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Bioremediation of anthracene contaminated soil in bio-slurry phase reactor operated in periodic discontinuous batch mode

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Abstract

Bioremediation of soil-bound anthracene was studied in a series of bio-slurry phase reactors operated in periodic discontinuous/sequencing batch mode under anoxic–aerobic–anoxic microenvironment using native soil microflora. Five reactors were operated for a total cycle period of 144 h (6 days) at soil loading rate of 16.66 kg soil/m^3/day at $30 \pm 2\,^{\circ}C$ temperature. The performance of the bioreactors was studied at various substrate loading rates (volumetric substrate loading rate (SLR), 0.1, 0.2 and 0.3 g anthracene/kg soil/day) with and without bioaugmentation (domestic sewage inoculum; 2×10^6 CFU/g of soil). Control reactor (without microflora) showed negligible degradation of anthracene due to the absence of biological activity. The performance of the bio-slurry system with respect to anthracene degradation was found to depend on both substrate loading rate and bioaugmentation. Application of bioaugmentation showed positive influence on the rate of degradation of anthracene. Anthracene degradation data was analysed using different kinetic models to understand the mechanism of bioremediation process in the bio-slurry phase system. Variation in pH/oxidation–reduction potential (ORP), soil microflora and oxygen consumption rate correlated well with the substrate degradation pattern observed during soil slurry phase anthracene degradation.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); Anthracene; Bio-slurry phase reactor; Periodic discontinuous batch process; Bioaugmentation

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) require serious consideration among the toxic pollutants because of their ubiquitous distribution, environmental persistence and potentially deleterious effects on human health. PAHs represent a large and diverse group of organic molecules having a broad range of properties, differing in molecular weight, structural configuration, water solubility, number of aromatic rings, volatility, sorption coefficients, etc. [1–5]. Various anthropogenic sources like gasoline and diesel fuel combustion, oil spills, former gas plant facilities, etc., contribute to the entry of PAH compounds into matrices [1,4,6–8]. The highly adsorptive nature and limited bioavailability may be attributed to their high organic partition coefficients, low aqueous solubility, and low vapor pressures [4]. Because of their stability and the presence of p-orbital, structures that create a thermodynamic barrier for their initial oxidation step, make it metabolically expensive [9–12]. Generally, PAH compounds get strongly adsorbed on to the soil particles [12–114] and desorption is a controlling factor for their efficient degradation [4,15–17].

Bioremediation is gaining wider approval as a feasible alternative treatment technology for the remediation of soils contaminated with PAHs. Bioremediation is considered to be a safe, efficient, eco-friendly and economic means of removing pollutants from contaminated soil without simply enacting transfer to another medium. Bioremediation technologies such as land-farming, bioventing, air sparging and bio-slurry reactor, were applied to remediation of PAH contaminated soils and sediments [5,12,17,18]. Several isolated microorganisms capable of mineralizing PAHs are reported to be both under aerobic and anaerobic microenvironment [12,19–24]. The efficacy of bioremediation process is mainly dependent on the activity of microorganisms in association with other factors, viz., nutrient availability, non- uniform spatial distribution of microorganisms and low bioavailability of the compounds to be degraded [25].

Even though *in situ* bioremediation was reported for remediation of soils, sludges and sediments contaminated with solvents,

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petroleum products, herbicides, pesticides and various other organic compounds [26–28], low permeability and heterogeneous nature of the soil and long duration of the process limit its use. Bioremediation by ex situ methods such as bio-slurry phase process was reported. Both the initial rates and overall extent of mineralization of the substrate were enhanced and are capable of treating high concentrations of organic contaminants in soils and sludges [4,29,30]. Bio-slurry phase systems utilize naturally occurring bacteria or inoculated strains having specific metabolic capabilities to convert hazardous organic compounds and offers maximum control by providing maximum degradation rate, at the same time minimizing the abiotic losses [4,5,12,31–38]. Rapid substrate degradation can be achieved in bio-slurry phase systems compared to *in situ* decontamination systems [31] due to enhanced mass transfer rates [4,12].

This communication reports experimental data pertaining to bio-slurry phase reactor performance with respect to anthracene degradation in soil matrix under both augmented and non-augmented conditions. Anthracene, a tricyclic aromatic hydrocarbon ($C_{14}H_{10}$) derived from coal tar, is a product of incomplete combustion. It has extensive natural and anthropogenic sources and is largely associated with particulate matter, soils and sediments.

2. Experimental design

2.1. Materials

Anthracene (crystalline blue–violet fluorescence flakes; molecular weight: 178.23; melting point: 216–218 °C; boiling point: 340 °C; density: 1.099 g/cm^3, octanol–water partition coefficient: 4.456; solubility: 0.065 mg/l) was purchased from Aldrich (99%) (Fig. 1). Soil used for horticulture activities in the premises of Indian Institute of Chemical Technology (IICT), Hyderabad was used as test soil in bioremediation studies (Table 1). Characteristically selected soil belongs to silt-loam category as per US department of agriculture triangular soil classification chart and composed of 23% of clay, 24% of sand, 1.1% of organic fraction and 51.9% of silt. The soil has 76% of finer particles (altogether silt, clay and organic fraction) with soil distribution coefficient (k_{sd}) of 26.649×10^{-7} m^3/g, bulk density (ρ_B) of 1.56 m^3/g and colony forming unit (CFU) of 2.36×10^4 CFU/g soil.

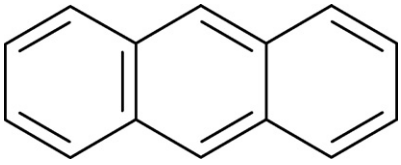

Fig. 1. Molecular structure of anthracene.

Table 1
Characteristics of experimental soil

Composition/properties of soil	Value
Sand (%)	26
Silt (%)	50
Clay (%)	24
Soil pH (1:5, w/v)	7.4
Organic composition (f_{oc}) (%)	0.9
Soil moisture content (%), field/air dry	20.1/1.9
Soil distribution coefficient (k_{sd}) (m^3/g)	26.66×10^{-7}
Bulk density (ρ_B) (m^3/g)	1.56

Table 2
Design criteria and operation details of bio-slurry phase reactor

Operation mode	Periodic discontinuous/sequencing batch mode
Total cycle period (h)	144 h [FILL (anoxic): 1 h; REACT (aerobic): 141 h; SETTLE (anoxic): 1 h; DECANT (anoxic): 1 h]
Reactor microenvironment (cyclic)	Anoxic (1 h); aerobic (141 h); anoxic (1 h); anoxic (1 h)
Total working volume (l)	0.5
Operating volume (l)	0.3
Soil loading rate (kg soil/m^3/day)	16.66
Slurry phase DO (mg/l)	2.0 ± 0.2 (air diffusion rate: 110 l/h)
Substrate loading rate (SLR, g anthracene/kg soil/day)	0.1, 0.2, 0.3
Slurry phase pH (operating)	7.0 ± 0.2
Operating temperature (°C)	30 ± 2

The selected soil sample did not contain anthracene even in traces. The soil after collection was sieved with 2 mm sieve for the removal of debris and air-dried (30 °C) prior to storing in closed containers at 4 °C to sustain biological activity. For impregnation of anthracene, a requisite amount of air-dried soil was completely soaked in known concentration of anthracene (dissolved in acetonitrile). The soil mixture was subjected to continuous stirring on horizontal shaker (20 rpm; 24 h; 30 °C) to facilitate uniform sorption of substrate onto the soil particles. Anthracene-impregnated soil was used for the preparation of soil slurry by adding either sterile distilled water (pH: 7.0; total dissolved solids: 24 mg/l) or combined domestic sewage (CDS; pH: 7.06, oxidation–reduction potential (ORP): −6 mV; CFU: 2×10^6 CFU/l) at a ratio of 1:10 (w/v). Apart from slurry preparation, CDS also acted as augmentation inoculum.

2.2. Bio-slurry reactor and operation

Five bio-slurry phase reactors having a total working volume of 0.5 l were used in bioremediation experiments. Reactors were fabricated using glass with inlet and outlet arrangement for both feed and recirculation feed. Air diffusers connected to sparger network using silicon tubing were provided at the bottom of reactor (1 mm above the reactor bottom) to facilitate uniform distribution of air through the system. This facility provided uniform distribution of air (∼100 l/h) throughout the reactor from bottom of the reactor towards up flow direction, which helped to keep slurry phase in continuous suspension during REACT phase operation. Bio-slurry phase reactor was operated in periodic discontinuous/sequencing batch mode with a single cycle length (retention time) of 144 h composing of FILL (1 h), REACT (141 h), SETTLE (1 h) and DECANT (1 h) phases (Table 2).

Five bio-slurry phase reactors were evaluated with different substrate loading rates (SLR) and microenvironment conditions as depicted in Table 3. Reactor A (SLR: 0.1 g anthracene/kg soil/day) acted as control, in which the soil was sterilized for 20 min at 103 °C to kill the resident soil native microflora. This reactor served as control to understand the volatile nature of anthracene (if any). Reactor B (SLR: 0.1 g anthracene/kg soil/day) was operated with only native soil microflora to assess the influence of mixed soil microflora on the anthracene degradation. Reactors C (SLR: 0.1 g anthracene/kg soil/day), D (SLR: 0.2 g anthracene/kg soil/day) and E (SLR: 0.3 g anthracene/kg soil/day) were operated in the presence of resident soil microflora augmented with combined domestic sewage. Except the augmentation and anthracene loading rates, all the reactors were operated for a period of 144 h in periodic discontinuous batch mode. Anthracene-impregnated soil was either mixed with sterile distilled water (Reactor A and B) or domestic sewage (Reactor C, D and E) in the ratio of 1:10 (w/v) (soil loading rate: 16.66 kg soil/m^3/day) to prepare the slurry. Solid loading rate was maintained constant throughout the experiments for all the systems studied.

All experiments were performed under passive sun light at room temperature (30 ± 2 °C). Air was supplied only during REACT phase operation. During this phase aerobic microenvironment prevailed while the other three phases (FILL, SETTLE and DECANT) were operated under profuse anoxic microenvironment. During operation, dissolved oxygen (DO) was maintained around 2.0 ± 0.2 mg/l during REACT phase. Slurry phase pH prior to feeding to reactor was adjusted to 7.0 using either 0.1N NaOH or 0.1N HCl. To sustain the requisite metabolic activity of the mixed microorganism in the systems, a fraction of treated slurry (10%) was retained in the reactor after DECANT phase operation and 90% volume of the untreated soil slurry was freshly loaded in each cycle operation. The reactors were not supplemented either with nutrients or trace metals and foaming was not observed during the reactor operation. The reactor volume was discarded safely after sampling. All the experiments were carried out in duplicate to get reliable data and the results presented represent an average of two independent measurements.

Table 3
Details of anthracene concentration and microflora during bio-slurry phase reactor operation

Reactor	Nature of the reactor	Nature of the microflora	SLR (g anthracene/kg soil/day)
A[+]	Non-augmented	Killed control	0.1
B[+]	Non-augmented	Native soil microflora	0.1
C[+]	Augmented	Native soil microflora augmented with domestic sewage	0.1
D[+]	Augmented	Native soil microflora augmented with domestic sewage	0.2
E[+]	Augmented	Native soil microflora augmented with domestic sewage	0.3

[+] Soil loading rate: 16.66 kg soil/m^3/day.

2.3. Analysis

The soil and aqueous phase samples were analyzed for anthracene using high performance liquid chromatography (HPLC) (column: C18 ODS, Solvent: 90% acetonitrile in water, flow rate: 1.0 ml/min, UV absorption: 282 nm). Twenty-five milligrams of air-dried soil was subjected to extraction using acetonitrile (50 ml) followed by agitation (100 rpm; 30 °C; 30 min) and sonication (5 min). The extraction was carried out three times and the resultant filtrate was separated (8000 rpm; 28 °C; 5 min) prior to analysis. The extraction procedure was validated based on the loading concentration of anthracene (96 ± 2% recover). Twenty microlitres of extracted filtered sample was injected (acetonitrile and water eluant (9:1)) in HPLC. Based on the area of standard anthracene obtained from chromatogram, the values were correlated. Apart from anthracene, pH and DO were also monitored during the reactor operation at predetermined time intervals as per the procedure outlined in standard methods of APHA [39]. Slurry phase pH was measured using pH meter (ELICO, India). DO and Oxygen consumption rate (OCR) were measured using DO probe (YSI 5100, USA). OCR of the bio-slurry was measured as per the procedure described elsewhere [35]. CFU was determined using plating procedure employing nutrient agar containing anthracene (glucose (2.5 g/l); anthracene 1 g/l; yeast extract 5 g/l; peptone 5 g/l; NaCl 2.5 g/l; agar 20 g/l) adopting serial dilution (10^{-4}) technique. CFU estimation was done regularly to evaluate anthracene degrading microbial diversity count during the course of the reactor operation. All the analytical estimates were carried out in duplicate and the average values were presented.

3. Results and discussion

3.1. Bio-slurry phase reactor performance

The performance of bio-slurry phase reactors with respect to anthracene degradation was evaluated estimating the substrate (anthracene) degradation efficiency (ξ_a) and substrate degradation rate (SDR; g anthracene/kg soil/day) as explained in Eqs. (1) and (2).

$$\xi_a(\%) = \left[\frac{C_o - C_t}{C_o}\right] \times 100 \quad (1)$$

$$\mathrm{SDR} = \mathrm{SLR} \times \left[\frac{C_o - C_t}{C_o}\right] \quad (2)$$

Where, C_o represents the initial anthracene concentration (g/kg soil), C_t denotes anthracene concentration (g/kg soil) at a given time 't' during the reactor operation and SLR represents substrate loading rate (g anthracene/kg soil/day). SDR provides the rate of anthracene degradation per unit time per kg of soil.

3.1.1. Non- augmented reactor

The antharcene degradation pattern of non-augmented bio-slurry phase systems with the function of cycle period for all the experimental variations studied are depicted in Fig. 2. Control systems (Reactor A; killed control; SLR: 0.1 g anthracene/kg

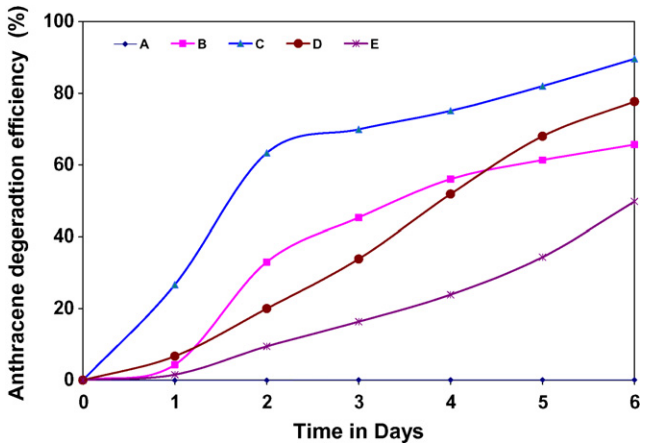

Fig. 2. Anthracene degradation efficacy during single cycle operation of bio-slurry phase reactor with all the experimental variations studied.

soil/day) showed relatively low or insignificant anthracene degradation. From the graph it can be observed that after 72 h, there was no change in the concentration of anthracene and after 92 h of operation, a significantly low anthracene removal (0.1%) was observed. Low anthracene removal observed after prolonged operation may be attributed to the volatilization/abiotic loss due to persistent aeration during the REACT phase of operation. Reactor B operated with resident/native flora (non-augmented; SLR: 0.1 g anthracene/kg soil/day) showed marked anthracene degradation, which is contrary to the control reactor performance. A gradual rise in the degradation efficacy (4.33%; 24 h) was observed with time and a maximum degradation (65.66%) was registered accounting for SDR of 0.065 g anthracene/kg soil/day at the end of cycle period (144 h). The degradation efficiency observed in the reactor B may be attributed to the consequence of the microbial metabolism by the resident/native soil microflora. Analysis of aqueous phase showed no traces of anthracene, which might be due to the hydrophobic nature of the anthracene. This observation suggested that the anthracene degradation in the bio-slurry phase might be confined to the soil or at soil–liquid interface.

3.1.2. Bioaugmented reactor

Reactors C, D and E were bioaugmented with domestic sewage and were also used to evaluate the anthracene loading rate on the bio-slurry phase performance. Enhanced degradation of anthracene was observed with the function of cycle period after augmentation (Fig. 2). Reactor C (SLR: 0.1 g anthracene/kg soil/day) showed rapid and effective degradation of anthracene compared to the corresponding non-augmented reactor (B). The reactor showed 63.3% of anthracene degradation efficiency accounting for an SDR of 0.063 g anthracene/kg soil/day within 24 h of the cycle operation. At the end of the cycle period about 89.6% degradation of anthracene (0.089 g anthracene/kg soil/day) was observed. The effective performance observed in the case of reactor C over reactor B might be attributed to the application of bioaugmentation strategy. Augmented reactor D, operated at a higher anthracene loading rate (SLR: 0.2 g anthracene/kg soil/day) compared to reactor C, showed relatively slow degradation rate initially (6.67%; 24 h). However,

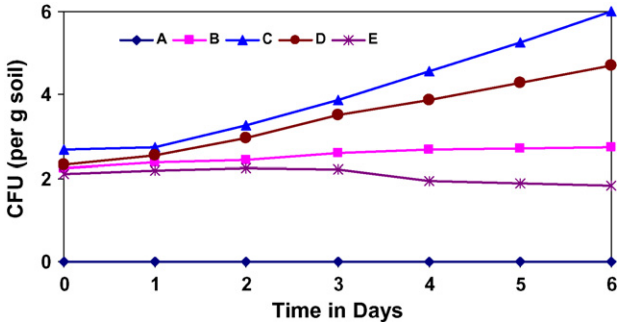

Fig. 3. Variation of CFU during single cycle operation of bio-slurry phase reactor with all the experimental variations studied.

with time the increase in degradation efficiency was observed and degradation of 77.7% (0.152 g anthracene/kg soil/day) was observed at the end of cycle period. Similarly, reactor E which was operated with highest substrate loading rate (SLR: 0.3 g anthracene/kg soil/day) among all the reactors studied, also evidenced an initial slow degradation efficiency (1.55%; 24 h) followed by enhanced efficacy (49.7%) accounting for an SDR of 0.149 g anthracene/kg soil/day at the end of the cycle period. Initial slow degradation observed in the case of reactors (D and E) operated at higher substrate loading rate compared to reactor C might be due to acclimatization of the microflora to the high substrate concentrations. By observing the degradation profile of anthracene, it can be believed that compared to non-augmented reactors, the bioaugmented reactors showed better, rapid and higher degradation efficiency.

Bioaugmentation is the application of indigenous or allochthonous wild type or genetically modified organisms to polluted hazardous waste sites or bioreactors in order to accelerate the removal of undesired compounds [35,40–45]. Bioaugmentation is believed to not only augment metabolic function but also influence the bioavailability of pollutants. It is presumed that applied bioaugmentation strategy was successful, if survival of the augmented culture in association to enhanced performance of the system was observed. Hence, CFU estimation was done regularly to count the number of anthracene degrading microbial diversity during the course of the reactor operation (Fig. 3). The purpose of colony count on nutrient agar plates containing anthracene, was to know the anthracene degrading population present in the mixed culture. It indicated the group of microflora capable of growing on anthracene, utilizing as sole carbon source. Anthracene degrading population represented a fraction of the total microflora. The linear pattern of graph with respect to reactor A indicated no microbial growth and hence no biological activity. Reactor C evidenced maximum variation in the CFU in comparison to other reactors. Reactor E showed a decreasing trend after 72 h, which might be due to substrate inhibition. Mixed microflora provided diverse microbial groups with wide metabolic properties.

3.2. Kinetics of anthracene degradation

The experimental data obtained during the bio-slurry phase reactor operation, was further examined to understand the kinetic

aspects of the anthracene degradation by employing zero, first and second order rate equations [3–5].

$$\frac{dC}{dt} = -k_0 \quad (3)$$

$$\frac{dC}{dt} = -k_1 C \quad (4)$$

$$\frac{dC}{dt} = -k_2[C]^2 \quad (5)$$

Integrated form of the above equations can be written as follows:

$$[C] = [C]_0 - k_0 t \quad (6)$$

$$\ln[C] = [C] - k_1 t \quad (7)$$

$$[C] = \frac{1}{[C] + k_2 t} \quad (8)$$

Where, C and t represents substrate concentration (g) and time taken for substrate degradation (day), respectively. k_0 (g/kg soil/day), k_1 (day^{-1}), k_2 (kg soil/g/day) specifies zero, first and second order rate constants, respectively. Rate constants were calculated from the slope of the straight line plot drawn from each of the rate equation drawn. The rate equations were also generated from the respective straight line plots (not keeping intercept at zero). Table 4 depicts data derived from the kinetic analysis output of the three equations along with R^2 values. Reactor A (control) did not follow any of the kinetic equation ($R^2 < 0.9$) applied, indicating the absence of the biological activity. However, reactors with biological activity showed reasonably good correlation with one or two equations studied. Reactor B operated with native soil microflora followed the second order rate equation ($R^2 = 0.98$), indicating the dependence on both substrate and substrate intermediates formed during the biodegradation process. The relatively less efficiency of the native microflora, favoring relatively slow degradation, was due to the accumulation of metabolic intermediates. While in the case of reactor C augmented with domestic sewage, followed the first order equation ($R^2 = 0.97$) indicating the substrate dependent nature of the process. On the contrary, reactors D and E with higher concentration followed zero order rate equation.

The half-lives for zero, first and second order were also calculated from linear regression analysis using the following equations [9–11]:

$$t_{1/2} = \frac{C}{2k_0} \quad (9)$$

$$t_{1/2} = \frac{0.693}{k_1} \quad (10)$$

$$t_{1/2} = \frac{1}{k_2 C} \quad (11)$$

In reactor B, which was non-augmented, the half-life was 3.56 h indicating that the microflora present is relatively less efficient in degrading anthracene (Table 4). With augmentation, reactor C showed considerable reduction in the half-life period (1.93 h) indicating the efficacy of bioaugmentation. In reactor D, half-life period of 2.71 h was observed suggesting that the mixed microflora was in acclimatization phase due to higher loading rate (SLR: 0.2 g anthracene/kg soil/day). In reactor E, the half-life period was 6.36 h, which was greater than the other two augmented reactors (C and D) due to the applied higher loading rate.

3.3. Bioprocess monitoring

Important parameters, viz., pH, DO, OCR and CFU were monitored during bio-slurry reactor operation at a predetermined time to understand the bioprocess of remediation. Slurry phase pH was regularly monitored during the reactor operation (Fig. 4). pH is one of the important parameters which influences the activity and type of microflora and substrate mobility. Visible change in pH values were not observed during control

Table 4
Kinetic data for anthracene degradation in soil phase

Reactor	Order of rate equation	Rate constant	Kinetic equation	Half-life (h)	R^2
A	0	6.1×10^{-3} g/kg soil/day	$C = 30.01 - 0.0061t$	2459.83	0.88
	1	2.0×10^{-4} day^{-1}	$\ln C = 3.40 - 0.0002t$	3465.00	0.75
	2	6.0×10^{-6} kg soil/g/day	$C = 30.03 + 0.000006t$	3753753.75	0.88
B	0	3.58 g/kg soil/day	$C = 32.93 - 3.58t$	4.60.00	0.93
	1	0.19 day^{-1}	$\ln C = 3.62 - 0.19t$	3.56.00	0.97
	2	0.01 kg soil/g/day	$C = 59.52 + 0.01t$	5221.93	0.98
C	0	4.19 g/kg soil/day	$C = 29.34 - 4.19t$	3.49	0.85
	1	0.36 day^{-1}	$\ln C = 3.70 - 0.35t$	1.93	0.97
	2	25.00 kg soil/g/day	$C = 26.11 + 25t$	1.04	0.86
D	0	8.30 g/kg soil/day	$C = 71.1 - 8.30t$	4.28	0.98
	1	0.25 day^{-1}	$\ln C = 4.53 - 0.25t$	2.71	0.94
	2	9.20×10^{-3} kg soil/g/day	$C = 476.19 + 0.009t$	51813.47	0.84
S					
E	0	7.37 g/kg soil/day	$C = 102.09 - 7.37t$	6.92	0.95
	1	0.11 day^{-1}	$\ln C = 4.69 - 0.10t$	6.36	0.90
	2	1.70×10^{-3} kg soil/g/day	$C = 126.58 + S\,0.002t$	74626.86	0.84

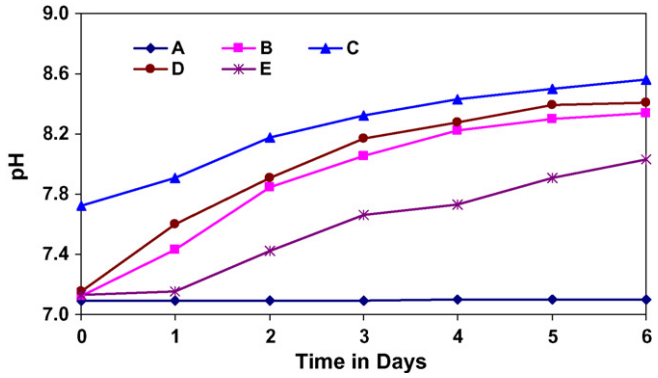

Fig. 4. Variation in pH during single cycle operation of bio-slurry phase reactor with all the experimental variations studied.

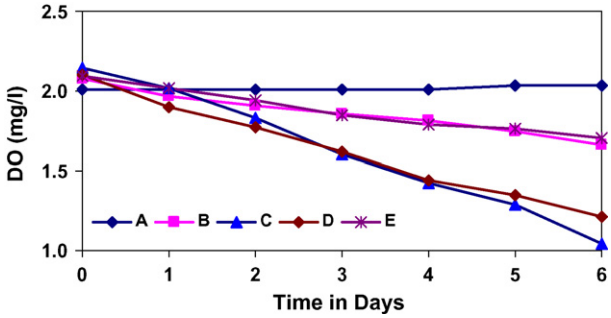

Fig. 5. Variation in DO during single cycle operation of bio-slurry phase reactor with all the experimental variations studied.

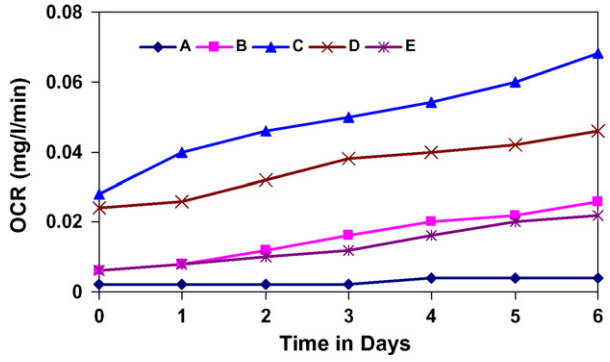

Fig. 6. Variation in OCR during single cycle operation of bio-slurry phase reactor with all the experimental variations studied.

reactor (A) operation, which may be due to the absence of microbial activity. A constant rise in the pH values was observed during operation of biologically active slurry phase systems. Reactors B, C and D showed an increase in the pH but the increase was more obvious in reactor C. In the case of reactor E, there was an increasing trend in pH but the change was comparatively less than the other reactors. In all the systems studied, alkaline pH values were observed in the slurry phase. The alkalinity in the slurry phase might be due to the alkaline nature of the byproducts formed during the anthracene degradation process. Degradation of anthracene by *Pseudomonas* sp. was reported by hydroxylate aromatic ring transformation to *cis*-1,2-dihydroanthracene-1,2-diol which was further converted to anthracene-1,2-diol [46–48]. This upon cleavage at meta position yielded 4-(2-hydroxynaph-3-yl)-2-oxobut-3-enoate which rearranged to form 6,7-benzocoumarin or be converted to 3-hydroxy-2-naphthoate, from which degradation proceeded through 2,3-dihydroxynaphthalene to salicylate [46]. At present, the only known productive pathway for bacterial degradation of anthracene, [46] proceeds through 3-hydroxy-2-naphthoic acid, 2,3-dihydroxynaphthalene and further through a pathway similar to the naphthalene degradation pathway [49]. Degradation from anthracene to 3-hydroxy-2-naphthoic acid proceeds through dioxygenation and dehydration, by which 1,2-dihydroxyanthracene was formed which is further cleaved by *meta*-ring cleavage to 2-hydroxy-3-naphthaldehyde and then to 2-hydroxy-3-naphthoic acid leading to side product 6,7-benzocoumarin [46–48,50]. Recently, it was also proposed that 6,7-benzocoumarin was an intermediate in a cometabolic pathway of anthracene before it was degraded by ring fission enzymes [51].

DO generally provides information about on-going biochemical activity taking place in the reactor with respect to microenvironment. Variation in the slurry phase DO can be seen in Fig. 5. At the beginning of the cycle period, the slurry phase DO in all the reactors was found to be around 2 mg/l. DO almost remained unchanged during the cycle period in the control reactor indicating absence of microbial activity in it. In reactor B, this was insignificant and could be attributed to the inability of the microflora to degrade the substrate effectively. In all the augmented reactors a decreasing trend in the DO was significantly observed. Reactor C showed a steep decline in the DO, which could be due to the compatibility of the microflora with the conditions present in the bioreactor and thus, effective degradation of the substrate. Reactor D also showed a decrease in the DO, which was comparable to reactor C. In case of reactor E even though decline in the DO was observed, the variation was not significant which indicated higher concentration of anthracene. OCR is a function of the organic degradation and the microbial growth in a given aerobic system and it also indicated the ability of the biomass in the bioreactor to degrade the substrate in the aerobic environment. Fig. 6 depicts the OCR profile of all reactors studied. The variation of OCR in reactor A was negligible suggesting absence of microbial activity in it. Among all the reactors evaluated, reactor C and reactor D showed a significant variation in the OCR indicating the prevailing active metabolic state of the respective systems and enhanced degradation. Both reactors B and E showed almost similar variation in the OCR values.

4. Conclusions

The study documented successful degradation of anthracene in bio-slurry phase reactors operated in periodic discontinuous batch mode. Anthracene degradation in bio-slurry phase system was found to depend on the applied substrate loading rate and the bioaugmentation. With higher substrate loading rate, a consistent reduction in the anthracene degradation efficiency was observed. Application of bioaugmentation strategy responded

positively on the substrate degradation rate. Combined domestic sewage (bioaugmented mixed culture) was found to be efficient in anthracene degradation. Variation of bioprocess parameters during reactor operation was observed to be dependent on the substrate loading condition.

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