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Short Communication

Biodegradation of oil in oily sludges from steel mills

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ABSTRACT

Lab-scale batch studies were conducted to determine the biodegradability of oil associated with oily sludge from a steel mill using two microbial cultures enriched in the laboratory. After 60 days of biodegradation the residual oil content in mill sludge was reduced from 4.5–5% to 2.7–3.0%, corresponding to 40–45% loss with respect to initial. The rate of degradation was different for the two enrichment cultures studied. Significant loss of oil was observed in the un-inoculated controls while loss in the azide killed controls was negligible. Bioavailability limitations and the presence of structurally complex high molecular weight hydrocarbons in lubricating oil are responsible for the slow rate of degradation. Significant loss of oil in un-inoculated controls indicated the presence of indigenous microorganisms in oily mill sludge. The association of biomass with sludge solids and presence of a high level of residual oil may adversely affect the recyclability of iron-fines associated with the sludge.

1. Introduction

Oily mill sludge generated from iron and steel plants comprises of dense slurry of iron fines associated with lubricating oil, water and other wastes including heavy metals. The oil in mill sludge is derived from lubricants and coolants used in the manufacturing of steel products and from the equipments used for steel making processes. Lubricating oil consists of about 5–20% (w/w) chemical additives in a base fluid which is a complex mixture of hydrocarbons including linear and branched paraffins, cyclic alkanes and aromatic hydrocarbons (Jirasripongpun, 2002).

The handling and disposal of oily sludges primarily involves reduction in the oil content as well as separation of water and other wastes from sludge. In contrast, to the physicochemical treatment methods for removal of oil, such as; incineration and solvent extraction, biodegradation offers a cost effective and environment-friendly technology (Vasudevan and Rajaram, 2001). The potential for biodegradation of oil by microbes is well established (Das and Mukherjee, 2007; Mohanty and Mukherji, 2008). It is widely recognized that effective degradation of oil containing various hydrocarbon group-types can be achieved by a microbial consortia rather than a pure culture (Rahman et al., 2002; Ward et al., 2003). Other factors, such as, temperature, aeration, pH, availability of nutrients and bioavailability of hydrocarbons may be optimized for enhancing the biodegradation rate. Bioavailability, which is affected by the type of hydrocarbons and presence of surfactants, is a critical factor affecting the rate and extent of degradation (Boopathy, 2000; Mohanty and Mukherji, 2007; Mukherji et al., 1998; Rahman et al., 2003). Free phase oil is more readily biodegraded by naturally occurring microbial cultures compared to the oil in oily sludge and oil associated with sorbents (Biswas et al., 2005). Although numerous studies have been reported biodegradation of oil associated with tank bottom sludge (Mishra et al., 2001; Mrayyan and Battikhi, 2005; Verma et al., 2006) only a few studies have reported biodegradation of lubricating oil associated with soil and sludges (Adesodun and Mbagwu, 2008; Jirasripongpun, 2002; Ward et al., 2003).

In the present study, enrichment and isolation of oil degrading microbial cultures was performed using sludge from a biological treatment plant and a mixed soil sample. Oily mill sludge was provided as a sole source of carbon and energy. The main objective was to study biodegradability of lubricating oil in oily mill sludge using the two enriched cultures and to determine the potential for recovering the iron fines that constitute the biosolids in mill sludge.

2. Methods

2.1. Source of oily mill sludge

The oily mill sludge used in this study was obtained from Bhilai Steel Plant, Steel Authority of India Limited (SAIL)-Bhilai, India. The studies were conducted with two samples of sludge, designated as

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sample 1 and sample 2, which were obtained from two different zones of a secondary settling tank. All samples were preserved at 4 °C.

2.2. Characterization of oily mill sludge

Characterization and analysis of oily mill sludge was performed with both wet as well as partially dry samples prepared by keeping the wet sludge overnight in an oven maintained at 45 °C. All the reagents used for the analysis of sludge were obtained from Merck and were of analytical grade with purity greater than 98%. Samples of oily mill sludge were characterized in terms of pH, moisture content, organic matter content and metal content. The pH was measured after mixing the sludge with water in the ratio 1:5 (Vasudevan and Rajaram, 2001). The moisture content was determined by oven drying at 103 °C. The organic matter content in the oily mill sludge was determined by loss on ignition at 600 °C before and after extraction of oil from the moisture-free sludge. The oxides present in sludge solids after heating at 100 °C and 600 °C was characterized using an X-ray diffractometer (XRD, Philips PW3040/60 X'pert PRO, The Netherlands) employing Cu/Kα radiation.

2.3. Quantification of oil in oily mill sludge

The oil in oily sludge was quantified gravimetrically after soxhlet extraction with dichloromethane as discussed by Biswas et al. (2005). The extracted oil was subjected to silica gel column chromatography as discussed by Vijay (2001). The extracted oil (0.5 g) pre-mixed with dry silica gel (3 g) was applied to a glass column (30 cm, L × 20 cm, ID) packed with silica gel (100–200 mesh size, activated at 100 °C). Each column was sequentially eluted with hexane (200 mL), toluene (200 mL) and methanol (100 mL) over 6 h, to obtain the aliphatic, aromatic and polar fractions, respectively. Column chromatography was also utilized for determining the change in composition of residual oil in the biodegradation rate studies. However, the oil applied to the column was reduced from 0.5 to 0.05 g due to the limited mass of residual oil available.

2.4. Enrichment and isolation of microorganisms from oily mill sludge

Laboratory experiments were conducted to enrich microorganisms from two source materials: sludge from a biological oxidation and dephenolization plant and a mixed soil sample obtained by mixing soil from the vicinity of the muck dump and secondary settling tank in a steel plant. Enrichment was conducted in 500 mL flasks containing 100 mL mineral medium (trace nutrients were not added) with 2 g oily mill sludge as the sole substrate (Mukherji et al., 2004). In the 4th and 5th transfer instead of sludge, oil extracted from oily mill sludge was added as substrate to observe the appearance of turbidity in the absence of sludge solids. Cultures enriched using the biological treatment plant sludge and the mixed soil samples were designated as BS and MS cultures, respectively. Isolation of pure cultures was carried out by spread plating and streak plating in nutrient broth plates. The isolates were identified based on 16S rDNA sequencing (Macrogen, Inc., Korea) followed by BLAST analysis (NCBI) and were identified based on closed match with available sequences.

2.5. Biodegradation studies with lubricating oil and oily mill sludge

Initially, the extent of biodegradation of oil extracted from steel mill sludge was tested over a period of 7 and 15 days using the BS and MS cultures. Its degradation was compared to that of lubricating oil used in 4-stroke diesel engines (Servo super multigrade, Indian Oil Corporation Ltd., Mumbai, India). The studies were setup in 500 mL flasks with 1% oil. After 7 and 15 days, liquid–liquid extraction (1:1) was performed using dichloromethane (DCM) by centrifuging the contents (Mohanty and Mukherji, 2006) and residual oil was estimated gravimetrically. Subsequently, biodegradation rate studies were conducted with mill sludge over 60 days. The BS culture was used with both the sludge samples 1 and 2 while the MS culture was used only with sludge sample 1. These studies were setup in 500 mL flasks containing 15% (w/v) oily sludge in 100 ml mineral medium provided as sole substrate. Since the oily sludge used was not presterilized, two types of controls were set-up to determine the abiotic losses, (i) un-inoculated controls and (ii) azide killed controls, containing 0.1% (w/v) sodium azide as bactericide. Experimental flasks were supplemented with 2 mL inoculum of freshly grown BS/MS culture. The flasks were incubated in a rotary shaker operated at 120 rpm and 30 °C. At each sampling time, the residual oil was extracted and quantified.

2.6. Recycling of iron fines from oily mill sludge

These studies were conducted using model mixtures of sludge and biomass. For preparing the model mixtures, both sludge and biomass were pre-dried at 100 °C. Moreover, the sludge used was soxhlet extracted with DCM for removing the oil. Subsequently, two techniques were attempted for separation of iron fines from the model mixtures, i.e., centrifugation at a low rpm, and magnetic separation. The model mixture was first suspended in water and mixed uniformly prior to centrifugation. Magnetic separation was achieved by holding a magnet above the dry model mixture. The mass of iron fines recovered was compared to that originally added to formulate the model mixture and a gain in weight was indicative of incomplete separation of biomass from the iron fines.

3. Results and discussion

The characteristics of oily mill sludge samples are provided in Table 1. The oil content determined by soxhlet extraction of dry sludge was 5.17% and 4.55% for sludge samples 1 and 2, respectively while organic matter determined by loss on ignition was in the range of 1.2–2.2%. The apparent inconsistency in these values may be explained by oxidation of iron present in the sludge solids. Heating the dried sludge after extraction of oil at 600 °C, resulted in weight gain of 3.1 ± 0.06 and 2.6 ± 0.05%, in sample 1 and 2, respectively. Elemental analysis by X-ray fluorescence spectroscopy revealed that 94–95% iron was present in the dry sludge solids (unpublished results). The XRD pattern of sludge samples revealed changes in the iron oxides present at 100 °C and 600 °C. While Fe_2O_3 (rhombic), $MgFe_2O_4$ (cubic) and $Fe_{0.95}O$ (cubic) were present at 100 °C, Fe_2O_3 (rhombic), Fe_3O_4 (cubic) and Fe_2O_3 (tetrahedral) were present at 600 °C (Biswal, 2007). Good recovery (approximately 98%) was achieved in silica gel chromatographic separation of extracted oil. The aliphatic, aromatic and polar components were estimated as 76%, 18% and 4%, respectively. The GC chromatogram of the aliphatic fraction (Biswal, 2007) primarily consisted of an unresolved complex mixture hump with very few resolved peaks and had the characteristic appearance of lubricating

Table 1
Characterization of oily mill sludges

Parameters	Sludge sample 1	Sludge sample 2
pH	6.74	6.94
Moisture (%)	32.04 ± 0.62	26.75 ± 0.12
Organic matter (LOI) (%)	2.18 ± 0.19	1.25 ± 0.07
Oil content (%)	5.17 ± 0.02	4.55 ± 0.05

oil chromatogram reported by Wang and Stout (2007) (Biswal, 2007).

Oil degrading cultures could be successfully enriched from the biological oxidation and dephenolization plant sludge and mixed soil samples and these enrichments were designated as BS and MS cultures, respectively. The pure cultures isolated from BS culture were identified as *Bacillus circulans* (99% match) and *Ochrobactrum intermedium* (99% match) while that from MS culture was identified as *Sphingomonas* sp. (98% match). The extent of degradation of oil extracted from mill sludge was similar for both the BS and MS cultures and was in the range of 25–27% and 40–45% after 7 and 15 days, respectively. In contrast, 40–50% degradation of diesel engine lubricating oil could be achieved within 7 days. The slower degradation rate of oil extracted from mill sludge may be attributed to differences in the nature of the hydrocarbon components or due to presence of biocides/biodegradation inhibitors in the oil used in steel mills. The loss in the controls devoid of cultures were less than 4% for diesel engine lubricating oil over 7 days and less than 3% for the extracted oil over 15 days. Jirasripongpun (2002) reported a maximum 34% degradation of lubricating oil extracted from steel mill wastes using Nocardia simplex culture over 30 days. In our study, use of bacterial consortium rather than a pure culture may have caused degradation at a higher rate.

Table 2 depicts the rate of decrease in residual oil in the mill sludge biodegradation rate studies. At the end of the 60 day long study, the residual oil in both sludge samples is about 2.7–3.0%. However, due to differences in the initial oil content, the percentage loss with respect to initial is 45% for sample 1 and 32% for sample 2. It appears that the residual oil at 2.7–3.0% is composed of components that are not easily degraded.

The differences in oily sludge degradation by the two cultures can be compared for degradation of sludge sample 1. The extent of loss over 60 days is comparable for the two cultures (41–45%), however the degradation rate is significantly different. In the study with BS culture, the rate of degradation is comparable to that of un-inoculated controls up to 35 days and the mass loss observed is 20%. With MS culture, maximum rate of degradation is observed between 15 and 32 days. At 32 days the residual oil is 3.45%, indicating 31.50% loss with respect to initial whereas the un-inoculated controls shows only 9.0% loss. Thus, use of MS culture may result in significant reduction in treatment time.

The un-inoculated control shows significant loss over time while the loss of oil is negligible, i.e., less than 3–5% in the controls containing azide. This confirms the presence of indigenous microorganisms in oily sludge. A pure culture of *Bacillus* sp. (99% match) could be isolated and identified from the un-inoculated controls. In the three biodegradation rate studies significant variability is observed in oil degradation rate by the indigenous cultures. This may be due to the non-homogeneous distribution of both the oil and oil degrading cultures in the sludge. Maximum degradation of oil by the indigenous cultures is observed for sludge sample 1, possibly since the oily sludge was pre-dried to 45 °C in this study while in the other studies, oily sludge was pre-dried at 70 °C. While some cultures were possibly killed at the elevated pre-drying temperature others could survive this high temperature and degrade oil under favorable condition. For pre-drying at <100 °C, the pre-drying temperature was found to have negligible effect on oil content in the sludge. Some decrease in pH was observed over the course of biodegradation in the experimental samples and the un-inoculated controls, while no pH drop was observed in the controls containing azide.

Results of the sludge biodegradation study revealed that the degradation rate is very slow and the maximum extent is less than 45% over 60 days. A similar extent of degradation was observed for free phase lubricating oil extracted from oily sludge over a period of only 15 days. The remaining 55% of the oil possibly contains high molecular weight hydrocarbons of complex structure. The slow rate of biodegradation of oil from oily sludge may be due to the slow rates of dissolution, desorption, or transport of hydrocarbons from the oil associated with sludge solids as suggested by various researchers (Biswas et al., 2005). The high concentration of iron in sludge solids may have inhibited the growth of cultures in the slurry systems and could be partly responsible for the slow degradation rate.

Color of the oil extracted from the controls at the beginning of the study was pale yellow. In contrast, the oil appeared brown for each of the experimental flasks extracted beyond 15 days. Change in the aliphatic, aromatic and polar fractions in residual oil based on column chromatography is illustrated in Fig. 1. At the end of the biodegradation study a significant reduction in the hexane eluted fraction was observed, such that for the 60 day experimental flasks, the hexane eluted fraction was 43% in sludge sample 1 with BS culture, 32% in sludge sample 2 with BS culture and 26% for sludge sample 1 with MS culture. For the un-inoculated controls (study with BS culture and sludge sample 1) extracted at 60 days the hexane eluted fraction was 66% while it was 76–77% in the beginning of the study. Thus, primarily the aliphatic fraction of lubricating oil is degraded by the bacterial cultures. The change in the toluene eluted aromatic fraction was not very significant indicating some biodegradation in this fraction. Biodegradation caused significant enhancement in the methanol eluted polar fraction. Thus, although some of the components are

Table 2
Decrease in residual oil in mill sludge with biodegradation

Sludge sample 1								Sludge sample 2			
BS				MS				BS			
%Residual oil				%Residual oil				%Residual oil			
Day	Control		Expt	Day	Control		Expt	Day	Control		Expt
	UN	Az			UN	Az			UN	Az	
0	5.13	5.10	5.03 ± 0.07	0	5.10	5.16	5.04 ± 0.06	0	4.43	4.43	4.42 ± 0.01
2	5.10	5.03	5.02 ± 0.08	5	–	5.03	4.76 ± 0.12	5	–	4.43	4.40 ± 0.01
4	5.00	5.00	4.80 ± 0.05	15	4.65	4.95	4.48 ± 0.03	10	4.43	4.43	4.13 ± 0.01
7	4.89	5.00	4.75 ± 0.07	25	4.71	5.04	3.92 ± 0.17	21	3.99	4.38	3.26 ± 0.01
12	4.72	5.00	4.62 ± 0.07	32	4.64	4.88	3.45 ± 0.09	28	4.04	4.40	3.33 ± 0.01
15	4.25	5.00	4.14 ± 0.14	39	4.49	4.99	3.27 ± 0.17	35	–	4.40	3.03 ± 0.05
28	3.70	5.01	4.18 ± 0.09	50	4.73	4.93	3.21 ± 0.02	42	3.39	4.40	3.05 ± 0.03
35	4.08	5.00	4.08 ± 0.19	55	–	4.91	3.08 ± 0.07	49	–	4.39	3.02 ± 0.03
60	4.65	4.95	2.73 ± 0.27	60	4.36	4.98	2.94 ± 0.14	60	3.40	4.39	2.99 ± 0.01

Where, UN = un-inoculated control, Az = azide killed control and Expt = samples inoculated with cultures.

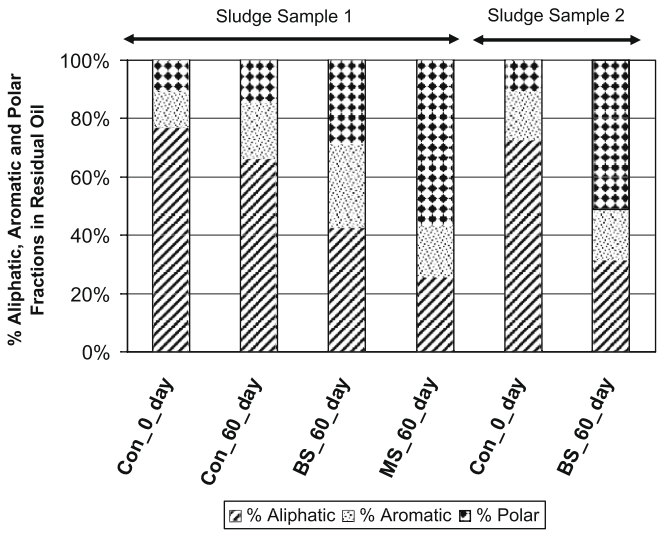

Fig. 1. Aliphatic, aromatic and polar fractions in residual oil after 60 days of biodegradation by BS and MS culture using sludge samples 1 and 2 and comparison with controls.

completely degraded, the more recalcitrant components are only partially transformed.

Separation of iron fines from sludge is necessary for reuse of this valuable resource. Initially, separation of iron fines in sludge from biomass was attempted by centrifugation at low speed (1000 rpm). However, the sludge biomass mixture settled out completely during centrifugation at low speed. Complete separation of iron-fines from the model mixture of iron-fines and biomass could not be achieved by magnetic separation. During the preparation of model mixtures containing dried biomass and sludge, the biomass adhered to the sludge solids. In the process of magnetic separation, some of the biomass was also separated along with the iron-fines. However, heating the model mixture in a muffle furnace at 600 °C, yielded iron-fines free of biomass. This is expected since biomass would be completely removed from the model mixture at this elevated temperature, and hence the mass of iron fines added to formulate the model mixture could be fully recovered using a magnet.

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