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Degradation of chlorpyrifos contaminated soil by bioslurry reactor operated in sequencing batch mode: bioprocess monitoring

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Abstract

Bioslurry reactor (SS–SBR) was studied for the degradation of chlorpyrifos contaminated soil using native mixed microflora, by adopting sequencing batch mode (anoxic–aerobic–anoxic) operation. Reactor operation was monitored for a total cycle period of 72 h consisting of 3 h of FILL, 64 h REACT, 2 h of SETTLE, and 3 h of DECANT with chlorpyrifos concentrations of 3000 μg/g, 6000 μg/g and 12000 μg/g. At 3000 μg/g of chlorpyrifos concentration, 91% was degraded after 72 h of the cycle period, whereas in the case of 6000 μg/g of chlorpyrifos, 82.5% was degraded. However, for 12000 μg/g of chlorpyrifos, only 14.5% degradation was observed. The degradation rate was rapid at lower substrate concentration and 12000 μg/g of substrate concentration was found to be inhibitory. Chlorpyrifos removal rate was slow during the initial phase of the sequence operation. Half-life of chlorpyrifos degradation ($t_{0.5}$) was estimated to be 6.3 h for 3000 μg/g of substrate, 17.5 h for 6000 μg/g and 732.2 h for 12000 μg/g. Process performance was assessed by monitoring chlorpyrifos concentration and biochemical process parameters viz., pH, oxidation and reduction potential (ORP), dissolved oxygen (DO), oxygen consumption rate (OCR) and microbial count (CFU) during sequence operation. From the experimental data obtained it can be concluded that the rate-limiting step with the bioslurry phase reactor in the process of chlorpyrifos degradation may be attributed to the concentration of substrate present in either soil or liquid phase. Periodic operations (SBR) by varying individual components of substrate with time in each process step place micro-organisms under nutritional changes from feast to famine and maintains a wide distribution in the population of micro-organisms resulting in high uptake of the substrate in the bioslurry reactor.

Keywords: Bioslurry reactor; Sequencing batch mode operation; Chlorpyrifos; Degradation; CFU

1. Introduction

Indiscriminate usage of synthetic agrochemicals is considered to be detrimental to sustainable development, due to their persistence and toxic nature. Chlorpyrifos [*o*,*o*-diethyl-*o*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate – $C_9H_{11}Cl_3NO_3PS$] is a broad spectrum organophosphorus insecticide, possesses low water solubility (1.39 mg/l), high soil sorption coefficient (849 ml/g) and vapor pressure (2.49 mpa (25 °C)). Chlorpyrifos (molecular weight – 350.62) has a melting/boiling point in the range of 41.5–44 °C. Chlorpyrifos used widely in direct soil application for the control of mosquitoes, flies, various crop pests in soil and on folige, household pests and aquatic larvae. It is also used on sheep and cattle for the control of ectoparasites. About 9.5 million kg of chlorpyrifos (active ingredient) was used per year during 1987–1998 in the USA alone [1]. Chlorpyrifos persists in soil for 60–120 days with degradation being primarily due to microbial action [2]. Products of biodegradation include 3,5,6-trichloro-2-pyridinol and which subsequently breaks down to organochlorine compounds and carbon diox-

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ide [3]. Although restrictions had to be placed on its use in public places by the end of 2001 [4], agriculture was almost not concerned, and the use of chlorpyrifos is expected to remain at 5–6 million kg per year for foreseeable future. Its use in Europe is also under examination [5], in particular because of its residual occurrence in food [6]. A complete phase out, if decided upon, will be planned to take as long as 20 years.

Bioremediation of hazardous hydrophobic organic molecules is of major concern, due to their persistent, toxic and carcinogenic nature [7,8]. Due to their hydrophobicity, the organic compounds are mainly associated with the non-aqueous phase liquids. In situ bioremediation techniques have been quite successfully used to remediate soils, sludges and sediments contaminated by explosives, petroleum hydrocarbons, petrochemicals, solvents, pesticides and other organic compounds [9–11]. However, low permeability and heterogeneous nature of soil and rapid treatment time required limit its application in some cases. In such cases, bioreactors are favored over in situ biological techniques. Bioreactors can be specially designed in a variety of configurations for maximizing the biological degradation while minimizing abiotic losses [12–14]. Bioslurry reactors are a relatively new application to environmental clean up. Bioslurry phase systems (liquid slurry treatment) utilize naturally occurring bacteria (native microflora) or inoculated strains having specific metabolic capabilities to convert hazardous organic compounds present in solid, liquid or sorbed forms to carbon dioxide and water. Bioslurry processes faster a heterogeneous, auto-catalytic reactions, in which the micro-organisms act as a catalysts in metabolizing the substrate.

Bioslurry systems can be maintained under aerobic or anaerobic conditions and an inoculum can be added if the soil being treated has low populations of organisms capable of degrading the chemicals of interest [15]. Bioslurry reactors allow for optimization of conditions conducive to biodegradation, mixing facilitates aeration and enhances the rate of chemical exchange between soil particles and the solution, and conditions can be further optimized for biodegradation by controlling pH and temperature, and by providing nutrients and other specialized amendments, such as surfactants [16]. Slurry phase bioremediation depends on various parameters such as type of soil, contaminant type and concentration, reactor design, solids loading rate, aeration, oxygen demand, temperature, surfactant, and nutrient addition. A good design would mean that solids are kept in suspension and not allowed to settle. However, high solid concentration reduces oxygen transfer rate. The configuration of a bioslurry reactor can have significant influence on the slurry phase homogeneity and therefore on the biological decontamination and detoxification processes [17]. Different modes of bioslurry operations have been tested in the laboratory and pilot scale which includes – batch systems [18,19], cyclic batch mode/sequencing batch reactor (SBR) [20–23], continuous flow reactor [24], and tanks-in-series [25]. Oxygen requirement, oxygen transfer rate, particle size, solid/liquid ratio (solid loading rate), reactor design, operating conditions, type of inoculum, nature of substrate, etc., have significant influence on the process of bioslurry reactor performance. Batch reactors are more appropriate when the volume of soil to be treated is relatively small and the contaminant present is relatively complex and inhibitory to biodegradation.

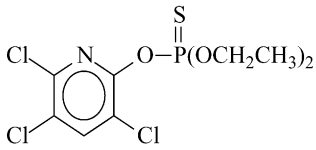

Fig. 1. Structure of chlorpyrifos.

Sequencing batch reactor (SBR) technology has been developed on the basic assumption that periodic exposure of the micro-organism to defined process conditions is effectively achieved in a fed batch system, in which exposure time, frequency of exposure and amplitude of the respective concentration can be set independently of any inflow conditions [26]. The SBR system is distinguished by the enforcement of controlled short-term unsteady state conditions leading in the long run to stable steady state with respect to composition and metabolic properties of the microbial population growing in the reactor by controlling the distribution and physiological state of the micro-organisms. Success of SBR technology depends upon the great potential provided by the possibilities of influencing the microbial system in the SBR and also upon the fact that SBRs are comparatively easy to operate and cost efficient. SBR process is known to save more than 60% of expenses required for conventional activated sludge process in operating cost [27–29].

The present study is an application of soil bioslurry–sequencial batch reactors (SS–SBRs) for treating chlropyrifos contaminated soil under anoxic–aerobic–anoxic condition. Process performance is assessed by monitoring chlorpyrifos concentration and biochemical process parameters viz., pH, oxidation reduction potential (ORP), dissolved oxygen (DO), oxygen consumption rate (OCR) and total microbial count (CFU) during sequence (batch) operation. The results obtained are presented and discussed in this communication.

2. Materials and methods

2.1. Chemicals

Technical grade chlorpyrifos (>97% purity, Excel Industries Limited, India) was used in SS–SBR degradation studies. The structural details of chlorpyrifos are shown in Fig. 1. All other chemicals and reagents used were of analytical reagent grade (AR). The solutions were prepared with double distilled (glass) water (pH 7.0 ± 0.2, TDIS-absent, CFU-absent, metals-below detectable limit).

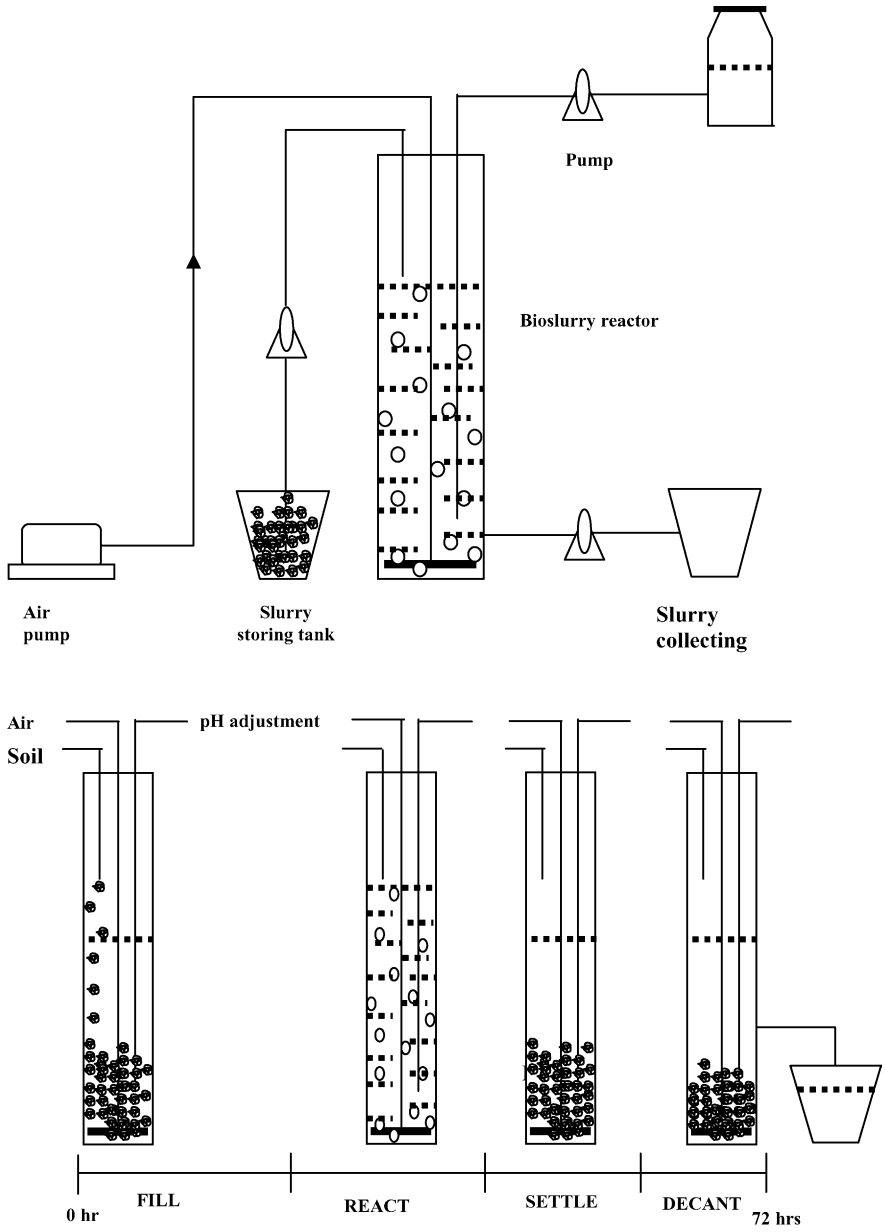

Fig. 2. Schematic representation of bioslurry reactor and sequence phase details.

2.2. Soil

Soil sample was collected from agricultural fields, where the soil profile was normally used for agricultural activities and has a previous history of chlorpyrifos application. The type and characteristics of the soil used in this investigation are shown in Table 1. The soil was a red, silty loam (as per US Department of Agriculture triangular soil classification chart) [30]. The selected soil matrix contained 24% clay, 26% sand, 0.9% organic fraction and 50% silt. Before using, the soil sample was passed through a 2 mm sieve to remove debris. The sieved fraction was partially air-dried (in fume hood for 24 h) and the moisture content estimated. The soil sample was stored at 4 °C to maintain biological activity prior to using in bioslurry experiments. The soil distribution coefficient ($k_{SD)}$ of the soil matrix used is found to be $26.649 \times 10^{-7} m^3/g$ [31] with an estimated bulk density (ρ_B) of $1.56\, m^3/g$.

2.3. Preparation of soil slurry

The air-dried soil was spiked with a known concentration of chlorpyrifos (3000 μg/g) dissolved in acetone and the soil was evaporated in a fume hood at room temperature (10 h). This was done to promote homogeneous sorption onto the soil particles. The chlorpyrifos impregnated soil particles were used for slurry preparation. Slurry was prepared with 1:30 (w/v) ratio (soil:sterilized water).

Table 1
Characterization of the soil matrix

Composition of soil	
Sand (%)	26
Silt (%)	50
Clay (%)	24
Soil pH (1:5 w/v)	7.4
Organic composition (f_{oc}) (%)	0.9
Soil moisture	
Field (%)	20.1
Air dry (%)	2.1
Soil distribution coefficient (k_{SD}) (m^3/g)	26.649×10^{-7}
Bulk density (ρ_B) (m^3/g)	1.56

2.4. Slurry phase reactor configuration

A SS–SBR having a total volume of 500 ml consisted of 'Pyrex' glass with a suitable inlet and outlet arrangement. Oxygen was supplied through air diffuser connected through silicon tubing to a well-distributed sparger network at the bottom. The sparger network arrangement consisted of four radial arms with equal distance from center and placed 1 mm from the bottom of the reactor. This arrangement facilitated a uniform diffusion of air from bottom of the reactor upwards, and also provided mixing.

2.5. Reactor operation

Schematic representation of reactor is depicted in Fig. 2. Reactor A acted as a killed control, in which the soil was sterilized for 20 min at 120 °C. This reactor served to understand the volatile nature of the substrate (if any) and sorption–desorption phenomenon of substrate during slurry phase operation. Reactor B_1 served as a biologically active SS–SBR, which contained soil microflora (3.68×10^4 CFU/g of soil). All other process parameters including application dose of substrate, cycle period, sequence phases, etc. were identical for both the reactors (Table 2). Initially reactors were operated with a total cycle time of 168 h (detention time of slurry) with a chlorpyrifos concentration of 3000 μg/g to understand the time required for total degradation of the substrate. Subsequently, throughout the experiment reactors were operated with 72 h of cycle period consisting of various phases as detailed in Table 2. The total cycle time was 72 h, consisting of 3 h of FILL, 64 h of REACT, 2 h of SETTLE and 3 h of DECANT phase. The reactor microflora was subjected to anoxic condition during the FILL, SETTLE and DECANT phase and aerobic condition during the REACT phase of reactor operation. The provision of anoxic phase in the sequence helps to suppress the use of oxygen and facilitates exposing the reactor microflora to diverse environmental conditions resulting in robustness to the microflora for adverse conditions. During the course of the experiments, reactors were operated at three different concentrations of chlorpyrifos (3000 μg/g (Reactor B_2), 6000 μg/g (Reactor B_3), and 12000 μg/g (Reactor B_4)). The impregnated soil was mixed with sterile distilled water in the ratio of 1:30 (w/v) (solid loading 3.33%) to prepare slurry, and slurry was introduced into the reactor with the indigenous microflora of the soil (Tables 3 and 4). Solid loading rate was maintained same through out the experiments. Initially after loading the slurry, the reactor mixture was subjected for air diffusion through sparger to mix the slurry homogeneously. During REACT phase, the slurry mixture was agitated with the help of air sparger arrangement by which the slurry was always in suspension. To sustain the requisite metabolic activity of the mixed microorganism in systems under SBR conditions, a fraction of treated slurry (10%) is retained in the reactor in the DECANT phase of each cycle and replaced with the remaining volume (90%) of untreated slurry in FILL phase to complete the cycle. The reactors were operated at room temperature (25 ± 2 °C) by diffusion air (atmospheric) to maintain aqueous phase DO in and around 2.5 mg/l. The pH of the slurry phase was maintained at 7.1 ± 0.1 with 1N NaOH and 1N HCl. The reactor was not supplemented with nutrients as the compound itself contains phosphorous and nitrogen source. The problem of foaming was not encountered during reactor operation.

To get the reliable data, all the experiments were studied in duplicates and the results presented here represent an average of two measurements.

Table 2
Sequence phase details of bioslurry phase reactors

Sequence phase	Cycle period (h)		Condition	Air supply/agitation
FILL	5	3	Anoxic	No
REACT	156	64	Aerobic	Yes
SETTLE	2	2	Anoxic	No
DECANT	5	3	Anoxic	No
Total cycle	168	72		

Table 3
Bioreactor operating conditions

Operation parameter	Value
Chlorprifos concentrations (μg/g)	3000, 6000, 12000
Slurry phase pH	7.1 ± 0.1
Slurry phase ORP (mV)	−30.0 to −32.0
Cycle period (h)	72
Temperature (°C)	25 ± 2
Operating volume (ml)	300
Solids loading (%)	3.33
Slurry phase DO (mg/l)	>2.5

2.6. Analytical methods

The reactors were monitored regularly for slurry phase pH, oxidation and reduction potential using pH meter (Denver, USA). The oxygen consumption rate was measured by monitoring dissolved oxygen consumption in oxygen (atmospheric) saturated bioslurry (100 ml) for a predetermined time. The DO was measured using a DO probe (YSI 5100, USA). All the analytical estimates were made in duplicates.

Table 4
Details of chlorpyrifos concentrations studied and soil CFU

Soil slurry reactors	Total cycle period (h)	Chlorpyrifos addition (μg/g)	Mixed soil micro flora	Micro-organisms present (CFU/g of soil)
A	168	3000	Absent	Nil (killed control)
B_1	168	3000	Present	3.68×10^4 (active)
B_2	72	3000	Present	3.91×10^4 (active)
B_3	72	6000	Present	3.89×10^4 (active)
B_4	72	12000	Present	3.74×10^4 (active)

2.7. Chlorpyrifos assay

Concentration of chlorpyrifos was estimated by employing gas chromatography (GC). For chlorpyrifos analysis, 2 g of slurry sample were collected, dissolved in 10 ml of toluene and the mixture was agitated for a period of 60 min at 100 rpm on a horizontal shaker at 25 °C. After separation of layers, the toluene extract was filtered through anhydrous sodium sulfate and evaporated by rotary evaporator (Buchi R 124, Switzerland) at 40 °C. One ml of toluene was added to the evaporate, and was analyzed by GC (Model 7540 Hewlett Packard) equipped with a FID detector (2 mm i.d. × 120 cm long borosilicate glass column). Temperatures of the injection block, oven and detector cell were 300, 220, and 300 °C respectively during operation. Gas flow rates of 60 ml/min (helium carrier gas), 40–43 ml/min (hydrogen) and 325 ml/min (air) were used.

2.8. Isolation of chlorpyrifos-degrading micro-organisms and CFU estimation

Soil samples were collected from the biologically active reactor after completion of cycle. The soil particles were resuspended in sterilized water and mixture was agitated on horizontal shaker at 100 rpm for 30 min (20 °C). The aqueous phase was centrifuged (4000 × *g*, 25 °C, 5 min) and the settled pellets were again resuspended in sterilized water and subsequently subjected to several successive dilutions (1:10) to reduce the diversity with in the mixed culture and were plated on selective medium (50 mg/l chlorpyrifos, 2% agar) and incubated at 30 °C for a period of 6 days. Four predominantly grown colonies were observed on agar plates after successive plating of the enrichment culture. The isolated micro-organisms were further subjected to morphological characterization. For studying the morphological structure of the bacteria the strain was grown in nutrient broth for 48 h at 30 °C. After harvesting the strain were resuspended in sterile double distilled water. The isolates were further studied for gram staining on air-dried and heat fixed smears prepared with 24–28 h old cultures.

The colony forming units (CFU) were counted on nutrient agar plates (3 days, 30 °C) by inoculation of 0.1 ml of bioslurry extracted biomass after several serial dilutions. CFU were counted periodically during the reactor operation, which represent the number of culturable heterotrophics in the aqueous and solid phases.

3. Results and discussion

3.1. Bioslurry reactor performance

The SS–SBRs were operated to degrade chlorpyrifos present in soil at varying substrate concentrations (3000, 6000, and 12000 μg/g) at room temperature (25 ± 2 °C). Biologically active reactor (Reactor B_1) was operated in sequencial batch mode having a 168 h of cycle period [5 h FILL, 156 h REACT, 2 h SETTLE, and 5 h DECANT] at chlorpyrifos concentration of 3000 μg/g to assess the extent of degradation and suitability of the reactor under aerobic condition with the native microflora. Killed control (Reactor A) was also operated with the same operation condition and substrate concentration to study the abiotic loss of substrate due to reactor operation. Chlorpyrifos degradation pattern during 168 h (7 days) of the cycle period for both the reactors is shown in the Fig. 3. The degradation profile of the killed control reactor was more or less uniform up to 168 h of the cycle period. Negligible abiotic loss of chlorpyrifos was observed in the control reactor. This observation suggests the mechanism responsible for chlorpyrifos removal in the slurry phase system may be entirely attributed to the biodegradation mechanism.

The active reactor (B_1) showed degradation of chlorpyrifos and was found to be dependent on the reaction time (Fig. 3). It can be seen from the figure that during initial 24 h of cycle period, the chlorpyrifos degradation was rel-

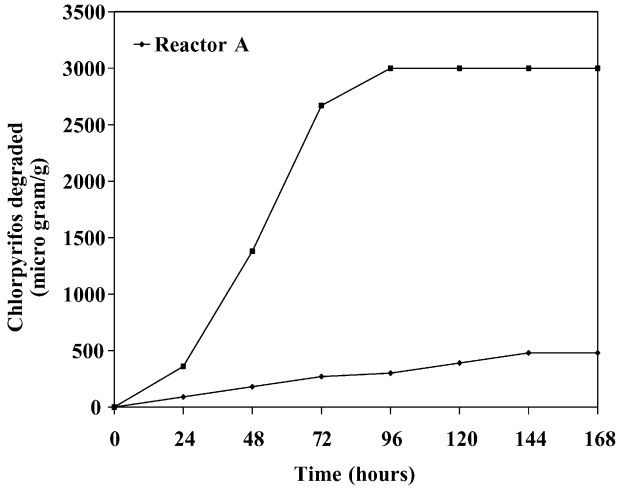

Fig. 3. Average chlorpyrifos degradation during the cycle operation.

Table 5
Morphological variation of microbial type in the soil matrix

Strain	Type	Gram staining
1	Cocci	+ve
2	Rod	+ve
3	Strepto cocci	+ve
4	Strepto cocci	+ve

atively low (12%), and increased after 24 h, approaching a maximum of 78% in 48 h. Thereafter, rates remained more or less constant for the remainder of the cycle time. From the graph, about 72 h of cycle period is fond to be sufficient for the maximum degradation of substrate under predefined operation conditions and subsequent operation of the reactor showed relatively negligible degradation of the substrate. The initially low chlorpyrifos degradation (lag phase) may be attributed to the acclimatization phase, which is a prerequisite for the native soil microflora to adjust to the new system environment. Unlike continuous systems, batch systems are normally associated with consistent lag phase. With increase in cycle period, the native microflora might have acclimatized to the new substrate (system) conditions facilitating a rapid degradation. The subsequent experiments of bioslurry phase reactor were limited to 72 h of the total cycle period including FILL and DECANT phase.

In order to understand the substrate mobility in slurry phase system, chlorpyrifos concentration in soil and water matrix was monitored periodically in control killed reactor (Reactor A) during the reactor operation (Table 5). The data pertaining to the desorption along with partitioning of chlorpyrifos is shown in graphical forms (Figs. 4 and 5). It can be inferred from the figure that the sorption of chlorpyrifos onto the soil particles (C_{soil}) was found to be relatively good. Relatively low desorption of chlorpyrifos was observed with increase in the contact time (cycle period) due to the continuos agitation (killed control). The desorption of substrate from soil particles was found to be relatively low and consistent with time (3% in 24 h) and remained more or less constant after 144 h of cycle period. It is evident from Figs. 4 and 5, that the soil partition was relatively low from soil to aqueous phase in the slurry system during reactor operation. It can be presumed from the desorption data, that chlorpyrifos sorption interaction on the studied soil matrix may be attributed to the chemical type of interaction as the agitation of the slurry phase showed relatively negligible diffusion of substrate into the aqueous phase. Also the hydrophobic nature of the substrate permits the substrate to adsorb on the soil particles rather than to diffuse in to the aqueous phase. It can be concluded from the experimental data that the degradation of chlorpyrifos taking place in the slurry phase reactor was mostly associated on the soil surface or in the solid–liquid interface. The desorbed substrate (relatively less) into the aqueous phase will be metabolized by the biomass in the aqueous suspension.

3.2. Reactor performance at various substrate concentrations

Subsequently, SS–SBRs were operated for 72 h of total cycle period with chlorpyrifos concentrations of 3000, 6000 and 12000 μg/g. The biodegradation pattern of chlorpyrifos understood as a function of time at various concentrations was shown in Fig. 6. The biodegradation of chlorpyrifos in slurry phase is found to dependent on the substrate concentration. It is evident from the graphs, that with increase in chlorpyrifos concentration, the chlorpyrifos removal rate was slow. As the concentration of substrate increases, the degradation rate is found to be reduced indicating an inverse relation-

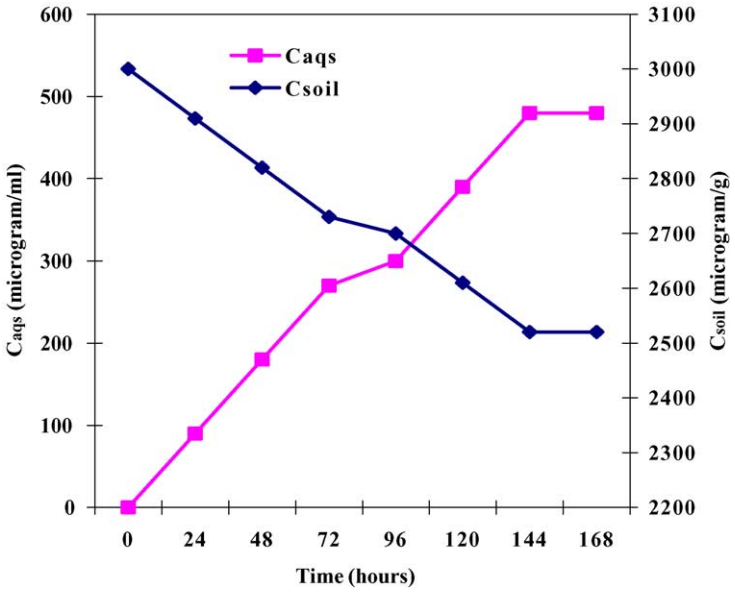

Fig. 4. Distribution of chlorpyrifos in slurry phase (Reactor A).

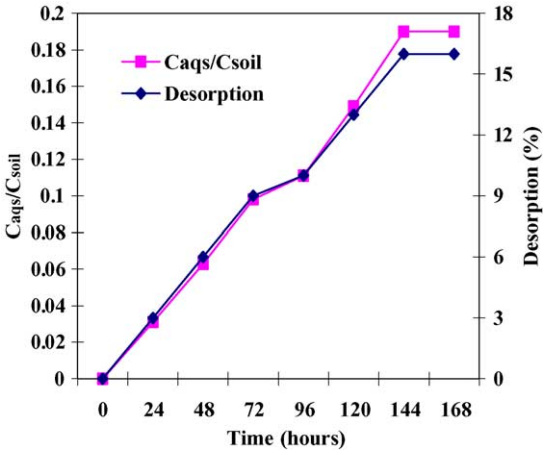

Fig. 5. Desorption and partition of chlorpyrifos with the function of time (Reactor A).

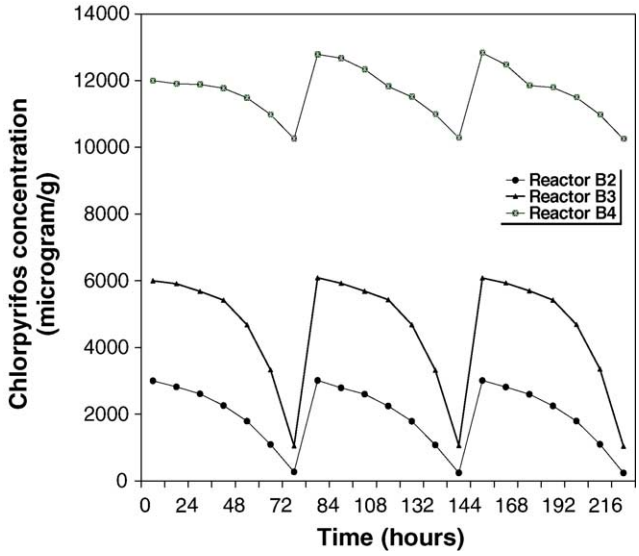

Fig. 6. Average chlorpyrifos degradation in three consecutive cycles in bioslurry reactor.

ship between concentration and rates of biodegradation. In reactor B_2, 91% of degradation was found after 72 h of the cycle period, whereas in the case of reactor B_3, 82.5% of the substrate was degraded. In contrast, in B_4 reactor, only 14.5% of the degradation was seen. Chlorpyrifos removal rate was comparatively slow during the initial phase of the sequence operation, and was found to be dependent on the chlorpyrifos concentration present in the slurry phase. With an increase in reaction period a relatively rapid removal of substrate was noticed. With an increase in sequence time, the native soil miccroflora might acclimatize to the new substrate conditions facilitating rapid removal of the organic substrate through mineralization. However, in case of 12000 μg/g of concentration, only 1740 μg/g of the chlorpyrifos was removed and relatively low degradation may be attributed to the high concentration of substrate leading to inhibition of the system. The relatively slow performance of the slurry phase reactor at higher concentration may be attributed to the presence of high substrate concentration gradients with relatively high concentration of the toxic compound. In this case, further increase in cycle period may facilitate enhanced acclimatization period by which the system efficiency may got improved, which cannot be ruled out.

3.3. Half-life period of degradation

From the experimental data, the bioslurry reactor operated in sequencial batch mode has shown rapid degradation rate. It is evident from, the applicability of bioslurry phase reactor to chlorpyrifos amended soil has yielded rapid degradation of substrate. Half-life of substrate is normally calculated based on assumption of the first order kinetics. It is reported that, the half-life for chlorpyrifos degradation ($t_{0.5}$) was ranging in between 60 and 120 days [2] in in situ soil decontamination system. Chlorpyrifos degradation half-lives ($t_{0.5}$) were reported to be in the range of 175–1576 days (1000 μg/g concentration) [32] in five different soil profiles, which is considered to be long. In present bioslurry phase reactors, total degradation of the substrate can be feasibly achieved within hours unlike days in in situ system. Chlorpyrifos was found to be rapidly degraded at ambient soil temperatures in soil slurry SBR systems with a half-lives ($t_{0.5}$) of 6.3 h for 3000 μg/g, 17.5 h for 6000 μg/g and 732.2 h for 12000 μg/g of substrate concentrations studied. Compared to the in situ bioremediation systems, bioslurry phase system showed enhanced degradation rate by several folds with native microflora due to prevailing controlled conditions, which can be simulated as required.

The SBR operation is considered to have significant flexibility in the operation, low construction and maintenance costs and controlled biological capability to enhanced degradation of toxic organic [26,28,11]. Batch systems generally requires longer reaction time and large reactor volumes, but the sequencing batch reactor offers the advantages of both continuos operation and with high biological activity associated with batch type system [21]. Batch systems are normally associated with consistent lag phase period relating to first-order (with respect to contaminants) and this may be due to the fact that the time required for acclimatization to the new environment will have considerable influence compared to the continuos flow operation. Juneson et al. [21] reported high degradation rates of *bis*(2-ethylhexyl) phthalate in a soil slurry SBR compared to batch system and attributed the enhance degradation due to higher microbial activity. Cassidy and Irvine [23] showed enhanced degradation of soil slurry contaminated with diesel fuel in sequencial batch operation condition compared to continuos mode. Periodic process induces controlled unsteady state conditions where in organisms have high substrate uptake capability due to altering feast and famine conditions and these systems are generally more robust to withstand the shock loads [28,26,33]. Enforced short-term unsteady state conditions coupled with periodic exposure of the micro-organisms to defined process conditions by controlling their physiological state (incorpo-

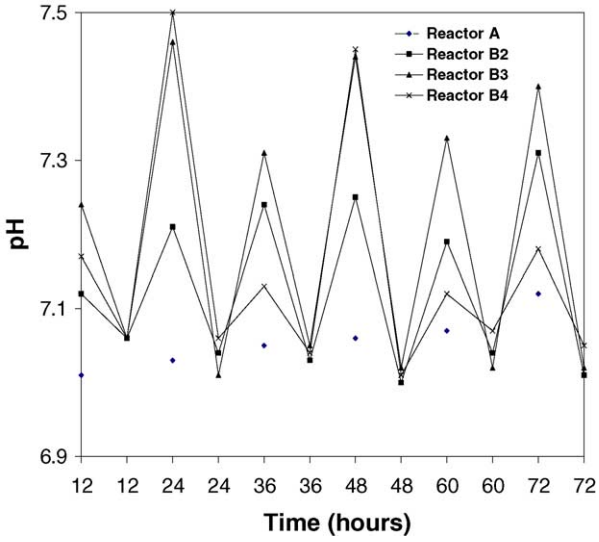

Fig. 7. Average variation of pH in bioslurry phase during cycle operation.

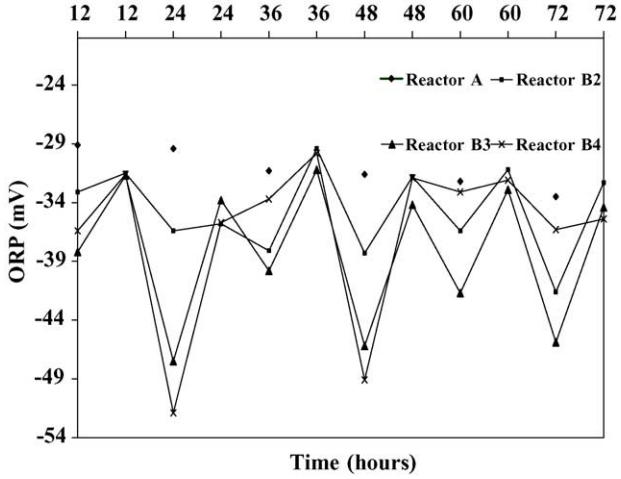

Fig. 8. Average variation of ORP in bioslurry phase during cycle operation.

rating required metabolic conditions) in SBR showed efficient performance.

3.4. Bioprocess monitoring

To understand the ongoing biochemical process during sequence operation of the cycle period, the process was monitored by determining slurry phase pH, ORP, DO oxygen consumption rate and colony forming units were monitored in slurry phase at predetermined time. Slurry phase pH was continuously monitored during reactor operation. The pH of slurry before loading was adjusted to near 7.0. Everyday a constant raise of pH at a rate of 0.44 ± 0.02 per day in the slurry phase was observed during the substrate degradation. The variation in pH during reactors operation was depicted in the Fig. 7. The pH in the slurry phase raised to alkaline range (>7.8) after operation of reactor for a period of 24 h. Slurry phase was adjusted to nearly neutral pH for every 24 h of operation using 1N HCl. A consistent increase in pH was noticed independent of the operated chlorpyrifos concentration. The raise in slurry phase pH may be attributed to the fact that, the chlorpyrifos during aerobic degradation tends to form as by-product 3,5,6-trichloro-2-pyridinol which specifically raises aqueous phase pH due to its alkaline nature. Variation of oxidation–reduction potential was also studied during the reactor cycle operation and same was shown with the function of cycle period in Fig. 8. ORP variation in slurry phase was comparable to the slurry phase variation of pH and profiles of both graphs are comparatively like mirror images.

DO is one of the important process parameters to have significant influence on the ongoing biochemical activity in any system of aerobic nature. Variation of DO in slurry phase during reactor operation with the function of cycle period was depicted in Fig. 9. During the starting of the REACT phase the slurry phase DO was 2.5 mg/l and remained unchanged up to 24 h of the sequence time. Comparatively substrate degradation was also found to be relatively low during this phase. This may be attributed to the acclimatization phase of the native microflora to the new system environment. With increase in cycle period a gradual reduction of DO was observed indicating the activation of biochemical process in utilizing the chlorpyrifos as substrate by microflora under aerobic condition. Decrease in DO is comparable with chlorpyrifos removal pattern by all the slurry phase systems studied here. The variation in the substrate concentration also has same pattern of DO depletion comparable to substrate degradation.

Oxygen consumption rate is one of the important factors that limit the capacity of any aerobic biological system in suspension. Oxygen transfer rate is a function of the organic degradation rate and the microbial growth rate in any system of aerobic condition. The metabolic activity of the microorganism is directly correlated with the electron transport system activity by measuring OCR. OCR is monitored to assess the ability of suspended slurry phase biomass to degrade complex substrate in the given aerobic environment. The OCR variation as a function of cycle time is shown in Fig. 9 for all

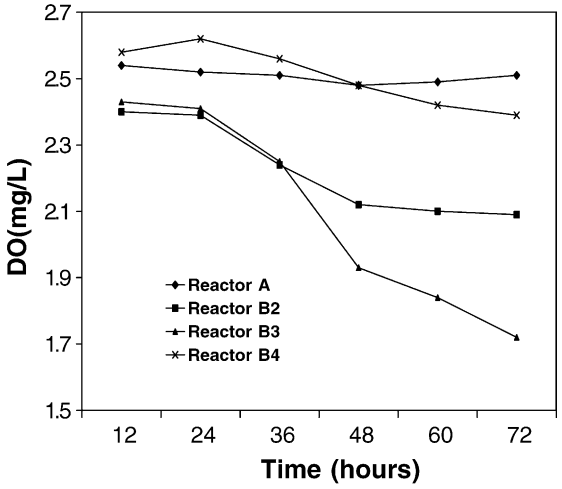

Fig. 9. Average variation of DO in bioslurry phase during cycle operation.

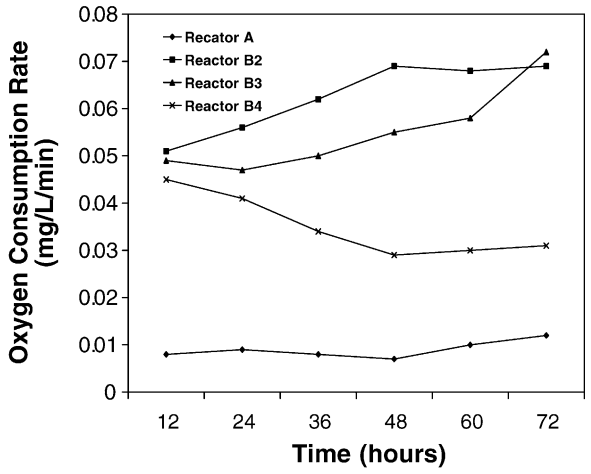

Fig. 10. Average variation of OCR in bioslurry phase during reactor operation with the function of cycle period.

the studied chlorpyrifos concentrations. The OCR curve of the killed control is more or less linear indicating negligible biological activity. The OCR profile of 3000 μg/g of chlorpyrifos concentration was found to be rapid in consumption or utilization of the aqueous phase DO. In case of 6000 μg/g of chlorpyrifos concentration, the consumption rate was found to increase after a lag phase of 24 h, which may be correlated to the degradation rate data. In case of 12000 μg/g of the chlorpyrifos concentration, OCR was not visible upto 48 hrs of cycle operation and increased thereafter. The OCR profiles are comparatively consistent with the respective degradation profiles shown in Fig. 10.

3.5. CFU variation

The microflora present in soil matrix was further isolated and studied for tentatively identifying the nature of bacteria present by adopting successive dilution technique and plating. Four predominant colonies grown on the plate were isolated and plated separately. The isolated colonies morphological details were depicted in the Table 5. All the colonies are of gram positive and the majority relates to Cocci (i.e., rod-shaped).

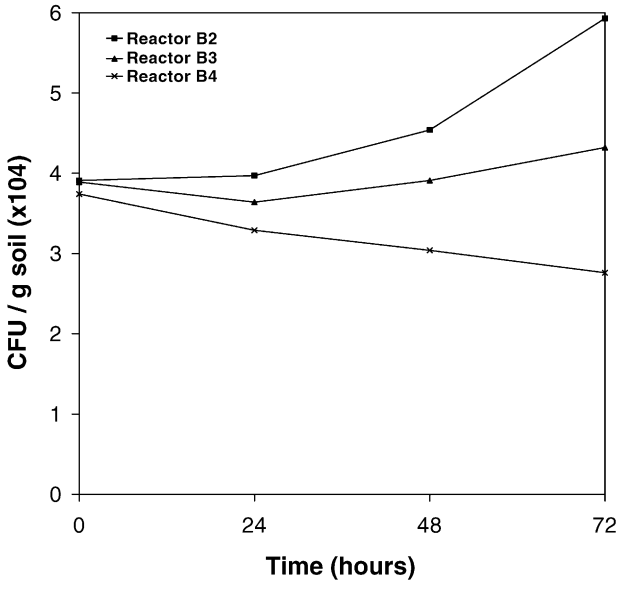

Fig. 11. Variation of CFU during reactor operation.

The growth of biomass in slurry reactor in terms of colony forming units was monitored during reactor operation (Fig. 11). The CFU values for all of the reactors increased by 2 orders of magnitude from time zero, indicating successful development of the microbial biomass in the soil slurry except in the case of 12000 μg/g of substrate concentration.

4. Conclusions

The study demonstrated successfully degradation of chlorpyrifos in bioslurry reactor operated with native microflora operated in sequencing batch mode (anoxic–aerobic–anoxic). Process monitoring indicted the performance of the slurry phase system is dependent on the substrate loading, HRT, oxygen consumption and microbial growth. Control reactor showed negligible removal of substrate due to the abiotic loss. Half rate of degradation calculated from the experimental data was found to be in hours rather than days in case of in situ bioremediation reported. Rapid degradation of the substrate in soil matrix was achieved in slurry phase system compared to the in situ decontaminate systems. Enforced short-term unsteady state conditions coupled with periodic exposure of the micro-organisms to defined process conditions in SBR showed efficient performance. Sorption experiments conducted on control reactor showed that the degradation of chlorpyrifos taking place in the slurry phase reactor was mostly associated on the soil surface or in the solid–liquid interface.

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