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Quantifying the effect of microbial consortium and alfalfa to accelerate the degradation of oily sludge

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Abstract

Microorganisms use oily sludge as a source of carbon. However their activity is retarded in oily sludge because of the presence of toxic hydrocarbons in oily sludge. Microbial activity in such soils can be enhanced once consortium is prepared from various genera of microbes capable of degrading hydrocarbons inoculated to oily sludge contaminated soils. The aim of this study was to evaluate the possibility of microbial consortium as the best remediation option of oily sludge contaminated soil. Furthermore the effectiveness of microbial consortium with alfalfa and fertilizer over a short period of time was also evaluated. Attention was paid to understand that consortium under alfalfa can increase the microbial population necessary to accelerate the remediation process. Microbial population was determined with colony forming units. Disappearance of hydrocarbons was determined with GC-FID. Remarkable differences in the percentage of degradation of n-alkanes (nC_{13} to *n*C_{29}) present in high concentration in oily sludge were observed when microbial consortium was added to such soil. Around 80% of *n*-alkanes were degraded in such soil. Whereas only 3% of *n*-alkanes were degraded in oily sludge contaminated soils. The rapid degradation of *n*-alkanes is more likely because alfalfa respond positively with consortium in the presence of fertilizer. Alfalfa increases the bacterial number to 100 folds and is directly linked to the increase in the degradation of hydrocarbons in oily sludge. This revealed that microbial consortium can enhance the degradation of hydrocarbons which is further increased with alfalfa and fertilizer in oily sludge contaminated soils.

Keywords: Oily sludge; Consortium; Microbial; Alfalfa; Hydrocarbons; Remediation.

1. Introduction

Until recently scant attention has been paid to the remediation of oily sludge contaminated soils by oil refineries worldwide more likely because of the presence of environmentally hazardous compounds in oily sludge injurious to flora and fuana and to some extent to the human health once exposed to such contamination. Use of microbial consortium to remediate oily sludge contaminated soil has been successfully adopted by oil refineries over the last decade. Nonetheless there are contradictory evidences in the previous literature regarding the use of microbial consortium alone or with fertilizer to remediate heavily oily sludge contaminated soils because of recalcitrant nature of some organic compounds present in oily sludge (Liu et al., 2010; Murray et al., 2013; Battikhi, 2014; He et al., 2014; Prakash et al., 2015). Therefore for complete remediation of oily sludge contaminated soils, green technology in association with microbial consortium has recently received attention (Muratova et al., 2008; Jublee and Suparna, 2014; Yong-Ming et al., 2014). Green technology means use of plants with consortium to remediate oily sludge contaminated soils. Plants with nitrogen fixing bacteria can provide enhance microbial activity and enough nutrients and oxygen for microbes to degrade hydrocarbons once grown over oily sludge (Abid et al., 2014; Adam et al., 2015; Liqiang et al., 2015). Alfalfa is considered to be suitable to remediate oily sludge more likely because of leguminous nature and increased microbial activity under the rhizosphere because of presence of nodules on roots. Kirk et al. (2005) reported that alfalfa cannot accelerate the rate of degradation of hydrocarbons once grown over oily sludge.

Kohat and Potwar Plateau are explored for oil over the last several years. During exploration several tons of oily sludge has been produced which is dumped in an open pit and causes severe environmental threat to the terrestrial and aquatic life. Therefore oil companies are looking for option to remediate such soils completely. The objective of this study was to introduce a relationship between microbial consortium and alfalfa as an

economically feasible and environmentally friendly technique to oil companies to remediate oily sludge contaminated soils. For that purpose a combination between alfalfa and consortium was established.

2. Materials and methods

2.1. Sample collection

Kal oil field in Potwar Plateau was selected for oily sludge. Around 5 kg of oily sludge was placed in zibber bag and was stored in the laboratory.

2.2. Colony forming units (CFUs)

Colony forming units (CFUs) per g of dry soil were determined after appearance of bacterial colonies on the nutrient broth agar media on 7, 14, 21, 28, and 60 days of incubation (Song and Bartha, 1990).

2.3. Microbial consortium preparation

For microbial consortium preparation, the previously isolated strains Bacillus cereus (Acc KF859972), Bacillus altitudinis(Acc KF859970), commomonas (Acc KF859971) belonging to family Comamonadaceae and Stenotrophomonas maltophilia (Acc KF859973) were used as consortium.

2.4. Mixing of soil with oily sludge

Control soil samples were collected within the vicinity of an oil field. Nearly 1.5 kg of triplicate soil sample was spread over the plastic sheet and was mixed with 450 g of oily sludge (sludge was added at the rate of 30 mg per g to soil). Nitrogen was added in the form of NH_4NO_3 whereas P was added as $(NH_4)_3PO_4$. The ratio of C:N:P was kept as 30:10:1.

Consortium was added to the oily sludge to provide an initial bacterial population of 106 bacteria cells per mL (Mishra et al., 2001).

2.5. Germination of alfalfa (Medicago sativa L)

Alfalfa (Medicago sativa L) seeds were procured from National Agriculture Research Centre-Islamabad-Pakistan. All seeds were washed with Clorox (10 %) and Ethanol (90%). For alfalfa pot experiment was conducted. Around 10 g of oily sludge alone (T1), oily sludge with consortium (T2), oily sludge with fertilizer (T3), oily sludge with alfalfa (T4), oily sludge with consortium+fertilizer (T5), oily sludge with consortium+nutrient+alfalfa (T6) was placed in the pot and was removed at 7, 14, 21, 28 and 60 days of incubation. The bacterial population and breakdown of hydrocarbons was determined with GC-FID.

2.6. Gas chromatography with flame ionization detector (GC-FID)

GC-FID-QC 2010 (Shimadzu) was used to carry out the analysis. Liquid samples were passed through capillary column of 30m×0.25mm ID coated with 5% phenyl and 95% methyl polysiloxane stationary phase (DB-5 MS, J & W scientific). Around 1µL of the liquid sample was injected to the injector via syringe with split/splitless. The temperature program of GCFID was 320°C for injector and detector whereas column oven temperature program was started from 75°C to 320°C final temperature with increase in temperature at 10°C/min with initial and final hold time of 30 minutes.

2.7. Reagents and standards

Dichloromethane was used as a reagent to extract hydrocarbons from crude oil. Phenantherene was used as external standard purchased from Fluka (Germany). For solid/liquid calibration standard standards a stock solution was prepared (Phenantherene was diluted with dichloromethane). Five dilutions such as 0.1, 0.2, 0.5, 1.0 and 1.5mg mL-1 were prepared and was placed in 2 mL vial and was analyzed with GCFID. The peak area of standard and each hydrocarbon as appeared on GC chromatogram was obtained by clicking on the program option of GCFID software. However the concentration of unknown hydrocarbons (mg per g) as appeared on GC chromatogram was determined from the response factor multiply by the dilution factor: The response factor of each hydrocarbon was calculated by dividing the peak area of external

standard with peak area of each unknown hydrocarbon.

The calibration curve was drawn from the peak area of each dilution of external standard obtained once run through GCFID. Nearly 5 calibration levels, i.e. 0.1, 0.2, 0.5, 1.0 and 1.5mg mL-1 was used to plot the calibration curve. Calibration curve was plotted against concentration (x-axis) with response area or peak area (y-axis). The calibration curve was best fitted to a linear curve using Microsoft Excel. The correlation coefficient (R) was 0.987.

3. Results

3.1. Soil physicochemical characteristics

Soil physicochemical characteristics are present in Table 1. The pH of soil was 7.5 and texture silty clay whereas oily sludge had pH 6.0 and texture clay loam in nature. The organic matter was 0.5% in soil it was around 20% in oily sludge. The greater content of increase in organic matter is more likely because of the difference in the carbon content between the soil and oily sludge.

3.2. Total hydrocarbons degradation

Table 2 shows the rate of degradation of total hydrocarbon during various incubation periods. Total Ion Chromatogram of GC-FID of oily sludge alone shows that n-alkanes from *n*C_{13} to *n*C_{34} were present in abundance. Traces of methyl branched hydrocarbons (Farnesane and Nor pristane) and isoprenoids such as pristane and phytane were present in oily sludge alone. The initial content of total hydrocarbons in oily sludge (T1) was 30.16 mg per g and was reduced to 17.32 mg per g at day 21 after when the extraction was made. Nearly 8.84 mg per g was remaining at 60 days of incubation. However, when microbial consortium was added to oily sludge (T2), total hydrocarbons were reduced to 12.63 mg per g at day 7. Nearly 50% of total hydrocarbons were degraded during short (7 days) period of time. Thereafter the rate of degradation of hydrocarbon was slow till 60 days. Nonetheless it was observed that when oily sludge was inoculated with microbial consortium the rate of degradation of total hydrocarbons was increased. However further increase in the degradation of hydrocarbons from oily sludge with consortium was reported with alfalfa over a short period of time. Alfalfa reduced the total hydrocarbon from 30.16 to 11.47 mg per g at day 7 of incubation. Thereafter further increase in the degradation of total hydrocarbons was not observed till the end of the incubation. Fertilizer addition to oily sludge with consortium had no effect on the rate of degradation of hydrocarbons over a short period of time. Virtually the same trend was observed with alfalfa and consortium. However the rate of degradation was increased at day 21 of incubation. Nearly 50% of oily sludge was degraded at day 21 when consortium with fertilizer was added to such soil. Alfalfa increased the degradation of hydrocarbons in oily sludge with microbial consortium and fertilizer at day 3.

3.3. Breakdown of n-alkanes

Table 3 shows the disappearance of n-alkanes from oily sludge with and without consortium, fertilizer and alfalfa duirng various incubation periods. The total ion chromatogram (TIC) extracted from GCFID of oily sludge from T1 to T6 composed of n-alkanes ranged from *n*C_{10} to *n*C_{34} with traces of isoprenoids such as pristane and phytane. Long carbon chain hydrocarbons (*n*C_{30}-*n*C_{34}) were found to be at low concentration whereas medium chain hydrocarbons (*n*C_{19}-*n*C_{29}) followed by short carbon chain hydrocarbons (*n*C_{13}-*n*C_{19}) were present at high concentration. Nearly 50% of *n*C_{19}-*n*C_{29} were found where 25% was *n*C_{13}-*n*C_{16} and 15% was *n*C_{17}-*n*C_{18}. Pristane and phytane were present at low concentration initially. Very little degradation of n-alkanes occurred in oily sludge alone (T1). More or less same concentration of short, medium and long carbon chain hydrocarbons were degraded from oily sludge only (T1) at 28 days. Thereafter *n*C_{10}-*n*C_{12} and *n*C_{30}-*n*C_{34} were disappeared completely at 60 days of incubation. Nearly 50% of *n*C_{19}-*n*C_{29}, *n*C_{13}-*n*C_{19}, were also degraded at 60 days.

When microbial consortium was added to

oily sludge (T2), nC_{10}-nC_{12} were negligible at short period of time. Only traces of nC_{30}-nC_{34} were present at day 3. Nearly 50% of nC_{13}-nC_{19}, nC_{19}-nC_{29} were degraded at day 3 thereafter further degradation was decreased and only traces were present at day 60. When fertilizer was mixed with oily sludge in which consortium (T3) was also present, degradation of all carbon chain hydrocarbons remained same. Nearly same amount of short, medium and long carbon chain hydrocarbons were degraded as was without consortium (T2). Alfalfa alone (T4) did not increase the degradation of any carbon chain hydrocarbons at day 3. More or less same amount of hydrocarbons were degraded at day 3 from T4. Consortium addition to oily sludge over which alfalfa was grown (T5) shows the disappearance of nC_{10}-nC_{12} at day 3. Around 50% of medium and long carbon chain hydrocarbons were disappeared at day 3. Thereafter the degradation process was slow and only minute amount of medium and long carbon chain hydrocarbons were present at 60 days. Fertilizer addition did not increase the process of degradation of any of the carbon chain and more or less same amount of short, medium and long carbon chain hydrocarbons were degraded with and without fertilizer (T6 and T5). Virtually the same trend was observed when alfalfa was grown over oily sludge with consortium and fertilizer. The rate of degradation of any of the carbon chain hydrocarbons were same at day 3. However it was observed that nC_{10}-nC_{12} and nC_{30}-nC_{34} were not present at day 3. Only 50% of medium and long carbon chain hydrocarbons were found at day 3. Thereafter the rate of degradation was slow and only traces were present at day 60 of incubation period.

3.4. Bacterial population

Table 4 represents the bacterial population in oily sludge treated soils (T1 to T6) during various incubation periods. Oily sludge alone had bacterial population of 2.1×104 initially. Bacterial population did not increase till 60 days. Inoculation of microbial consortium to oily sludge increased the bacterial population from 2.1×104 to 3.1×106 at day 3. Fertilizer (T3) increased the bacterial population from 2.1×104 to 4.1×105 at day 3 which was 10 fold greater than bacterial population present naturally in oily sludge (T1). Bacterial population remained same in T4 and T2 whereas 10 to 100 fold increase in bacterial population was observed in T4 than T5 and T1. Thereafter bacterial population remained same till 60 days. Alfalfa increase the bacterial population to 10 fold between days 3 to 14 after when the extraction was made. Bacterial population remained same with alfalfa till 60 days. The same trend was observed when fertilizer was added to oily sludge with consortium and alfalfa (T6). However fertilizer addition alone increase the bacterial population to 10 fold at day 7 and remained same till 60 days.

Table 1. The physicochemical characteristics of soil and sludge.

Sample type	**pH**	**OM(%)**	**Texture**
Soil	7.5	0.5	Silty clay
Sludge	6.0	20	Silty clay

Table 2. Total degradation of hydrocarbons from oily sludge contaminated soil.

Treatments	Days of incubation after when the extraction was recorded				
	7	14	21	28	60
Oily sludge (T1)	30.16	26.16	17.32	9.96	8.84
Oily sludge with consortium (T2)	12.63	12.03	11.72	10.05	7.80
Oily sludge with fertilizer (T3)	30.52	27.83	20.29	19.37	10.90
Oily sludge+consortium+fertilizer (T4)	21.36	12.83	12.33	10.09	9.83
Oily sludge+consortium+alfalfa (T5)	11.47	9.066	8.10	5.40	4.40
Oily sludge+consortium+fertilizer+alfalfa (T6)	22.44	13.92	10.97	8.96	5.51

Table 3. The rate of degradation of n-alkanes (nC_{10} to nC_{34}) and isoprenoids from oily sludge and treated soils over an incubation period of 3 to 60 days.

TREATMENT	n-alkanes	7	14	21	28	60
Oily sludge (T1)	nC_{13}-nC_{16}	6.67	5.95	4.08	2.48	1.56
	nC_{17}-nC_{18}	4.7	3.36	2.4	1.38	1.27
	nC_{19}-nC_{29}	12.65	10.26	7.46	4.13	3.7
	nC_{30}-nC_{34}	2.7	2.12	1.11	0.58	1.04
	Pristane	1.54	1.06	1.28	0.75	0.77
	Phytane	1.54	1.05	0.76	0.40	0.43
		30.16	26.16	17.32	9.96	8.84
Oily sludge with consortium (T2)	nC_{13}-nC_{16}	2.91	2.60	2.66	2.46	1.19
	nC_{17}-nC_{18}	1.67	2.13	1.60	1.39	1.10
	nC_{19}-nC_{29}	4.97	5.0	5.10	4.32	3.85
	nC_{30}-nC_{34}	0.99	0.90	0.8	0.48	0.5
	Pristane	0.86	0.9	0.8	0.79	0.68
	Phytane	0.52	0.5	0.5	0.41	0.37
		12.63	12.03	11.72	10.05	7.80
Oily sludge with fertilizer (T3)	nC_{13}-nC_{16}	6.52	5.2	4.62	4.46	1.80
	nC_{17}-nC_{18}	4.17	3.2	2.70	2.61	1.17
	nC_{19}-nC_{29}	12.12	12.01	8.07	7.72	4.33
	nC_{30}-nC_{34}	1.95	1.75	1.07	1.10	1.63
	Pristane	2.8	2.9	2.2	2.05	1.03
	Phytane	1.65	1.62	1.2	1.1	0.69
		30.52	27.83	20.29	19.37	10.90
Oily sludge+consortium+ Fertilizer (T4)	nC_{13}-nC_{16}	4.71	2.69	2.35	2.67	2.53
	nC_{17}-nC_{18}	3.22	1.68	1.60	1.74	1.16
	nC_{19}-nC_{29}	9.86	5.68	6.23	4.65	3.11
	nC_{30}-nC_{34}	1.57	0.94	0.90	0.85	0.70
	Pristane	1.06	1.01	1.02	1.02	0.64
	Phytane	0.65	0.61	0.60	0.60	0.36
		21.36	12.83	12.33	10.09	9.83
Oily sludge+consortium+fertilizer +alfalfa (T5)	nC_{13}-nC_{16}	5.01	2.6	1.80	1.80	0.88
	nC_{17}-nC_{18}	2.74	1.68	1.17	0.59	0.71
	nC_{19}-nC_{29}	9.64	5.75	4.33	4.03	2.21
	nC_{30}-nC_{34}	1.82	1.08	1.63	0.87	0.51
	Pristane	1.83	1.35	1.03	0.90	0.61
	Phytane	1.11	0.83	0.69	0.54	0.37
		22.44	13.92	10.97	8.96	5.51
Oily sludge+consortium+alfalfa (T6)	nC_{13}-nC_{16}	1.99	1.33	1.32	0.85	0.80
	nC_{17}-nC_{18}	1.69	0.61	0.60	0.45	0.40
	nC_{19}-nC_{29}	5.67	3.74	3.71	1.64	1.60
	nC_{30}-nC_{34}	0.7	1.71	0.79	1.75	1.60
	Pristane	0.76	0.469	0.47	0.20	0.1
	Phytane	0.48	0.31	0.29	0.34	0.2
		11.47	9.066	8.10	5.40	4.40

Table 4. Bacterial population in oily sludge treated soils (T1 to T6) at various incubation period.

Treatment	Days of incubation after when the extraction was made					
	3	7	14	21	28	60
Oily sludge (T1)	2.1×10^4	5.1×10^4	6.1×10^4	6.1×10^4	6.0×10^4	6.0×10^4
Oily sludge with consortium (T2)	3.1×10^6	5.9×10^7	6.2×10^7	6.0×10^7	5.9×10^7	6.2×10^7
Oily sludge with fertilizer (T3)	4.1×10^5	6.1×10^6	6.3×10^6	6.5×10^6	6.7×10^6	7.1×10^6
Oily sludge with alfalfa (T4)	3.7×10^6	6.3×10^7	6.5×10^7	6.9×10^7	7.1×10^7	7.2×10^7
Oil sludge+consortium+alfalfa (T5)	5.1×10^7	6.9×10^8	7.5×10^9	7.8×10^9	7.0×10^9	7.1×10^9
Oily sludge+consortium+fertilizer+alfalfa (T6)	6.7×10^7	6.5×10^8	6.610^9	6.7×10^9	6.5×10^9	6.6×10^9

4. Discussion

The present study was aimed to develop a rapid and environmentally friendly method to remediate oily sludge contaminated soils in such a manner that can be applicable commercially. The results of the study revealed that microbial consortium inoculated to oily sludge (T2) increased the disappearance of total hydrocarbons than oily sludge alone treatment (T1) at day 3. This is in accordance with the findings of Roy et al. (2014) who found that consortium increased the degradation of total hydrocarbons than without consortium. This suggests that microbial consortium can degrade hydrocarbons rapidly. However in this study further increase in the degradation of total hydrocarbons was noted when alfalfa was grown over oily sludge with consortium (T5). It was observed that nearly 80% of total hydrocarbons were disappeared at day 3 from T5. This contradict with the findings of Muratova et al. (2008). They reported that rapid degradation of oily sludge was observed with rye grass whereas alfalfa slows the process of degradation. Nearly 50% of total hydrocarbons were degraded with ryegrass whereas only 10% of hydrocarbons were degraded with alfalfa. The response of alfalfa differs from Muratova et al. (2008) and of this study is more likely because Muratova et al. (2008) did not study the effect of consortium and alfalfa on the degradation of oily sludge. This suggests that alfalfa further improves the rate of degradation of total hydrocarbons with microbial consortium at day 3. Fertilizer addition did not increase the degradation of total hydrocarbons in oily sludge with or without alfalfa. This suggests that there was enough nutrients were present for microbes to degrade total hydrocarbons in oily sludge.

The degradation of total hydrocarbons is directly related to the increase in bacterial population in oily sludge. It was noted in this study that bacterial population was 104 initially in oily sludge which was not enough for complete degradation of hydrocarbons. Inoculation of microbial consortium to oily sludge increased the bacterial population to 100 fold thus accelerate the rate of degradation of total hydrocarbons at day 3. The result of this study is in agreement with the findings of Roshanak et al. (2014) and Thavamani et al. (2012). They found that consortium addition increased the bacterial population to 10 fold in oily sludge. Alfalfa further increase the bacterial population to 100 fold at day 3. This increase continued till 60 days. The increase in bacterial population increases the degradation of hydrocarbons in oily sludge.

The alfalfa growth was directly related to the disappearance of toxic hydrocarbons such as nC13-nC16. Once such hydrocarbons were disappeared the rate of degradation was increased and oily sludge started to rehabilitate.

5. Conclusion

Microbial consortium emerge as a low cost and economical technique to rehabilitate oily sludge contaminated soils. Furthermore the efficiency of microbial consortium can be enhanced with alfalfa and gave encouraging results even at day 3. This suggests that microbial consortium with alfalfa can successfully be used in future to remediate oily sludge. It was also observed that 80% of oily sludge was disappeared with consortium and alfalfa at day 3. However, fertilizer addition did not accelerate the degradation of total hydrocarbons and suggested that enough nutrients were available for microbes to degrade hydrocarbons from oily sludge. Consortium and alfalfa maintained the bacterial population up to 106 or more enough for degradation through the incubation period.

Therefore it is recommended to the oil refineries to adopt consortium with alfalfa to remediate moderate oily sludge contaminated soils.

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