Songklanakarin J. Sci. Technol.
41 (1), 12-20, Jan. – Feb. 2019

http://www.sjst.psu.ac.th

Original Article

Abundance and diversity of hydrocarbon utilizing bacteria in the oil-contaminated soils throughout a remedial scheme using compost amendment

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Received: 14 August 2016; Revised: 24 July 2017; Accepted: 17 September 2017

Abstract

Since hydrocarbon utilizing bacteria (HUB) play a role in the biodegradation of hydrocarbons in oil-contaminated soil, the abundance and diversity of HUB over the course of soil remediation is indicative of the hydrocarbon bioremediation performance. This research experimented with uncontaminated soil, 5% (w/w) oil-contaminated soil (with no compost amendment), and 5% (w/w) oil-contaminated soil amended with chicken manure compost. The entire experiment lasted 84 days. The findings indicated that the introduction of chicken manure compost into the soils increased the abundance and diversity of the bacterial communities and thereby the biodegradation performance. The higher numbers of microbes in the compost-amended soil relative to the non-amended soils could be attributed to the nutrients in the compost, which promoted the bacterial growth. The experimental results are expected to provide useful insights for further study and the application of poultry waste to the bioremediation of petroleum-contaminated soils.

Keywords: hydrocarbon utilizing bacteria, PCR-DGGE, contaminated soil, used lubricating oil

1. Introduction

The disposal of hydrocarbons into the soil causes enormous damage to the ecosystem. Furthermore, the accumulation of pollutants in animal or plant tissues leads to mutations or even death (Yakubu, 2007). Bioremediation is among the commonly used methods to treat hydrocarbon-contaminated soils, whereby the microbes are utilized to degrade the toxic hydrocarbon contaminants.

Bioremediation is an environmentally-friendly and cost-effective means of cleaning up contaminated soil. Examples of bioremediation methods are aeration of contaminated soils (natural attenuation) and the addition of organic matter (compost) or nutrients (bio-stimulation) to stimulate the activities of indigenous or exogenously added microorganisms (bioaugmentation) to accelerate the process of contaminant elimination (Adetutu *et al.*, 2013; Aleer *et al.*, 2011; Bundy *et al.*, 2004).

Animal waste is an economical source of nitrogen, phosphorus, and potassium as well as other nutrients for plant growth (Okafor *et al.*, 2016). In addition to the soil-enriching benefits, animal waste products from livestock and poultry are good candidates for the bioremediation of soil contamination. Naowasarn and Leungprasert (2016) carried out a batch-scale experiment to determine the effectiveness of chicken manure in the biodegradation of used lubricating oil in soil concentrations of 5%, 10%, and 20%. The highest total petroleum hydrocarbon (TPH) reduction efficiency (>60%) was achieved at 5% oil contaminated soil that was remediated with chicken manure compost. In addition, the study also aimed to determine the effects of oil concentration on biodegradation of used lubricating oil.

The biodegradation rates could be accelerated simply by increasing the populations of indigenous bacteria in the oil-contaminated soil (Berekaa, 2013). In addition, bio-stimulation techniques can be used to increase the activity of the indigenous bacteria by adjusting the environmental

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parameters (Zhang *et al.*, 2011). Thus, a deeper understanding of the bacterial community of the polluted site is useful for developing and optimizing bioremediation strategies.

This research investigates the bacterial abundance and diversity in a full-scale composting process of soils contaminated with used lubricating oil at a 5% (w/w) concentration with and without the chicken manure compost. In addition, the hydrocarbon utilizing bacteria (HUB) were determined using the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique. The research results are expected to be of use for the future application of poultry waste-based bioremediation in petroleum-contaminated soils.

2. Materials and Methods

2.1 Synthetic uncontaminated soil

The initial soil samples were 0-30 cm of fresh uncontaminated top soil without used lubricating oil obtained at the Faculty of Agriculture, Kasetsart University, Bangkok. The soils were dried indoors to reduce the moisture content to less than 5% and then passed through a 2-mm mesh sieve to remove the debris. The initial soil was 28% sand, 20% silt, and 52% clay. The soil was modified to be similar to the Eastern Seaboard Industrial Estates region of Thailand by adding sand in a ratio of 1:1 (soil:sand, w/w dry wt) for sandy clay loam with 64% sand, 10% silt, and 26% clay. The addition of sand (Naowasarn & Leungprasert, 2016) contributed to the lower soil bulk density, higher porosity and oxygen diffusion, and the formation of water-stable aggregates. Moreover, microbial influence is most pronounced in sandy soils where soil microorganisms produce a readily available C source for the rapid stabilization of aggregates (Bronick & Lal, 2005). Table 1 tabulates the physicochemical properties of the experimental soils.

2.2 Synthetic contaminated soil and compost material

2.2.1 Synthetic contaminated soil

The hydrocarbon-contaminated soil was prepared by mixing the synthetic uncontaminated soil with used lubricating oil to obtain a concentration of 5% (w/w). Prior to the experiment, the synthetic contaminated soils were stored in plastic tote boxes at room temperature (30±2 ºC) for 14 days.

2.2.2 Compost material

In this research, the chicken manure was collected from the Department of Animal Science, Kasetsart University, Bangkok. The chicken manure was stored in polythene bags and transported to the laboratory. The compost material was a mixture of chicken manure and charred chaff in a ratio of 1:0.2 (manure:chaff, w/w dry wt) with the C/N ratio of 30:1. The compost material was then mixed with the synthetic contaminated soil in a ratio of 3:1 (soil:compost, w/w dry wt) (Wong *et al.*, 2002). The physicochemical properties of the chicken manure and charred chaff are tabulated in Table 1.

2.3 Composting reactors and experimental setup

Unlike our previous study (Naowasarn & Leungprasert, 2016) which was batch-scale experiments that employed seven 3 L cylindrical composting reactors, this current research deployed three 20 L cylindrical composting reactors of 20 cm and 65 cm in diameter and height, respectively. The first and second reactors were filled with the synthetic contaminated soil (S1) and the synthetic uncontaminated soil mixed with the compost (S2), respectively. The S1 and S2 reactors were the control reactors. The third reactor was filled with the synthetic contaminated soil mixed with the compost (S3). The mixture ratios of the experimental composting reactors are provided in Table 2.

Table 1. The physicochemical properties of the experimental soils, chicken manure and charred chaff.

Parameter	Synthetic uncontaminated soil	Synthetic uncontaminated soil + 5% oil	Synthetic uncontaminated soil + Compost	Synthetic uncontaminated soil + 5% oil + Compost	Composting material	
					Chicken manure	Charred chaff
Moisture content (%)	0.59	28.49	30.76	31.10	2.16	43.96
pH	7.16	7.49	6.26	6.82	6.32	5.73
Ec (ds m^{-1})	0.36	2.51	2.43	2.57	0.009	1.57
Total Nitrogen (mg kg^{-1} of dry weight)	594	975	2,469	2,784	8,977	224
Total Phosphorus (mg kg^{-1} of dry weight)	1,199	1,309	27,799	22,729	12,159	185
Total Carbon (mg kg^{-1} of dry weight)	31,344	4,2143	72,817	82,820	228,110	211,390
TPH[a] (mg kg^{-1} of dry weight)	ND	47,809	ND	39,431	ND	ND
THB[b](log CFU kg^{-1})	6.13	6.96	6.79	6.69	8.47	6.78
HUB[c] (log CFU kg^{-1})	5.67	6.77	6.58	6.92	7.61	6.34

[a]TPH denotes total petroleum hydrocarbons
[b]THB denotes total heterotrophic bacteria
[c]HUB denotes hydrocarbon utilizing bacteria
ND denotes not detectable

Table 2. Mixture ratios of the experimental composting reactors.

Composting reactor	Dry weight (kg)				Mixture ratio (soil:manure:chaff)
	Soil	Chicken manure	Charred chaff	Total	
S1(control)	14.03	-	-	14.03	-
S2 (control)	10.35	2.87	0.57	13.79	1 : 0.28 : 0.06
S3	10.65	2.82	0.56	14.03	1 : 0.26 : 0.05

S1, synthetic uncontaminated soil + 5% oil; S2, synthetic uncontaminated soil + compost; S3, synthetic uncontaminated soil + 5% oil + compost

To minimize the heat loss, the reactors were wrapped with aluminum foil. The reactors were also fitted with three sampling ports at the heights of 12.5 (L), 32.5 (M) and 52.5 cm (U) from the base. The reactors were aerated by an ACO-318 electrical magnetic air pump at the rate of 0.4 m^3 kg VS^{-1} day^{-1} (Figure 1). Moreover, the moisture content in all three reactors was maintained at approximately 30% to keep the mixtures in solid phase for the entire composting period, by which air fed through deionized water was continually supplied from the reactor base.

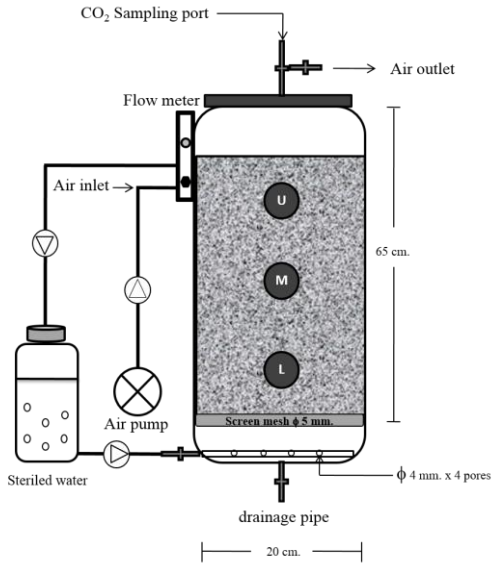

Figure 1. Schematic of the experimental composting reactors, where U, M, and L are the upper, middle and lower sampling ports, respectively.

2.4 Sampling

Approximately 60 g of homogenized samples were collected through each of the three sampling ports of the three reactors (S1, S2, and S3). The sample collection for the bacterial count was carried out at the start (day 0) and every 14 days (days 14, 28, 42, 56, and 70) to termination day 84. Meanwhile, the samples for the PCR-DGGE analysis were collected on days 0, 42, and 84. The samples were freeze-stored at −20 °C for further analysis (Chang *et al.,* 2010; Ferrera-Rodríguez *et al.,* 2013).

2.5 TPH estimation

The TPHs in the contaminated soil were measured every 14 days using the Soxhlet extraction method, whereby 150 mL of acetone/n-hexane (1:1, v/v) as the extraction agent was added into 5 g of the compost samples drawn from the three sampling ports (lower, middle, and upper ports) of the S1 and S3 reactors. Since the S2 reactor was filled with the uncontaminated soil, no TPH measurements were taken. The extraction solution was then evaporated using a rotary evaporator (Buchi Rotavapor, R-114) and the residual TPHs were determined gravimetrically (Mishra *et al.*, 2001).

In this research, TPH removal was calculated by

$$\text{TPH removal (\%)} = (C_1 - C_2)/C_1 \times 100$$

where C_1 is the concentration (mg kg^{-1}) of the TPHs in the pre-remediated contaminated soil, and C_2 is the concentration (mg kg^{-1}) of the TPHs in the post-remediated soil.

2.6 Enumeration of THB and total HUB bacteria

This research utilized the pour plate method for the bacterial count. First, 10 g of the compost samples of days 0, 14, 28, 42, 56, 70, and 84 from the three reactors were mixed with 90 mL of distilled water. Then, 1 mL of the mixture was diluted with 9 mL of distilled water (10-fold serial dilution).

THB were enumerated using nutrient agar (HiMedia). The medium was prepared according to the manufacturer's specifications. The aliquots (1 mL) were plated in triplicate and incubated for 24 h at room temperature (30±2 °C) prior to the colony counts (Subathra *et al.,* 2013). Meanwhile, total HUB were enumerated using oil agar (Zajic & Supplisson, 1972) prepared by mixing K_2HPO_4 (1.8 g), NH_4Cl (4.0 g), $MgSO_4{\cdot}7H_2O$ (0.2 g), KH_2PO_4 (1.2 g), $FeSO_4{\cdot}7H_2O$ (0.01 g), NaCl (0.1 g), agar (20 g), and used lubricating oil (1 mL) in 1000 mL of distilled water. The pH of the medium was adjusted to 7.4 before autoclaving. The oil agar plates were incubated at 30±2 °C for 5 days before the colony counts. The average mean colony counts were recorded and the log colony forming unit (log CFU) per kilogram of the samples were calculated.

2.7 DNA extraction

The genomic DNA was extracted directly from the compost samples (0.25 g each) at days 0, 42, and 84 from the three reactors using the FavorPrep Soil DNA Isolation Mini kit (Favorgen Biotech) according to the manufacturer's protocol.

2.8 PCR-DGGE

The genomic DNA extracts from the compost samples were used as templates for PCR amplification of V3 region of 16S rDNA. The universal primers GC-341F (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACG GGGGGCCTA-CGGGAGGCA GCAG-3′) and 518R (5′-ATTACCGCG GCTGCTGG-3′) were used to target the bacteria (Muyzer *et al.,* 1993). PCR amplification was carried out in a 50 µL solution containing 2 µL of purified DNA extract from the compost samples, 1xTop*Taq* PCR buffer, 3 mM $MgCl_2$, 200 µM dNTP mixture, 10 pmol of each primer, and 1 U *Taq* polymerase (Qiagen, Germany). The temperature cycle for the PCR was 30 cycles of 50s of denaturation at 95 ºC, 1 min of annealing at 60 ºC, a 50 s extension at 72 ºC, and the final primer extension of 7 min at 72 ºC. The number of base pairs (~200 bp for the domain bacteria) was determined by electrophoresis on 1.0% agarose gel. The gel was then stained with ethidium bromide and the DNA bands were visualized and photographed with a UV transilluminator.

Denaturing gradient gel electrophoresis (DGGE) was performed using the DCode Universal Mutation Detection System (BioRad Laboratories, USA). An 8% polyacrylamide gel with a 40–60% gradient was prepared using 7M urea and 40% formamide as the denaturants. After the polymerization, 20 µL of the PCR product was loaded into a well containing polyacrylamide gel. The DNA extracts were then run at a constant voltage of 80 V and 60 ºC for 10 h in the 1x TAE buffer. The gel was stained for 30 min in the 1xTAE containing ethidium bromide solution and then rinsed with distilled water for 5 min and photographed with the UV transilluminator.

2.9 Sequencing of DNA

The bands on the DGGE gels were excised aseptically, incubated in 30 µL of MilliQ water, and stored overnight at 4 ºC. The eluted DNA was then re-amplified with the primer pair (314F and 518R). Each product band was sequenced by a DNA sequence analyzer (AITbiotech Pte, Singapore). The DNA sequences were manually identified using the BLAST software on the NCBI website (http:// www.ncbi.nlm.nih.gov/) and compared against the GenBank references.

2.10 Bacterial community diversity

The relative band intensities on the DGGE profile were determined and the Shannon-Weaver diversity (*H′*) calculated (Shannon, 2001). Specifically, $H' = -\Sigma P_i \, LN \, P_i$, $P_i = n_i/N$, where n_i is the intensity of band i in the lane and N is the total intensity of all bands in the lane.

3. Results and Discussion

3.1 The removal of TPH by used lubricating oil

The highest TPH removal efficiency (>60%) was achieved under the 5% (w/w) contaminated compost condition. The 5% oil contamination was used based on previous research (Naowasarn & Leungprasert, 2016). Figure 2 compares the percentages of TPH removal relative to the composting time (14, 28, 42, 56, 70, and 84 days) of the S1 and S3 reactors. Since the S2 reactor contained the uncontaminated soil, it was excluded from the TPH removal analysis. The results indicated higher TPH removal efficiency in the S3 reactor (with the chicken manure compost) from day 14 to termination day 84 compared with the S1 reactor (without the compost). No significant difference was observed in the TPH removal between the three sampling ports of the same reactor. The higher TPH removal of the S3 reactor could be attributed to the higher nutrient contents in the chicken manure, e.g., nitrogen, which promoted the bacterial growth. Similar results were observed by Ros *et al.* (2010) and Chijioke-Osuji *et al.* (2014) in the hydrocarbon-contaminated soils amended with sewage sludge compost and agricultural wastes, respectively.

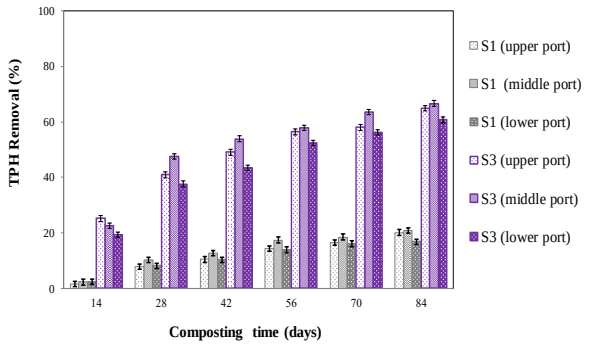

Figure 2. Percentages of TPH removal in the sampling ports relative to the composting time of synthetic contaminated soil (S1) and the synthetic contaminated soil mixed with the compost (S3). The bars indicate standard error (n=3). Note: The reactors had lower, middle, and upper sampling ports. The S2 reactor was filled with the *uncontaminated* soil.

3.2 Enumeration of bacteria

The initial counts of THB and HUB in the three reactors were almost identical, probably due to the low moisture content (<5%) of the synthetic soils and no stimulation for the bacteria growth. During the composting process compared with the S1 and S2 reactors, the THB and HUB populations in the S3 reactor increased rapidly during the first 28 days of the 84-day experimental period (Figures 3A and B). The THB and HUB counts in the S3 reactor were in the range of 10.39-10.87 log CFU kg^{-1} and 9.97-10.82 log CFU kg^{-1}, respectively. Moreover, the samples from the middle sampling port of the S3 reactor exhibited the highest bacterial numbers. The highest was significantly higher (P=0.01) than in the upper and lower sampling ports. The findings indicated that the hydrocarbons in the soils served as the carbon source for the microbial growth and thus resulted in the higher log CFU for both THB and HUB in the S3 reactor compared with the S2 reactor.

3.3 Microbial community analysis

The microbial communities in the samples of the three reactors drawn on days 0, 42, and 84 were determined using the PCR-DGGE technique. Figure 4 illustrates the

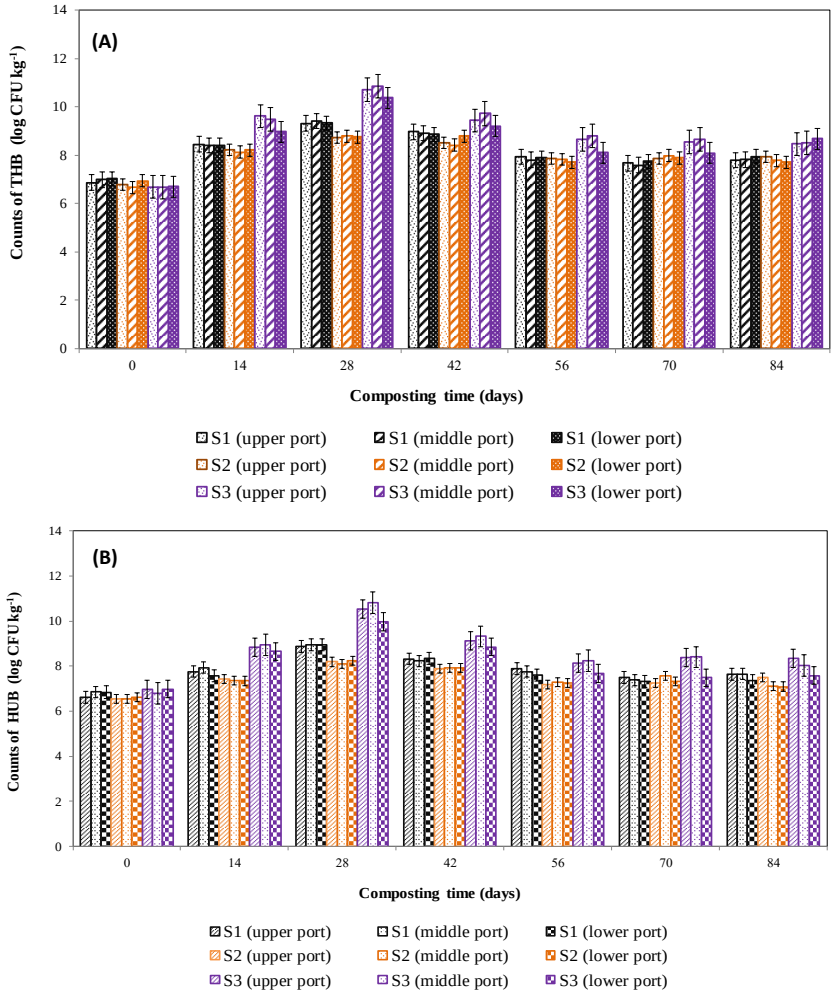

Figure 3. Bacterial counts in the sampling ports relative to the composting time of synthetic contaminated soil (S1), the synthetic uncontaminated soil mixed with the compost (S2) and the synthetic contaminated soil mixed with the compost (S3). The bars indicate the standard errors (n=3). Note: The reactors had lower, middle, and upper sampling ports. (A) total heterotrophic bacteria, (B) hydrocarbon utilizing bacteria.

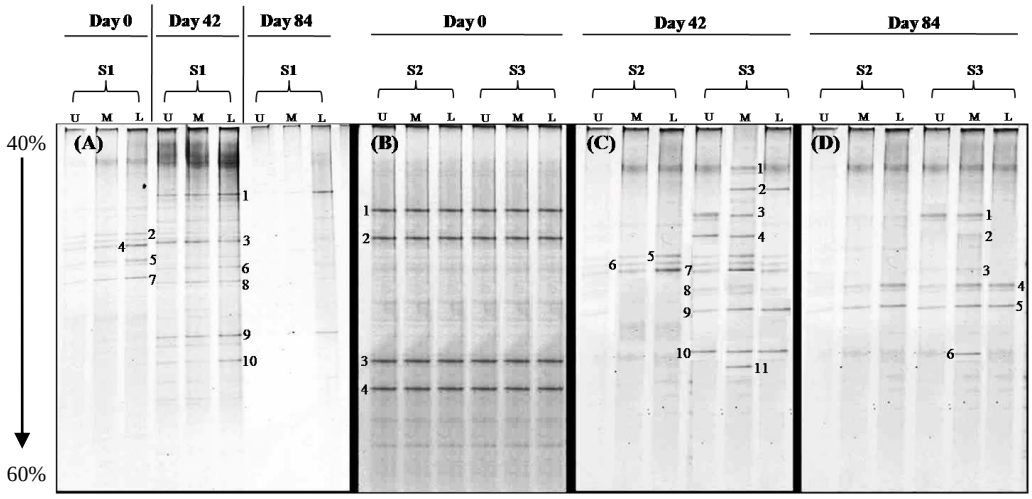

Figure 4. DGGE profiles of 16S rDNA gene fragments of the compost samples on days 0, 42, and 84 of the S1, S2, and S3 reactors, where U, M and L are the upper, middle, and lower sampling ports. Note: The DGGE bands were determined and are described in Table 1.

DGGE profiles. The dominant bands were excised and sequenced and their sequence identities tabulated in Table 3. The results showed the greatest bacterial abundance in the S3 reactor on day 42, compared with the other two reactors.

In the S1 reactor, 10 visible bands were detected that included *Desulfovibrio longus*, *Bacillus bataviensis*, *Gordonia polyisoprenivorans*, *Bacillus boroniphilus*, *Bacillus vireti*, *Cellulomonas sp.*, *Bacillus macauensis*, *Nocardia asteroides*, *Mycobacterium rhodesiae*, and *Thermomonospora curvata* (Figure 4A). Meanwhile, in reactors S2 and S3 on day 0, four visible bands were identified that included *Bacillus sp.*, *Clostridium celatum*, *Anoxybacillus flavithermus*, and *Bacillus cytotoxicus* (Figure 4B). On day 42, 11 visible bands were identified that included *Pseudomonas putida*, *Lysinibacillus sphaericus*, *Advenella kashmirensis*, *Caulobacter sp.*, *Shewanella oneidensis*, *Geobacter lovleyi*, *Dyadobacter fermentans*, *Starkeya novella*, *Pseudomonas stutzeri*, *Mycobacterium chubuense*, and *Rhodomicrobium vannielii* (Figure 4C). On day 84, six visible bands were detected that included *Brevibacillus borstelensis*, *Flavobacterium psychrophilum*, *Pseudomonas monteilii*, *Micavibrio aeruginosavorus*, *Rhodomicrobium vannielii*, and *Balneola vulgaris* (Figure 4D).

The *Bacillus* species were dominant in all reactors on day 0 but non-existent on day 42 and at termination day 84. The findings could be attributed to the operational and environmental factors, e.g., the soil types, the physico-chemical properties, and the hydrocarbon concentrations (Adetutu *et al.*, 2013; Das & Chandran, 2011). According to Reddy *et al.* (2011) and He *et al.* (2013), the *Bacillus* species were the main bacterial components of the microflora that degraded the organic matter. The species are also the dominant hydrocarbon degraders and play a major role in degrading polycyclic aromatic hydrocarbons (PAHs).

In this research, the differences in the microbial diversity were determined by the Shannon-Wiener index (*H′*) that was calculated according to the number and relative abundance of the terminal restriction fragments amplified from the DGGE profiles. On day 42, the *H′* index rose to 2.17, 1.06, and 3.49 from 1.45, 0.86, and 0.86 on day 0 and decreased at termination day 84 to 0.46, 0.69, and 1.96 for S1,

Table 3. Sequence identification of bands excised from the DGGE gel, where (A), (B), (C) and (D) correspond to the profiles in Figure 3.

DGGE band no.	Closest GenBank relative		% Identity	Phylogenetic group
	Strain or species	Accession no.		
(A)				
1	*Desulfovibrio longus* DSM 6739	NZ ATVA01000016.1	100	Gammaproteobacteria
2	*Bacillus bataviensis* LMG 21833	NZ AJLS01000166	97	Firmicutes
3	*Gordonia polyisoprenivorans* VH2	NC 016906.1	98	Actinobacteria
4	*Bacillus boroniphilus* JCM 21738	NZ_BAUW01000204	95	Firmicutes
5	*Bacillus vireti* LMG 21834	NZ ALAN01000208.1	97	Firmicutes
6	*Cellulomonas sp.* JC225	NZ HE978589.1	86	Actinobacteria
7	*Bacillus macauensis* ZFHKF-1	NZ AKKV01000038	94	Firmicutes
8	*Nocardia asteroides* NBRC 15531	NZ BAFO02000004	100	Actinobacteria
9	*Mycobacterium rhodesiae* NBB3	NC 008726.1	98	Actinobacteria
10	*Thermomonospora curvata* DSM 43183	NC 013510.1	94	Actinobacteria
(B)				
1	Bacillus sp. 1NLA3E	NC 021171.1	97	Firmicutes
2	Clostridium celatum DSM 1785	NZ KB291630.1|	92	Firmicutes
3	Anoxybacillus flavithermus WK1	NC 011567.1	93	Firmicutes
4	Bacillus cytotoxicus NVH 391-98	NC 009674.1	99	Firmicutes
(C)				
1	Pseudomonas putida KT2440	NC 002947.3	95	Gammaproteobacteria
2	Lysinibacillus sphaericus C3-41	NC 010382.1	99	Firmicutes
3	Advenella kashmirensis WT001	NC 017964.1	95	Betaproteobacteria
4	Caulobacter sp. K31	NC 010338.1	98	Alphaproteobacteria
5	Shewanella oneidensis MR-1	NC 004347.2	98	Gammaproteobacteria
6	Geobacter lovleyi SZ	NC 010814.1	95	Deltaproteobacteria
7	Dyadobacter fermentans DSM	NC 013037.1	93	Bacteroidetes
8	Starkeya novella DSM 506	NC 014217.1	94	Alphaproteobacteria
9	Pseudomonas stutzeri A1501	NC 009434.1	96	Gammaproteobacteria
10	Mycobacterium chubuense NBB4	NC 018027.1	98	Actinobacteria
11	Rhodomicrobium vannielii ATCC 17100	NC 014664.1	96	Alphaproteobacteria
(D)				
1	Brevibacillus borstelensis AK1	NZ APBN01000004.1	97	Firmicutes
2	Flavobacterium psychrophilum JIP02/86	NC 009613.3	100	Bacteroidetes
3	Pseudomonas monteilii SB3101	NC 023076.1	97	Gammaproteobacteria
4	Micavibrio aeruginosavorus ARL-13	NC 016026.1	93	Alphaproteobacteria
5	Rhodomicrobium vannielii ATCC 17100	NC 014664.1	91	Alphaproteobacteria
6	Balneola vulgaris DSM	NZ AQXH01000010.1	95	Bacteroidetes

S2, and S3, respectively (Figure 5). The bacterial diversity largely increased in the S3 reactor, compared with the other two reactors. According to He *et al.* (2013), effective composting requires a certain level of interactions between the microbial species. In fact, the bacterial diversity in the S2 reactor was lower than in the S1 reactor. This observation was attributable to the lack of a carbon source for the growth of hydrocarbon-degrading microbes.

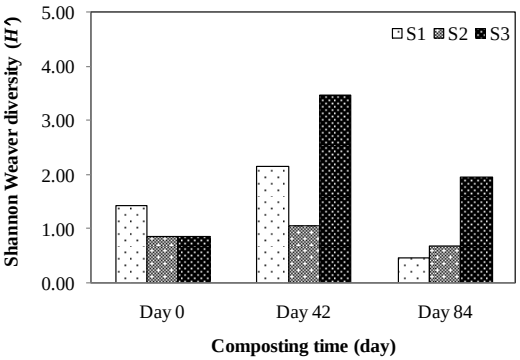

Figure 5. The Shannon-Weaver (H') index of microbial diversity relative to the composting time of synthetic contaminated soil (S1), the synthetic uncontaminated soil mixed with the compost (S2) and the synthetic contaminated soil mixed with the compost (S3).

3.4 Phylogenetic grouping analysis

A total of 31 bands were identified in the three reactors which were categorized into seven phyla, namely Bacteroidetes, Actinobacteria, Firmicutes, Deltaproteobacteria, Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. On day 0 the phylum Firmicutes was predominant in all three reactors as no microbial activity had been stimulated (Figure 6). According to Reddy *et al.* (2011), the absence of microbial activity could lead to the accumulation of metabolites as a result of the oxidation of hydrocarbons and subsequently reduce the viability of several hydrocarbon degraders.

In addition, in the S1 reactor, Actinobacteria and Gammaproteobacteria accounted for 83% and 17%, respectively, of the total on day 42. In the S2 reactor, Gammaproteobacteria, Deltaproteobacteria, and Bacteroidetes were the dominant groups at 33% each. Meanwhile, in the S3 reactor, Gammaproteobacteria and Alphaproteobacteria were the dominant groups at 27% each followed by Betaproteobacteria, Deltaproteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes at 9% each.

The phylogenetic grouping analysis on day 42 revealed a significant increase in the soil microbial diversity in the S3 reactor with the highest number of seven phyla compared with two and three phyla in the S1 and S2 reactors. This was probably due to the activation of the bacterial community through the available nutrient input (Baek *et al.*, 2007). In addition, Gammaproteobacteria and Alphaproteobacteria were the most abundant phyla in the S3 reactor.

Besides Gammaproteobacteria, its members *Pseudomonas* and *Shewanella* were dominant on day 42 but declined on day 84. Evidently, the phylum Gammaproteobacteria was capable of surviving on the contaminants for growth. In addition, specific adaptive mechanisms enabled the hydrocarbon-tolerant bacteria to grow in the presence of toxic hydrocarbons (Cheema *et al.*, 2015; Lăzăroaie, 2009). Consistent with Reddy *et al.* (2011), Gammaproteobacteria are gram negative bacteria that are both aerobic and facultative anaerobes known for degrading petroleum compounds.

According to Covino *et al.* (2016), *Pseudoxanthomonas* sp. is an effective degrader of PAHs as well as other high-molecular-weight compounds. In addition, certain species of *Pseudomonas* are capable of degrading *n*-alkanes and PAHs in the Alpine soils (Margesin *et al.*, 2003). The introduction of nutrients could also stimulate the co-degradation of alkanes in the soil and alter the sorption properties of alkanes to the soil particles. For instance, several *Pseudomonas* species in the soils are well known to produce biosurfactants like rhamnolipids (*P. aeruginosa* [Kumar *et al.*, 2008] and *P. fluorescens* [Abouseoud *et al.*, 2008]) that enhance hydrocarbon degradation.

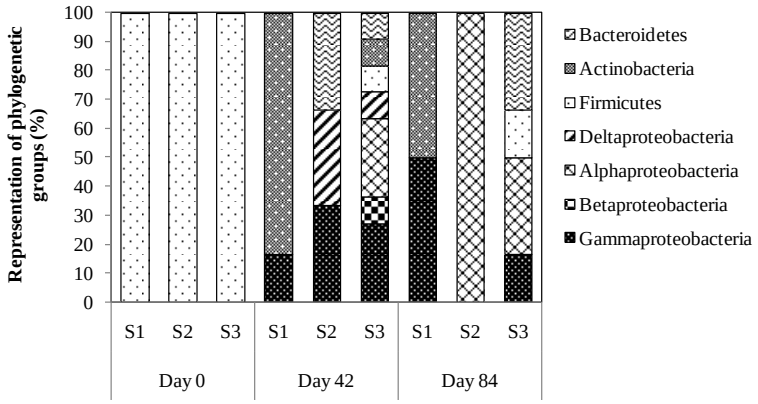

Figure 6. The percentage of representation of phylogenetic groups in the bioremediation process relative to the composting time of the synthetic contaminated soil (S1), the synthetic uncontaminated soil mixed with the compost (S2), and the synthetic contaminated soil mixed with the compost (S3).

The low percentages of the phyla Betaproteobacteria, Deltaproteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes indicated the lower levels of diversity and abundance. Certain members, i.e. *Geobacter* (belonging to Deltaproteobacteria) and *Mycobacterium* (Actinobacteria), were able to degrade hydrocarbons. Their presence was crucial to the hydrocarbon degradation, consistent with Wallisch *et al.* (2014) who reported that *Mycobacterium* played a role in the degradation of alkanes with a chain length between C5 and C16.

At termination day 84, in the S1 reactor, Gammaproteobacteria and Actinobacteria were the dominant groups at 50% each, while in the S2 reactor, Alphaproteobacteria was dominant at 100%. In the S3 reactor, Alphaproteobacteria and Bacteroidetes at 33% each were the dominant groups followed by Gammaproteobacteria and Firmicutes at 17% each. The findings were consistent with Reddy *et al.* (2011) who reported that Gammaproteobacteria showed a high intensity on day 0 and low intensity on days 5 and 10 with TPH/PAH degradation.

4. Conclusions

This experimental research has investigated the abundance and diversity of hydrocarbon utilizing bacteria (HUB) over an 84-day remediation period of oil-contaminated soils with and without the chicken-manure compost amendment. The experiments were carried out using three 20 L large-scale reactors given the C/N ratio of 30:1, the soil/compost ratio of 3:1, and the aeration rate of 0.4 m^3 kg VS^{-1} day^{-1}. The contents of the three experimental reactors were the synthetic 5% (w/w) oil-contaminated soil (S1), the uncontaminated soil with compost amendment (S2), and the 5% oil-contaminated soil with compost amendment (S3). The findings indicated that chicken manure is a potential source of nutrients for microbial activity and the indigenous bacteria are capable of utilizing hydrocarbons as a source of carbon and energy which is potentially useful in soil hydrocarbon pollution response action. Moreover, the successive degradation strategy was found to be useful when the bacterial communities (i.e. *Pseudomonas* and *Mycobacterium* spp.) removed most of the alkanes and partially removed the aromatic hydrocarbons. Since the entire experimental scheme of this current research was restricted to 84 days, a longer-term observation of a larger scale is thus recommended to further validate the method. For future remediation, this study can be usefully applied to other petroleum products. Nevertheless, long-term monitoring experiments need to be performed, especially on a field scale. Furthermore, information on the microbial community and environmental conditions, such as soil properties and oil concentration, is a key factor to be considered in the bioremediation of oil-contaminated areas.

Acknowledgements

The authors would like to extend deep gratitude to Thailand's King Prajadhipok and the Queen Rambhai Barni Memorial Foundation for the research grants.

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