodegradation efficiency for optimal bioremediation. Consequently, pure wastewater sources provide a more specific and concentrated nutrient composition that more effectively supports oil-degrading microorganisms. Mixing of different wastewater sources may result in competition among microorganisms for resources and may contain inhibitory substances that impede bioremediation. Different environmental conditions and disruption of potential microbial synergies can also affect treatment efficiency. Therefore, the use of wastewater sources tailored to the needs of oil-degrading microorganisms is critical for optimizing bioremediation results (Amenorfenyo et al. 2019).

However, for the BVTc treatments, the introduction of air into the system improved toxic conditions that increased biodegradation by providing an aerobic environment sufficient to stimulate and regenerate the autochthonous microorganism‘s activities (Couto & García-Frutos 2016) while the presence of wastewaters in the bioventing system acts as biostimulants, and thus provides enough nutrient levels to boost the growth of the microbial community leading to high energy demand by oil-degrading microbes, which enhanced hydrogenase and increased TPH biodegradation (Agarry & Latinwo 2015). Bioventing stimulates the indigenous microbial community by adequate air (oxygen) supply to enhance the aerobic degradation of biodegradable contaminants (Byun et al. 2005) through the oxidation process (Troquet et al. 2003). Muskus Morales et al. (2013) reported a higher biodegradation efficiency with bioventing more than bioaugmentation in a crude oil treatment, while Agarry and Latinwo (2015) reported greater %TPH removal with combined bioventing and organic nutrient than bioventing used alone during the treatment period. The study by Thomé et al. (2014) buttresses the assertion that nutrient supplementation to a bioventing system renders greater TPH removal than a single utilization of each approach. This is evident in the present study, where air-injection + wastewater was able to increase the TPH removal efficiencies by 16.36%, 3.4 % and 12.42%, which represents 74.75%, 60.02% and 61.47% removal efficiencies (for BVTc-2, BVTc-3, BVTc-4, respectively) when compared to BSTc treatments (BSTc-1, BSTc-5 and avg. Mixed substrates – treatments without air-injection) while bioventing without nutrient recorded 54.93% removal efficiency. Also, the overall average removal efficiency recorded for BVTc treatment was 10.36% greater than BSTc treatment (52.43%), which correlates with the study by Møller et al. (1996), which demonstrated that the addition of nutrients to bioventing rendered an appreciable increase in the rate of degradation of TPH. Unlike biosimulation treatment, bioventing involves the supply of oxygen to facilitate microbial metabolism for oil degradation. In this case, the competition and inhibition effects observed with effluent mixtures may not be as significant because the focus is on oxygen availability rather than nutrient composition. Bioventing relies on aeration to increase the activity of indigenous oil-degrading microorganisms in the soil. While oxygen is essential to their metabolic processes, the ability of the microbial community to utilize nutrients from the injected air may be less affected by competition and inhibitory substances than with effluent-based bioremediation. In addition, studies have shown that supplementing wastewater (in bioventing treatment) leads to an increase in soil microbial counts (Agarry & Latinwo 2015; Chen et al. 2019).

The findings of this study and the ability to achieve an enhanced bioventing biodegradation with nutrient addition were in contrast with the investigation by Dupont et al. (1991) which reported that the addition of nutrients to the BVTc system was insignificant for the increased rate of biodegradation of TPH contaminated soil. However, further study by Bulman et al. (1993) demonstrated that the addition of nutrients to bioventing rendered an appreciable increase in the rate of degradation, which showed that the ability of nutrient amendment to boost TPH removal efficiency is dependent on soil type and nature of nutrient required for a successful bioventing process since some additives (nutrients) may trigger an increase in toxicity or hinder bioremediation process (Frutos et al. 2010).

However, soil type was reported as a crucial factor that influences the rate of degradation as Haghollahi et al. (2016) recorded the highest TPH reduction rate with sandy soil (70%) and a very low rate with clay soil (23.5%) during the study period. The degradation efficiency was increased to 57% by mixing clay with sandy soil. This low removal rate observed with clay soil is attributed to the low availability of oxygen in the soil environment since the increase in the volume of available oxygen increases the rate of degradation. Sandy soil has low total porosity with large individual pores, which facilitates faster oxygen absorption into and through the soil and the transport of carbon dioxide out of the soil, which is attributed to the appreciable biodegradation recorded in the present study. Soils like clay with small pores have slower absorption of oxygen into the soil and diffusion of carbon dioxide from the soil (Haghollahi et al. 2016). However, soil modification with organic wastes like BWW and MWW has shown an improvement in soil porosity and water holding capacity (Liu et al. 2018), reduction in soil ecotoxicity, and increase in soil pH and organic nutrients, which are indispensable for an improved rate of biodegradation (Liu et al. 2018).

In this study, TPH loss due to abiotic factors (sunlight, temperature, light, wind and water) were not taken into cognizance because they often played a marginal role in extracting petroleum hydrocarbons (Wild & Jones 1993). Similarly, Agnello et al. (2016) reported that abiotic factors facilitated the desorption of pollutants to enhance the bioavailability of petroleum and, hence, minimally contribute to direct TPH removal (Sun et al. 2013). Also, volatilization was not considered in the process because the contaminant has less volatile components and considering low operating temperature and low flow intensities, these components have a negligible effect, according to the findings of Fingas (2004) and Ma et al. (2014). To further reaffirm the above-stated, Jia et al. (2016) noted that the removal of crude oil in soils in the sets of treatments was caused by volatilization and biodegradation. Still, biodegradation was the most effective removal process, accounting for more than 58% of the total removal and reached the highest removal efficiency with nutrient adjustment, as reported by Jia et al. (2016). The non-responsive nature of some hydrocarbons to biological degradation accounts for the low removal efficiency of this recalcitrant group of hydrocarbons, which occurs at difficult intervals in the same treatment environment, hence reducing the TPH removal efficiency. According to Adams et al. (2017), the biological method mechanism is targeted at degrading mainly aliphatic compounds, cyclic hydrocarbons, aromatics, and other heavy hydrocarbons which are resistant to bioattenuation. The difference in initial TPH concentration with bioattenuation indicates the microbial population degradation of the hydrocarbons (Agarry & Latinwo 2015; Adams et al. 2017) which was supported by the GCMS study, which showed a substantial reduction in TPH concentration due to microbial degradation and the formation of oxidized hydrocarbons, such as alcohols, acids, carboxylic acids and esters, and the formation of low concentration amine compounds due to the presence of organic nutrients in the soil (Adams et al. 2017). However, the decrease in removal efficiencies suggests the depletion of nutrients needed by microbes (Boopathy 2000).

In agreement with the present BSTc study, which recorded TPH average removal efficiency of 52.43% with 50 g/kg TPH initial concentration of contaminated soil after 28 days, Mohajeri et al. (2017) recorded a 43% TPH average removal efficiency using an organic substrate with 60 g/kg TPH contaminated soil while 3 and 30 g/kg contaminated soil recorded 53.22% and 58.36% average removal efficiencies after 90 days study period. The result indicated that a high concentration of crude oil affects the bioremediation efficiency, presumably due to the toxicity of excess crude oil (> 30 g/kg) to the microbial community, which inhibits or lowers metabolism, as reported by Mohajeri et al. (2017). To buttress the above-stated, Ofoegbu et al. (2014) reported that the rate of biodegradation of crude oil contaminated soil is dependent on the volume of contaminants or the degree of contamination. Thus, the higher the contaminant concentration, the slower the rate of biodegradation and vice versa, hence, the significant variation in removal efficiencies of organic amendments. In addition, the present bioventing study which recorded 54.93% (BVTc) and 65.41% (BVTc and wastewater amendment) average TPH removal efficiency after 28 days correlated with the study by Lee and Swindoll (1993), where bioventing with organic matter amendment boosted > 60% removal efficiency after 70 days study period. Also, the study by Balba et al. (1998) and Agarry and Latinwo (2015) reported 64.2% and 61.7% biodegradation efficiencies with bioventing after 12 months and 28 days treatment period, respectively, while appreciable removal efficiency of > 75% was recorded with bioventing and organic amendment as reported by Agarry and Latinwo (2015). However, the control treatment was unable to appreciably reduce the concentration below the toxic level due to a lack of nutrients, which bolsters the positive effect of wastewater and air injection as a potential bioremediation strategy for the treatment of crude oil-contaminated soils.

## CONCLUSION

The findings demonstrated that the introduction of wastewater can stimulate the microbial environment, increase the mineralization rate and promote the activities of microorganisms for effective biodegradation, as evidenced by the appreciable removal efficiencies recorded, which showed that bioremediation of petroleum-contaminated soil could be promoted by optimizing soil physicochemical properties using organic substrate only. The bioventing process provided sufficient oxygen in the treatment system, which induced and adjusted the aerobic environment to enhance microbial activities for effective TPH removal. Still, the combined application of bioventing with wastewater improved the removal efficiency to achieve an average TPH removal efficiency of 62.79% as against 52.43% recorded for BSTc treatment as BVTc recorded appreciable efficiency with BWW than MWW amendment. This study demonstrated the feasibility of a combined wastewater and bioventing strategy in the remediation of crude oil-contaminated soil having significantly reduced the TPH concentration in the soil. Given the proven success and remarkable efficiency of this process, it can be readily extended to treat larger quantities of contaminated soil directly at affected sites, offering significant benefits to oil companies in oil-producing regions seeking efficient soil remediation solutions. Based on the scope of work, we have established the efficiency of wastewater in crude oil-contaminated soils. While we focused on the TPH, it is worth noting that the application of the soils in various activities could further serve as a guide towards understanding other changes, if any, that may have also affected the soil. Changes may be influenced by any other activities owing to the nature of the samples containing microorganisms.

## REFERENCES

Aburto-Medina A., Adetutu E.M., Aleer S., Weber J., Patil S.S., Sheppard P.J., Ball A.S., Juhasz A.L. (2012): Comparison of indigenous and exogenous microbial populations during slurry phase biodegradation of long-term hydrocarbon-contaminated soil. Biodegradation, 23: 813–822.

Adams F.V., Niyomugabo A., Sylvester O.P. (2017): Bioremediation of crude oil contaminated soil using agricultural wastes. Procedia Manufacturing, 7: 459–464.

Adekunle A.A., Adekunle I.M., Badejo A.A., Alayaki F.M., Olusola A.O. (2017): Laboratory scale bioremediation of crude oil impacted soil using animal waste compost. Tehnički glasnik, 11: 45–49.

Adekunle I.M. (2011): Bioremediation of soils contaminated with nigerian petroleum products using composted municipal wastes. Bioremediation Journal, 15: 230–241.

Agarry S.E., Ogunleye O.O. (2012): Box-Behnken design application to study enhanced bioremediation of soil artificially contaminated with spent engine oil using biostimulation strategy. International Journal of Energy and Environmental Engineering, 3: 1–14.

Agarry S., Latinwo G. (2015): Biodegradation of diesel oil in soil and its enhancement by application of bioventing and amendment with brewery waste effluents as biostimulation-bioaugmentation agents. Journal of Ecological Engineering, 16: 82–91.

Agarry S.E., Owabor C.N., Yusuf R.O. (2010): Bioremediation of soil artificially contaminated with petroleum hydrocarbon oil mixtures: evaluation of the use of animal manure and chemical fertilizer. Bioremediation Journal, 14: 189–195.

Agnello A.C., Bagard M., Van Hullebusch E.D., Esposito G., Huguenot D. (2016): Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. Science of the Total Environment, 563: 693–703.

Al-Kindi S., Abed R.M.M. (2016): Effect of biostimulation using sewage sludge, soybean meal, and wheat straw on oil degradation and bacterial community composition in a contaminated desert soil. Frontiers in Microbiology, 7: 240.

Alexander M. (1999): Biodegradation and Bioremediation. San Diego, Gulf Professional Publishing.

Amanchukwu S., Obafemi A., Okpokwasili G. (1989): Hydrocarbon degradation and utilization by a palm-wine yeast isolate. FEMS Microbiology Letters, 57: 151–154.

Amenorfenyo D.K., Huang X., Zhang Y., Zeng Q., Zhang N., Ren J., Huang Q. (2019): Microalgae brewery wastewater treatment: Potentials, benefits and the challenges. International Journal of Environmental Research and Public Health, 16: 1910.

Balba M., Al-Daher R., Al-Awadhi N., Chino H., Tsuji H. (1998): Bioremediation of oil-contaminated desert soil: The Kuwaiti experience. Environment International, 24: 163–173.

Barathi S., Vasudevan N. (2003): Bioremediation of crude oil contaminated soil by bioaugmentation of *Pseudomonas fluorescens* NS1. Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering, 38: 1857–1866.

Bassey E., Inyang J. (2012): Characterization of brewery effluent fluid. Journal of Engineering and Applied Sciences, 4: 67–77.

Benyahia F., Embaby A.S. (2016): Bioremediation of crude oil contaminated desert soil: Effect of biostimulation, bioaugmentation and bioavailability in biopile treatment systems. International Journal of Environmental Research and Public Health, 13: 219.

Boopathy R. (2000): Factors limiting bioremediation technologies. Bioresource Technology, 74: 63–67.

Bremner J.M., Mulvaney C.S. (1982): Nitrogen-total. In: Page A.L., Miller R.H., Keeney D.R. (eds.): Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America, Madison: 595–624.

Bulman L.T., Newland M., Wester A. (1993): In situ bioventing of a diesel fuel spill. Hydrological Sciences Journal, 38: 297–308.

Byun I.-G., Nam H.-U., Song S.K., Hwang I.-S., Lee T.-H., Park T.-J. (2005): Monitoring of bioventing process for diesel-contaminated soil by dehydrogenase activity, microbial counts and the ratio ofn-alkane/isoprenoid. Korean Journal of Chemical Engineering, 22: 917–921.

Chaillan F., Chaineau C., Point V., Saliot A., Oudot J. (2006): Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. Environmental Pollution, 144: 255–265.

Chen F., Li X., Zhu Q., Ma J., Hou H., Zhang S. (2019): Bioremediation of petroleum-contaminated soil enhanced by aged refuse. Chemosphere, 222: 98–105.

Chijioke-Osuji C.C., Ibegbulam-Njoku P.N., Belford E.J.D. (2014): Biodegradation of crude oil polluted soil by co-composting with agricultural wastes and inorganic fertilizer. Journal of Natural Science Research, 4: 28–39.

Chorom M., Hosseini S., Motamedi H. (2010): Bioremediation of crude oil polluted soil as affected by sewage-sludge. In: Proc. 19th World Congress of Soil Science: Soil Solutions for a Changing World, Brisbane, Aug 1–6, 2010: 4–7.

Couto N., García-Frutos F.J. (2016): Biological Techniques to remediate petroleum hydrocarbons in contaminated environments. Soil Remediation: Applications and New Technologies: 139: 148–157.

Deckers J., Nachtergaele F., Spaargaren O. (2003): Tropical soils in the classification systems of USDA, FAO and WRB. In: Evolution of Tropical Soil Science, Brusel, March 6, 2002: 79–94.

Dupont R.R., Doucette W.J., Hinchee R.E. (1991): Assessment of *in situ* Bioremediation Potential and the Application of Bioventing at a Fuel-contaminated Site. Stoneham, Butterworth Publishers: 262–282.

Fingas M.F. (2004): Modeling evaporation using models that are not boundary-layer regulated. Journal of Hazardous Materials, 107: 27–36.

Frutos F.J.G., Escolano O., García S., Babín M., Fernández M.D. (2010): Bioventing remediation and ecotoxicity evaluation of phenanthrene-contaminated soil. Journal of Hazardous Materials, 183: 806–813.

Haghollahi A., Fazaelipoor M.H., Schaffie M. (2016): The effect of soil type on the bioremediation of petroleum contaminated soils. Journal of Environmental Management, 180: 197–201.

Hinchee R.E. (2017): Bioventing of Petroleum Hydrocarbons. Handbook of Bioremediation (1993). Boca Raton, CRC Press.

Hinchee R.E., Arthur M. (1991): Bench scale studies of the soil aeration process for bioremediation of petroleum hydrocarbons. Applied Biochemistry and Biotechnology, 28: 901–906.

Hinchee R.E., Downey D.C., Aggarwal P.K. (1991): Use of hydrogen peroxide as an oxygen source for in situ biodegradation: Part I. Field studies. Journal of Hazardous Materials, 27: 287–299.

Huling S.G., Bledsoe B.E., White M.V. (1990): Enhanced bioremediation utilizing hydrogen peroxide as a supplemental source of oxygen: A laboratory and field study. Final Report, August 1987–November 1989. U.S. Department of Energy, Office and Scientific and Technical Information. Available on https://www.osti.gov/biblio/6934160.

Jia J., Zhao S., Hu L., Wang Y., Yao L., Liu Y., Yuan Z. (2016): Removal efficiency and the mineralization mechanism during enhanced bioventing remediation of oil-contaminated soils. Polish Journal of Environmental Studies, 25: 1955–1963.

Joo H.-S., Ndegwa P.M., Shoda M., Phae C.-G. (2008): Bioremediation of oil-contaminated soil using Candida catenulata and food waste. Environmental Pollution, 156: 891–896.

Lee M.D., Swindoll C.M. (1993): Bioventing for in situ remediation. Hydrological Sciences Journal, 38: 273–282.

Ling C.C., Isa M.H. (2006): Bioremediation of oil sludge contaminated soil by co-composting with sewage sludge. Journal of Scientific and Industrial Research, 65: 364–369.

Liu Q., Li Q., Wang N., Liu D., Zan L., Chang L., Gou X., Wang P. (2018): Bioremediation of petroleum-contaminated soil using aged refuse from landfills. Waste Management, 77: 576–585.

Ma Y., Zheng X., Anderson S., Lu J., Feng X. (2014): Diesel oil volatilization processes affected by selected porous media. Chemosphere, 99: 192–198.

Mbah G.C., Obahiagbon K.O. (2018): Kinetics of bioremediation of crude oil contaminated soil using organic and inorganic particulates. Petroleum Science and Technology, 36: 9–15.

Mohajeri L., Zahed M.A., Abdul Aziz H., Hasnain Isa M. (2017): Assessment of bioaugmentation and biostimulation efficiencies for petroleum contaminated sediments. Environmental Energy and Economic Research, 1: 89–98.

Møller J., Winther P., Lund B., Kirkebjerg K., Westermann P. (1996): Bioventing of diesel oil-contaminated soil: Comparison of degradation rates in soil based on actual oil concentration and on respirometric data. Journal of Industrial Microbiology, 16: 110–116.

Muskus Morales A.M., Santoyo Muñoz C., Plata Quintero L.S. (2013): Evaluation of natural attenuation, bioventing, bioaugmentation and bioaugmentation- bioventing techniques, for the biodegradation of diesel in a sandy soil, through column experiments. Gestión y Ambiente, 16: 83–94. (in Spanish)

Ofoegbu R.U., Momoh Y.O.L., Nwaogazie I.L. (2014): Bioremediation of crude oil contaminated soil using organic and inorganic fertilizers. Journal of Petroleum & Environmental Biotechnology, 6: 198.

Okop I.J., Ekpo S.C. (2012): Determination of total hydrocarbon content in soil after petroleum spillage. In: World Congress on Engineering, London, July 4–6, 2012, Vol. 3: 2–6.

Olsen S.R., Sommers L.E. (1982): Phosphorus. In: Page A.L. (ed.): Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America, Madison: 403–430.

Osuji L., Egbuson E., Ojinnaka C. (2005): Chemical reclamation of crude-oil-inundated soils from Niger Delta, Nigeria. Chemistry and Ecology, 21: 1–10.

Oudot J., Merlin F., Pinvidic P. (1998): Weathering rates of oil components in a bioremediation experiment in estuarine sediments. Marine Environmental Research, 45: 113–125.

Shahsavari E., Poi G., Aburto-Medina A., Haleyur N., Ball A.S. (2017): Bioremediation approaches for petroleum hydrocarbon-contaminated environments. In: Enhancing Cleanup of Environmental Pollutants. Vol. 1: Biological Approaches. Springer: 21–41.

Sun Y., Wang Z., Fu P., Jiang Q., Yang T., Li J., Ge X. (2013): The impact of relative humidity on aerosol composition and evolution processes during wintertime in Beijing, China. Atmospheric Environment, 77: 927–934.

Talinli I., Anderson G. (1992): Interference of hydrogen peroxide on the standard COD test. Water Research, 26: 107–110.

Thomé A., Reginatto C., Cecchin I., Colla L.M. (2014): Bioventing in a residual clayey soil contaminated with a blend of biodiesel and diesel oil. Journal of Environmental Engineering, 140: 06014005.

Troquet J., Larroche C., Dussap C.-G. (2003): Evidence for the occurrence of an oxygen limitation during soil bioremediation by solid-state fermentation. Biochemical Engineering Journal, 13: 103–112.

Varjani S.J., Upasani V.N. (2017): Crude oil degradation by *Pseudomonas aeruginosa* NCIM 5514: Influence of process parameters. Indian Journal of Experimental Biology, 55: 493–497.

Wang S.-Y., Kuo Y.-C., Hong A., Chang Y.-M., Kao C.-M. (2016): Bioremediation of diesel and lubricant oil-contaminated soils using enhanced landfarming system. Chemosphere, 164: 558–567.

Wild S., Jones K. (1993): Biological and abiotic losses of polynuclear aromatic hydrocarbons (PAHs) from soils freshly amended with sewage sludge. Environmental Toxicology and Chemistry, 12: 5–12.

Zhang K., Wang S., Guo P., Guo S. (2021): Characteristics of organic carbon metabolism and bioremediation of petroleum-contaminated soil by a mesophilic aerobic biopile system. Chemosphere, 264: 128521.

Received: July 13, 2023
Accepted: March 27, 2024