



Cultivation of a bacterial consortium with the potential to degrade total petroleum hydrocarbon using waste activated sludge

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Abstract

Waste activated sludge was aerobically treated to demonstrate multiple uses such as cultivating an oil degrading bacterial consortium; studying the influence of a bulking agent (peat moss) and total petroleum hydrocarbon concentration on bacterial growth and producing a soil conditioner using waste activated sludge. After 30 days of incubation, the concentration of oil-degrading bacteria was 4.3×10^8 CFU g⁻¹ and 4.5×10^8 CFU g⁻¹ for 5 and 10 g of total petroleum hydrocarbon, respectively, in a mixture of waste activated sludge (1 kg) and peat moss (0.1 kg). This accounts for approximately 88.4 and 91.1%, respectively, of the total heterotrophic bacteria (total-HB). The addition of bulking agent enhanced total-HB population and total petroleum hydrocarbon-degrading bacterial population. Over 90% of total petroleum hydrocarbon degradation was achieved by the mixture of waste activated sludge, bulking agent and total petroleum hydrocarbon. The results of physico-chemical parameters of the compost (waste activated sludge with and without added peat moss compost) and a substantial reduction in *E. coli* showed that the use of this final product did not exhibit risk when used as soil conditioner. Finally, the present study demonstrated that cultivation of total petroleum hydrocarbon-degrading bacterial consortium and production of compost from waste activated sludge by aerobic treatment was feasible.

Key words

Bulking agent, Oil degrading bacteria, Total Petroleum hydrocarbon, Waste activated sludge

Introduction

Waste activated sludge or biosolids are organic by-products from wastewater treatment plants that contain valuable nutrients, but also cause pollution problem (Song *et al.*, 2009). Ocean acts as a sink for disposal of waste activated sludge. Furthermore, traditional methods like incineration and landfill could be also considered, but these methods also adversely affect the environment due to the hazardous pollutants (heavy metals), and pathogenic organisms present in waste activated sludge. Utilization of waste activated sludge for agricultural purpose is the best alternative for sludge disposal because it recycles both nutrients and organic matter (Singh and Agrawal, 2008). However, use of waste activated sludge as fertilizer can

cause accumulation of heavy metal in soil (Alonso *et al.*, 2002) and risk spread pathogens in soil (Dacera *et al.*, 2009). Song *et al.* (2009) proved that immobilization of heavy metals in waste activated sludge using phosphate amendment is a possible approach to its use as a fertilizer while inhibiting heavy metal accumulation in soils. Therefore, the composting of waste activated sludge after heavy metal immobilization is an attractive method for recycling and reusing sludge.

Moreover, the reduction of pathogens in waste activated sludge is required after metal immobilization to reduce health risks to the public related to biosolid handling and beneficial use. However, pollution by petroleum products is a serious environmental problem. Petroleum products have entered

ecosystems in large volumes through numerous routes, and they pose a long-term threat to all life forms on earth. Total petroleum hydrocarbon is a mixture of carcinogenic organic chemicals, such as benzene, toluene, naphthalene and benzopyrene, from crude oil. Many studies have examined the breakdown of petroleum products by microorganisms (Margesin *et al.*, 2007; Riffaldi *et al.*, 2006). This bioremediation technique has been shown to be viable and cost-effective and has become a valuable alternative to chemical and physical treatments (Aislabie *et al.*, 2006). To this end, several microorganisms with the ability to degrade contaminants from crude oil have been identified (Antic *et al.*, 2006; Joseph and Joseph, 2009). Joseph and Joseph (2009) successfully separated oil from petroleum refinery sludge using bacterial strains isolated from petroleum-contaminated soils. Margesin *et al.* (2007) reported that microorganisms could be the primary degraders of total petroleum hydrocarbon in contaminated ecosystems. Generally, microorganisms capable of utilizing petroleum hydrocarbons produce enzymes to metabolize total petroleum hydrocarbon when exposed to total petroleum hydrocarbon-contaminated ecosystem, ultimately becoming hydrocarbon-utilizers (Margesin *et al.*, 2007). This process may depend on the chemical composition of hydrocarbons and the species of microorganisms present in the particular ecosystem.

Direct application of microbial consortium of cultivated degraders to petroleum contaminated area with low numbers of indigenous total petroleum hydrocarbon-degrading microorganisms is of great use because it can shorten the remediation time (Marquez-Rocha *et al.*, 2001). Until now, oil-contaminated soil, marine water and sediments have been used to isolate oil-degrading bacteria. Although, degradation of petroleum products using these isolates has been successfully demonstrated in many studies, a review of the literature shows lack of information on isolation of oil degraders from waste activated sludge. Therefore, waste activated sludge, which harbours a variety of microorganisms and contain high level of nutrients for microbial growth (Namkoong *et al.*, 2002), waste activated sludge used to cultivate total petroleum hydrocarbon degraders in the present study.

Several reports have confirmed that microbial consortia are better at degrading petroleum hydrocarbons than individual strains (Facundo *et al.*, 2001; Akoachere *et al.*, 2008). Moreover, several laboratory and field experiments have demonstrated that inorganic and organic fertilizers have several positive effects on total petroleum hydrocarbon decontamination in soil ecosystem (Delille *et al.*, 2004; Margesin *et al.*, 2007; Xu and Obbard 2004). Owing to high moisture content, it is difficult to use waste activated sludge to cultivate a total petroleum hydrocarbon-degrading bacterial consortium. Therefore, waste activated sludge should be mixed with bulking agents, which absorb moisture, to provide appropriate degree of sponginess and aeration (Jolanun and Towprayoon, 2010; Rhykerd *et al.*, 1999; Yamada and Kawase,

2006). Rhykerd *et al.* (1999) reported that bulk oil-contaminated soil was more efficiently decontaminated than non-bulked soil. Further, mixing waste activated sludge with a bulking agent decreases concentration of toxic metals (Lakshmi Priya *et al.*, 2011; Yamada and Kasase, 2006). Therefore, peat moss waste activated sludge used as a bulking agent in the present study due to its ability to retain water.

In light of the above, the objective of the present study was to assess the potential of bacterial consortium for degradation of total petroleum hydrocarbon in waste activated sludge; to study the influence of bulking agent and total petroleum hydrocarbon concentration on bacterial growth rate, and to assess the potential of modified waste activated sludge as soil conditioner.

Materials and Methods

Sample collection, storage and analysis : Waste activated sludge was used in the study, originated from a wastewater treatment plant located in Ulsan, South Korea, and was used as both, inocula and media to cultivate total petroleum hydrocarbon-degrading microorganisms. Waste activated sludge samples were placed in a sealed plastic container to maintain their original moisture content and were transferred to laboratory for immediate use. Commercially available peat moss was used. Waste activated sludge tested in the present study showed following characteristics: pH 6.3; 2.3 mS conductivity; 75% moisture; 23.4% organic carbon; 3.5% total nitrogen; 6.7 C/N ratio; 2.3% total P; 254 g kg⁻¹ total solids; and 84 g kg⁻¹ volatile solids. Total concentration of heterotrophic bacteria (total-HB) was 1.6x10⁸ CFU g⁻¹.

Experimental setup and preparation of biosolids : The experiment was conducted in aerobic fixed-bed reactors. The reactors consisted of an air pump (6 l h⁻¹), a CO₂ removal bottle (0.5 l of 50% NaOH), a humidifier (0.5 l distilled water), an air flow meter, a cultivation reactor (5 l capacity) and a trap (0.5 l of 5% NaOH) to collect the CO₂ produced during biodegradation (Fig. 1). CO₂ in the incoming air was removed by CO₂-removal bottle containing 50% NaOH. Loss of water from biosolids was prevented by passing air supplied from the air pump through distilled water. After CO₂ was removed, the humidified air was transferred to a 5 l fermentation vessel (an air-tight acrylic glass vessel) through a perforated plate placed at the bottom of fermentation vessel to ensure that the humidified air was supplied uniformly. Newly generated CO₂ was continuously trapped in 5% NaOH. The experiment included of seven reactors (R1 to R7) containing the following media: 1 kg waste activated sludge alone (R1), 1 kg waste activated sludge+50 g peat moss (R2), 1 kg waste activated sludge+100 g peat moss (R3), 1 kg waste activated sludge+100 g peat moss+5 g total petroleum hydrocarbon (R4), 1 kg waste activated sludge+100 g peat moss+10 g total petroleum hydrocarbon (R5), 500 g peat moss+5

g total petroleum hydrocarbon (R6) and 1 kg autoclaved waste activated sludge+5 g total petroleum hydrocarbon (R7). The waste activated sludge was autoclaved at 121°C for 15 min and used as autoclaved waste activated sludge in reactor R7. The experiment was maintained at 23±1 °C, and all the vessels were completely closed to avoid CO₂ leakage.

Sample analysis : Temperature change of the cultivating material was monitored daily using thermometers fixed inside each reactor. While the reactors were operating, cultivation material was collected every other day to measure pH of the waste activated sludge (1:2.5 waste activated sludge water suspensions). CO₂ trap alkaline solution was detached from the reactor for 10 min to avoid allowing a significant amount of environmental CO₂ to enter the reactor during sampling. Microbial growth was monitored in the reactors through changes in pH of the alkaline solution that trapped microbe-produced CO₂. Once every two days, the CO₂ trapped in the alkaline solution was determined based on the change in pH of the solution without detaching the solution from the reactor. CO₂ content was determined as per the formula of moore (2005).

At the end of growth period, samples were collected from the reactors and analyzed for the number of total-HB, total petroleum hydrocarbon-degrading bacteria and total total petroleum hydrocarbon concentration. To analyze total petroleum hydrocarbon, 1 g of the dried sample was mixed with 20 ml extraction solution (S316, HORIBA) in 50 ml conical tube. Then, samples were extracted by vibration (Vortex-2 Genie) and centrifuged (HA-1000) for 15 and 20 min, respectively. Finally, the extracted samples were analyzed using oil analyzer (OCMA-300 HORIBA).

Total-HB were counted according to the pour plate method, and the results were expressed in colony forming units per gram of wet waste activated sludge. A phosphate-buffered, pH-neutral mineral salt medium containing purified agar, yeast extract (10 mg l⁻¹) and total petroleum hydrocarbon as the carbon source was used for culturing total petroleum hydrocarbon-degrading bacteria in waste activated sludge collected from the reactors after 30 days of incubation. The composition of mineral salt medium was as follows: 3.5 mg l⁻¹ Na₂HPO₄·2H₂O; 2 mg l⁻¹ KH₂PO₄; 1 mg l⁻¹ (NH₄)₂SO₄; 0.05 mg l⁻¹ Ca(NO₃)₂·4H₂O; 0.01 mg l⁻¹ ammonium iron(III) citrate and 0.02 mg l⁻¹ MgSO₄·7H₂O. Supernatant of waste activated sludge (1 g of sludge mixed with 100 ml sterile distilled water) was used to prepare ten-fold serial dilution series in sterile distilled water up to 10⁻⁸ dilution. Each dilution waste activated sludge shaken by hand, and 1 ml portion was dispensed into sterile, empty petri plate. The mineral salt medium was poured onto the sludge solution, and the plate was swirled to distribute the inocula and agar. A small piece of filter paper containing 20 µl total petroleum hydrocarbon was placed on the lid of Petri dish. Control plates were maintained without any carbon source. Total petroleum hydrocarbon-degrading bacteria

were counted on all the plates after 6 days of incubation at 37 °C.

For compost study, the samples were removed after 30 days from reactors R1, R2 and R3 and then air-dried and analyzed for pH (1:2.5 soil water suspension), electrical conductivity (EC) (1:2.5 soil water suspension filtrate), organic carbon (OC) (Jackson, 1973), total nitrogen (N), moisture and total and volatile solids (APHA, 2012). *Escherichia coli* was enumerated on commercially available Petrifilm *E. coli* count plates (3M Company, St. Paul, MN, USA). Significant differences of the results were statistically analysed by one-way ANOVA followed by Duncan's Multiple Range Test.

Results and Discussion

The cumulative amount of CO₂ evolved in different reactors is shown in Fig. 2. For total petroleum hydrocarbon concentration effects, CO₂ production rate was compared between the reactors R3, R4 and R5. CO₂ production was slightly lower up to 15 days in 'total petroleum hydrocarbon added' reactors (R4 and R5) when compared to 'without total petroleum hydrocarbon added' reactor (R3), and thereafter CO₂ production was stable and almost equal in all the reactors (Fig. 2). The effect of bulking agent and total petroleum hydrocarbon addition on CO₂ production rate was compared between the results of R1, R3 and R4. CO₂ production rate was positively influenced by addition of peat moss (R3) and peat moss with total petroleum hydrocarbon (R4) reactors when compared to R1 (waste activated sludge alone). The influence of waste activated sludge on CO₂ production rate was compared between the results of reactors with waste activated sludge added (R4) and without waste activated sludge added (R6). CO₂ production was higher in R4 (1 kg waste activated sludge+100 g peat moss+5 g total petroleum hydrocarbon) when compared with R6 (500 g peat moss+5 g total petroleum hydrocarbon). CO₂ production was relatively lower in the autoclaved waste activated sludge (R7) than that of other reactors with waste activated sludge added. The influence of bulking agent on CO₂ production rate was compared between the reactors R1, R2 and R3. CO₂ production was significantly influenced by addition of peat moss, as shown by comparison of the results of R1, R2 and R3 reactors; the differences between the reactors follow the order R3 (100 g peat moss) >R2 (50 g peat moss) >R1 (absence of peat moss). The results of the present study showed that addition of two different concentrations of total petroleum hydrocarbon (5 g and 10 g) to waste activated sludge did not affect CO₂ production. However, addition of peat moss and total petroleum hydrocarbon greatly influenced CO₂ production rate. There was no CO₂ production in the reactor without waste activated sludge addition (R6), and low CO₂ evolution rate in the reactor with added autoclaved waste activated sludge (R7) is supporting evidence to the above findings. Whether total petroleum hydrocarbon was added or not, CO₂ evolution at early stages of the reactors (up to 16 days) was dramatically increased, perhaps due to degradation of total petroleum hydrocarbon in

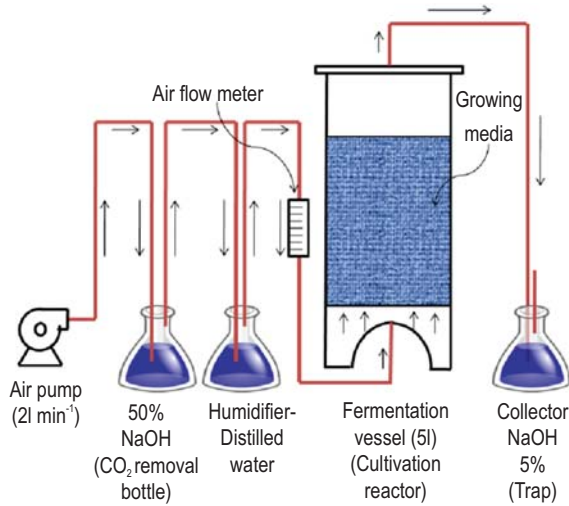


Fig. 1 : Schematic representation of the experiment conducted in aerobic fixed-bed reactor

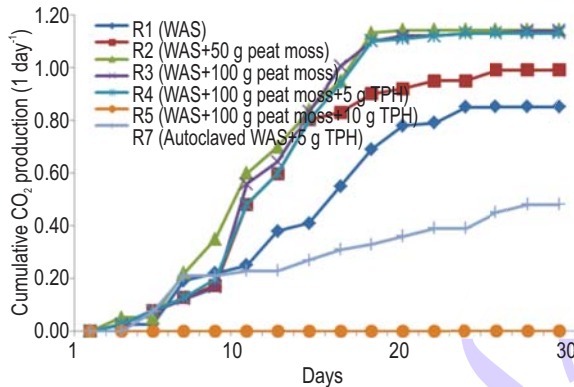


Fig. 2 : Microbial production of CO₂ in different reactors R1 to R7

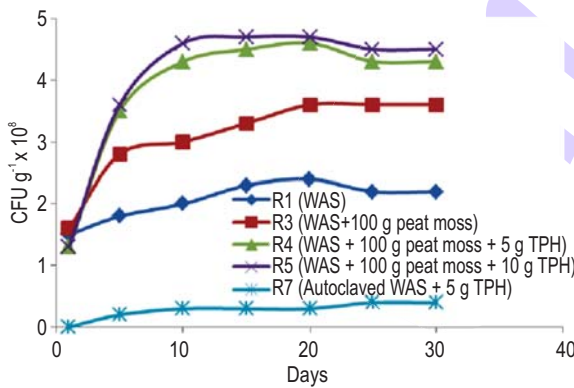


Fig. 3 : Density of total heterotrophic bacteria population in different reactors R1, R3, R4, R5 and R7

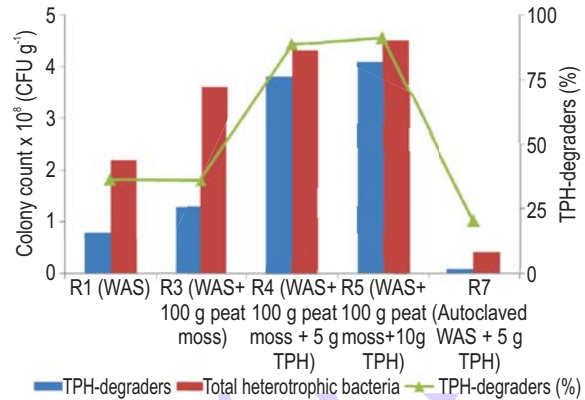


Fig. 4 : Total petroleum hydrocarbon degraders in different reactors R1, R3, R4, R5 and R7 after 30 days

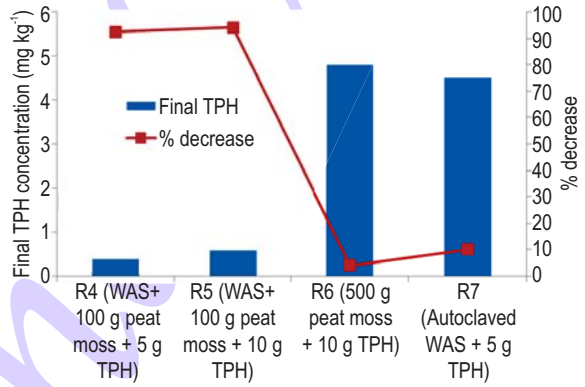


Fig. 5 : Total petroleum hydrocarbon removal efficiency for different reactors R4, R5, R6 and R7

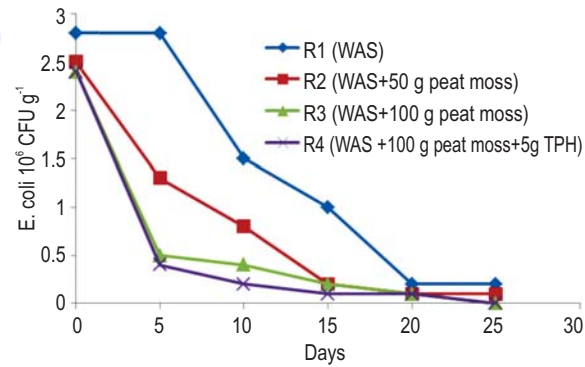


Fig. 6 : E. coli density in different reactors R1, R2, R3 and R4

total petroleum hydrocarbon-added reactors (R4 and R5) and availability of readily degradable organic matter in waste activated sludge without total petroleum hydrocarbon-added reactors in the same period. CO₂ production rate is an indicator of microbial activity (Kao *et al.*, 2001), because CO₂ is a by-product of degradation of organic compounds. Namkoong *et al.* (2002) reported that addition of organic amendments with oil-contaminated soil produced more CO₂ than without addition of organic amendments. Furthermore, addition of peat moss (R2 and R3), as a bulking agent, resulted in higher CO₂ production rates and enhanced microbial growth (present study; Fig. 2), which has also been reported by Rhykerd *et al.* (1999).

Prior to addition of total petroleum hydrocarbon, waste activated sludge contained 1.6×10^5 CFU total-HB g⁻¹. Total-HB population showed an initial increase (10 days) and thereafter remained stable (next 20 days) with minor decrease in all the reactors except the reactor containing autoclaved waste activated sludge (R7) (Fig. 3). To measure the effect of total petroleum hydrocarbon concentration total-HB densities were compared between the reactors R3, R4 and R5. Total-HB densities were relatively lower in R3 (without total petroleum hydrocarbon) (3.6×10^8 CFU g⁻¹ DS) than in R4 and R5 (with total petroleum hydrocarbon) (4.3×10^8 CFU g⁻¹ DS in R4 and 4.5×10^8 CFU g⁻¹ DS in R5) at the end of the experiment.

The effect of bulking agent and total petroleum hydrocarbon addition on total-HB population was compared between the results of R1, R3 and R4 reactors. Total-HB population was comparatively higher in waste activated sludge containing peat moss and 10 g of total petroleum hydrocarbon (4.3×10^8 CFU g⁻¹ DS, R4) at the end of the experiment. The order of total-HB population in the remaining reactors was R3 (3.6×10^8 CFU g⁻¹ DS) > R1 (2.2×10^8 CFU g⁻¹ DS) on the 30th day of the experiment. Total-HB density was relatively lower in the autoclaved waste activated sludge (R7) than in other reactors. Increase in microbial population in initial days was probably due to availability of nutrients in fresh media, and the subsequent decline was probably due to reduction in available nutrients, as they were consumed by bacteria (Sarkar *et al.*, 2005). In addition, the observed increase in bacterial population that resulted from addition total petroleum hydrocarbon (R3 and R4) is in line with the findings published by Khan *et al.*, (2006). Furthermore, waste activated sludge containing peat moss and total petroleum hydrocarbon (R3 and R4) produced higher bacterial population than waste activated sludge alone (R1). It was evident from Fig. 3 that increased microbial activity in the presence of bulking agents (Rhykerd *et al.*, 1999; Chaw and Stoklas, 2002) and total petroleum hydrocarbon (Sarkar *et al.*, 2005; Chorom *et al.*, 2010) has been previously reported. A possible explanation for this result is that microorganisms use carbon as an energy source for reproduction (Ogboghodo *et al.*, 2004).

Total petroleum hydrocarbon-degrading bacterial growth was relatively lower than that of total-HB; however, the population

of total petroleum hydrocarbon-degraders were higher in total petroleum hydrocarbon-added waste activated sludge reactors (R4 and R5) than in waste activated sludge alone reactors (R3) (Fig. 4). After 30 days of incubation, the concentration of oil-degrading bacteria was 4.3 and 4.5×10^8 CFU g⁻¹ for 5 and 10 g of total petroleum hydrocarbon, respectively, in the mixture of waste activated sludge (1 kg) and peat moss (0.1 kg), which accounts for approximately 88.4% and 91.1%, respectively, of the total-HB. Only 36% of the total-HB was converted to total petroleum hydrocarbon degrading bacteria in the reactors containing waste activated sludge without addition of total petroleum hydrocarbon (R1 and R3). Reactor four (R4) contained maximum total petroleum hydrocarbon-degrading bacteria, followed by R3 and R1 (4.3×10^8 , 3.6×10^8 and 2.2×10^8 CFU g⁻¹), respectively. The results showed that only 36% of the THB in R1 and 88% of the total-HB in R3 and R4 were total petroleum hydrocarbon degraders. The effect of total petroleum hydrocarbon concentration (5 and 10 g) on total petroleum hydrocarbon degradation by total-HB were tested and are presented in Fig. 5. After 30 days, both, R4 and R5 reactors achieved over 90% total petroleum hydrocarbon degradation, significantly higher than that in R6 and R7 (<10% total petroleum hydrocarbon degradation). R5 reactor degraded 94.1% total petroleum hydrocarbon after 30 days, slightly higher than that in R4 (92.4%).

Generally, the percentage of THB converted to total petroleum hydrocarbon-degrading bacteria reflects the extent of microbial acclimation and hydrocarbon degradation activities in an oil-contaminated environment (Sarkar *et al.*, 2005). A decreased total petroleum hydrocarbon concentration due to increased total petroleum hydrocarbon degraders, in oil-contaminated soils treated with waste activated sludge, was reported by Sarkar *et al.* (2005). In the present study, addition of total petroleum hydrocarbon (5 g in R3 and 10 g in R4) resulted in an increase in the number of total petroleum hydrocarbon-degrading bacteria. Over 90% of total petroleum hydrocarbon degradation at the end of the study in the reactors with waste activated sludge, peat mass and total petroleum hydrocarbon added, indicates that total petroleum hydrocarbon-degraders in these reactors had the ability to utilize and degrade the total petroleum hydrocarbon efficiently. Various studies have proved that endogenous bacteria in oil-contaminated soil can reduce total petroleum hydrocarbon concentration (Chiu *et al.*, 2009; Chorom *et al.*, 2010; Ekpo and Udofia, 2009). Similarly, inoculated bacterial culture isolated from petroleum refinery sludge (exogenous) can also reduce total petroleum hydrocarbon concentration in oil contaminated soil (Joseph and Joseph, 2009; Lin *et al.*, 2011; Mukherjee and Bordoloi, 2011). Total petroleum hydrocarbon-degraders greater than 80% in total petroleum hydrocarbon-added waste activated sludge reactors (R4 and R5) (Fig. 2C and 3C) might be due to the ease with which the mixed bacterial population with broad enzymatic capacities in waste activated sludge metabolizes added total petroleum hydrocarbon in R4 and R5, Riffaldi *et al.* (2006).

Table 1 : Physico-chemical analysis of waste activated sludge (WAS) before and after aerobic composting

Parameter	Waste activated sludge (WAS)	Peat moss	After 30 days incubation		
			Reactor 1	Reactor 2	Reactor 3
pH	6.30	4.30	7.10	7.15	6.88
Conductivity (mS)	2.30	2.00	2.30	2.60	2.60
Moisture (%)	75.00	0.15	75.00	62.00	61.00
Organic carbon (%)	23.4	64.00	31.20	44.00	44.00
Total nitrogen (%)	3.5	1.26	1.30	2.90	2.90
C/N ratio	6.7	50.60	24.0	15.00	15.00
Total solid (g kg ⁻¹)	254	746	254	323	300
Volatile solids (g kg ⁻¹)	84	651	84	155	117
<i>E. coli</i> (10 ⁶ CFU g ⁻¹ DS)	2.8	NA	0.2	0.1	0.1

NA- Not analyzed

The initial temperature of cultivation reactors was 21±0.2 °C, and all the reactors (R1 to R7) reached a maximum temperature of 24 °C after 10 days. Thereafter, the temperature remained constant. Initially, waste activated sludge-containing reactors (R1, R2, R3, R4, R5 and R7) had a pH range of 5.9 to 6.6. This value gradually increased and reached maximum of 7.4 after 25 days. There was no pH change in R6 throughout the study. Table 1 shows the physico-chemical characteristics of reactors R1, R2 and R3. The pH of the reactors waste activated sludge initially acidic (6.3), and with the progress of decomposition, they gradually became neutral (6.9 to 7.2). The electrical conductivity value of peat moss added reactors (R2 and R3) was high (2.6 mS) during last day of the experiment as compared with initial value. The moisture content differed significantly between the reactors (75 to 61%). After 25 days of incubation, compared with initial day, the organic carbon content increased by 133% in R1 and 188% in R2 and R3. However, the total N content was reduced to 37% in R1 and 82% in R2 and R3. The C/N ratio was found between 15% (R2 and R3) and 24% (R1). The initial total P in all reactors (2.3%) was reduced to 2.1% after 25 days of incubation. There was no difference between the initial and final value of TS and VS in reactor R1; however, R2 and R3 showed significant higher TS and VS after 25 days of incubation. Initially, growth of *E. coli* between the reactors was 2.5 to 2.8 10⁶ CFU g⁻¹ (Fig. 6). The growth rate was then reduced with increasing incubation days (Fig. 6). Reduction was higher in peat moss added reactors (R2 and R3) and total petroleum hydrocarbon added reactors (R4 and R5) as compared with that in reactors without added peat moss (R1) and total petroleum hydrocarbon. The number of *E. coli* was reduced to 0.2x10⁶ CFU g⁻¹ (7.1% of the initial value) in R1 and 0.1x10⁶ CFU g⁻¹ (3.5% of the initial value) in R2 and R3.

During the cultivation of total petroleum hydrocarbon-degrading bacteria and composting of waste activated sludge, heat was generated as a by-product of microbial breakdown of organic materials in waste activated sludge. In general, a well-maintained aerobic fermentation system for organic wastes, such as a composting system, can reach temperatures up to 64°C.

However, the smaller temperature range observed in all the reactors, used in this study, might be due to water vaporization in the reactors caused by continuous aeration of the system. The pH increase in all waste activated sludge containing reactors (R1 to R7, except R6) might be due to ammonia produced by microbial degradation of proteinaceous substances contained in waste activated sludge (Elango *et al.*, 2009). For the compost study, it was assumed that all the parameters in waste activated sludge increased their original values after being mixed with peat moss (50 and 100 g) in R2 and R3 (Table 1). The content of volatile solids decreased from the expected range in the original value (R2 and R3) at the end of the experiment probably due to loss of organic matter through microbial degradation in peat moss added reactors. The measurement of compost conductivity is an indication of soluble salt content. Conductivity values between 2.3 to 2.6 mS cm⁻¹ were found in all the reactors indicating low salinity content. A reduction in C/N ratio to less than 20 in peat moss added reactors (R2 and R3) indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes (Khairakpam and Bhargava, 2009). Fresh waste activated sludge showed high density of *E. coli* (2.8 x 10⁶ CFU g⁻¹). Generally, pathogenic microorganisms are destroyed by the temperature generated during the composting process (Conter *et al.*, 2007). However, the present study showed that reactor temperature was between 21 to 24°C during the experimental period. Meanwhile, a substantial reduction of *E. coli* was observed at the end of the experiment in all the reactors (R1, R2 and R3) due to *E. coli* losing the ability to form colonies during aeration in starved condition (Velasco-Velasco *et al.*, 2004). Reduction of *E. coli* from final compost indicates good sanitization during composting. Therefore, use of final compost product in the present study with low salinity does not exhibit risk when used as soil conditioner.

The results of the present study showed that oil-degrading bacteria were abundant in waste activated sludge (greater than 80% of the THB). Furthermore, addition of a bulking agent (peat moss) enhanced the cultivation of total petroleum

hydrocarbon degraders. Total petroleum hydrocarbon degradation level of over 90% was achieved in this study. Finally, the present study demonstrated that cultivation of total petroleum hydrocarbon-degrading bacterial consortium and production of compost from waste activated sludge by aerobic treatment is feasible.

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