Biodegradation (2012) 23:673–682
DOI 10.1007/s10532-012-9543-z

ORIGINAL PAPER

Surfactant mediated enhanced biodegradation of hexachlorocyclohexane (HCH) isomers by *Sphingomonas* sp. NM05

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Received: 12 August 2011 / Accepted: 21 January 2012 / Published online: 3 February 2012

Abstract Environmental biodegradation of several chlorinated pesticides is limited by their low solubility and sorption to soil surfaces. To mitigate this problem we quantified the effect of three biosurfactant viz., rhamnolipid, sophorolipid and trehalose-containing lipid on the dissolution, bioavailability, and biodegradation of HCH-isomers in liquid culture and in contaminated soil. The effect of biosurfactants was evaluated through the critical micelle concentration (CMC) value as determined for each isomer. The surfactant increased the solubilization of HCH isomers by 3–9folds with rhamnolipid and sophorolipid being more effective and showing maximum solubilization of HCH isomers at 40 μg/mL, compared to trehalose-containing lipid showing peak solubilization at 60 μg/mL. The degradation of HCH isomers by *Sphingomonas* sp. NM05 in surfactant-amended liquid mineral salts medium showed 30% enhancement in 2 days as compared to degradation in 10 days in the absence of surfactant. HCH-spiked soil slurry incubated with surfactant also showed around 30–50% enhanced degradation of HCH which was comparable to the corresponding batch culture experiments. Among the three surfactants, sophorolipid offered highest solubilization and enhanced degradation of HCH isomers both in liquid medium and soil culture. The results of this study suggest the effectiveness of surfactants in improving HCH degradation by increased bioaccessibility.

Keywords Biosurfactants · Hexachlorocyclohexane (HCH) · *Sphingomonas* sp.

Introduction

Biosurfactants are a structurally diverse group of surface active molecules synthesized by microorganisms. These molecules reduce surface and interfacial tension in aqueous solutions containing hydrocarbon. This property of biosurfactant makes them potential candidates for enhancing solubilization and biodegradation of persistent environmental pollutants. In last two decades, enhanced biodegradation by microorganisms using biological and chemical surfactants for hydrophobic toxic pollutants has been extensively studied. It includes chemically synthesized

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ethoxylated alcohols, sulphonates, Triton, Brij 35 and sodium dodecyl sulphate (Lajoie et al. 1997; Fava and Di Gioia 1998; Billingsley et al. 1999), and enzymatically produced, e.g. cyclodextrins (Schwartz and Bar 1995; Bardi et al. 2000; Wang et al. 1998). Among others, the biosurfactants that can enhance hydrocarbon biodegradation include heteropolysaccharides, fatty acids, phospholipids, and glycolipids such as rhamnolipids and sophorolipids (Pacwa-Płociniczak et al. 2011).

Chlorinated solvents and pesticides are widespread contaminants of soil and groundwater due to their persistence and recalcitrance. These chemicals undergo biomagnifications and pose severe health hazards to humans and animals. Therefore, the development of potent clean up methodologies for these compounds from various environmental compartments such as sites of manufacture, application, storage, obsolete samples and accidental spillage sites is necessary. Biosurfactants have been previously reported to aid the degradation of pesticides by microorganisms. Biodegradation of endosulfan isomers in soil and flask culture conditions was studied using a biosurfactant, isolated from *Bacillus subtilis* MTCC1427. This has enhanced the rate of biodegradation by 30–45% under both the conditions (Awasthi et al. 1999). A biosurfactant produced by *Pseudomonas aeruginosa* WH-2 increased aqueous phase partitioning of different isomers of HCH-muck and showed higher emulsification of HCH and *n*-hexadecane (Sharma et al. 2009). In a recent report, pentachloro- and tetra-chloroethylenes were found to be solubilized up to 1,900 mg/L in two surfactants, and was degraded using nano bimetallic Fe–Ni particles in less than 75 h (Sivaram and Cumaraswamy 2011). In another study, rhamnolipid was found to potentially facilitate the biodegradation of diesel fuel co-contaminated with toxic phenol, 4-chlorophenol (4-CP) or 2,4-dichlorophenol (2,4-DCP). Interestingly, these findings also showed that the rhamnolipids reduces toxicity of 4-CP and 2,4-DCP towards the hydrocarbon-degrading cells (Chrzanowski et al. 2009). Effect of rhamnolipid biosurfactants on hydrocarbon degraders in presence of toxic chlorinated phenols (Chrzanowski et al. 2011) and its role in biodegradation of diesel/biodiesel was also investigated (Owsianiak et al. 2009). In another report, rhamnolipid ‘JBR 425’ could remove 99% of 30–100 mg of 2,4-DCP in a bioreactor concurrent with stimulation of the bacterial growth (Uysal and Türkman 2005). This property of rhamnolipids is of particular interest in using biosurfactants for microbial treatment of hydrocarbon-rich wastewaters co-contaminated with toxic compounds. The study of effect of rhamnolipid mixture and Triton X-100 on the extent of biodegradation of trifluralin, atrazine, and coumaphos in liquid culture demonstrated that while both the surfactants failed to influence the atrazine degradation, they enhanced the degradation of coumaphos (Mata-Sandoval et al. 2001). Urum et al. (2006) demonstrated improvement in crude oil elimination in presence of synthetic surfactant sodium dodecyl sulfate (SDS) and rhamnolipid compared to saponins. Also, sophorolipid showed a higher soil washing efficiency than nonionic surfactants except Tween 80 which has high hydrophilic-lipophilic balance (HLB) (Kang et al. 2010). Lai et al. (2009) studied the ability of two biosurfactants, rhamnolipid and surfactin, and two synthetic surfactants, Tween 80 and Triton X-100 in removal of total petroleum hydrocarbon (TPH) from soil. The study indicated that biosurfactants being more efficient in TPH removal as compared to synthetic surfactants. Franzetti et al. (2008) used an emulsan from *Gordonia* sp. to remediate hydrocarbon contaminated soils. In view of a need to develop a combination of biosurfactant and degrading microorganisms for bioremediation of persistent chlorinated toxicant such as HCH, this study reports the effect of three different biosurfactant on solubilization and biodegradation of four HCH isomers in liquid culture and in soil bound matrix.

Materials and methods

Chemicals

Hexachlorocyclohexane-isomers (HCH) (α, β, and δ) of analytical grade were purchased from Riedel-deHaën (Seelze, Germany). γ-HCH was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Chemicals for culture media were purchased from Difco™ (Sparks, MD, USA). Chemicals for buffer, media preparations were purchased from Qualigens and Hi-media chemicals (Mumbai, India). Solvents used were of highest purity and chromatographic grade from Merck and Qualigens fine chemicals (Mumbai, India).

Biosurfactants

The biosurfactants used in this study were: rhamnolipid-mixture “JBR 425” from *P. aeruginosa* purchased from Jeneil Biosurfactant Company (USA), the sophorolipid “Sopholiance” from *Candida bombicola* purchased from Soliance (France) and the trehalose tetraester from *Rhodococcus erythropoli*s B7g (Frank et al. 2010). The trehalose tetraester from *R. erythropoli*s B7g was isolated and purified at Institute of Biosciences of Technische Universitat Bergakademie Freiberg (Frister 2006). The rhamnolipid-mixture “JBR 425” is a 25% aqueous solution of mono- and di-rhamnolipid surfactants. All the aforementioned biosurfactants contain sugar unit (rhamnose, sophorose, and trehalose, respectively) connected to alkyl chains by ester or ether bonds.

Microorganism

A *Sphingomonas* sp. strain NM05 (MTCC8061, Microbial Type Culture collection and GenBank Chandigarh, India) characterized for its ability to utilize and degrade all the major isomers of HCH was used for the present study. The strain NM05 was isolated from a dumping site near a HCH manufacturing industry in Lucknow, India. This bacterium is a novel species belonging to the *Sphingomonas*, harboring *lin*ABCDE genes similar to those found in other HCH degraders (Manickam et al. 2008; Lal et al. 2010). However, so far we have not investigated its ability to produce any surfactant and resultant contribution to HCH degradation.

Solubilization of HCH isomers

Solubilization experiments for HCH isomers were carried out using three biosurfactant as described above. Based on the previous biodegradation experiment using strain NM05 (Manickam et al. 2008), the initial concentration of HCH isomers for these experiments was selected to exceed 10fold of its maximum solubility. Five different concentrations of the test surfactants were used ranging from 20 to 100 µg/mL. Aqueous solution of mineral salt medium [MSM (per L) KH_2PO_4, 170 mg; Na_2HPO_4, 980 mg; $(NH_4)_2SO_4$, 100 mg; $MgSO_4$, 4.87 mg; $FeSO_4$, 0.05 mg; $CaCO_3$, 0.20 mg; $ZnSO_4$, 0.08 mg; $CuSO_4 \cdot 5H_2O$, 0.016 mg; H_3BO_3, 0.006 mg; pH 6.8]; with 20, 40, 60, 80 and 100 µg/mL (200, 400, 600, 800 and 1,000 mg/L) of respective surfactant were prepared. In a screw cap culture vials 10 mL of each solution was placed on a gyratory incubator shaker (New Brunswick Scientific, Innova 4230, Edison, NJ, USA) run at constant temperature of 30°C at 240 rpm for 72 h.

Biodegradation of HCH isomers under liquid culture conditions

The MSM, having same composition as described for dissolution was used for the biodegradation of HCH isomers. In 500 mL Erlenmeyer flasks, 50 mL MSM containing HCH at the final concentration of 200 ppm or 0.68 mol L^{-1} (197.8 mg/mL) was added. With 40 µg/mL of each test surfactant NM05 was grown in presence of HCH isomers till mid log phase, harvested by centrifugation at 12,000 rpm, 5 min, 4°C, washed two times using MSM. The cells were resuspended in the MSM and added to the experimental flasks to give initial OD_{600} of 0.1. Two parameters such as the surfactant concentration and the initial inoculum size of strain NM05 were kept constant. Two controls were run in parallel, one having MSM, HCH and surfactant without any NM05 inoculum and the second one with all ingredients except surfactant. All the flasks were placed in a gyratory shaker at 240 rpm with constant temperature of 30°C throughout the experiment. The experiment was initiated by using inoculum of strain NM05.

Analysis of HCH isomers

All the samples for HCH quantification was analyzed in a gas chromatography (GC) equipped with electron capture Ni^{63} detector (Perkin-Elmer Model Clarus 500, Norwalk, CT, USA). Experimental samples were extracted twice with acetone: hexane (1:1), extracts were pooled and the solvents were evaporated at room temperature (25–28°C). The residues were redissolved in 1 mL of hexane (HPLC grade) and further diluted in volumetric flasks. The following conditions were used for GC analysis using Perkin-Elmer Clarus 500 Model having DB5 column with 30 m/250 µm diameter, oven temperature 170/250°C and, the mobile phase carrier flow of N_2 gas (IOLAR grade) 1 mL/min. The detector and injector temperature were 375 and 250°C, respectively. For solubilization of the HCH in presence of biosurfactants, the samples vials were centrifuged (Sigma 3K18) at 3,000 rpm for 10 min and filtered (0.45 µm Millipore, Milex HV) after 72 h. For the degradation analysis of HCH isomers in aqueous conditions, the flasks were harvested in triplicate after every 2 days interval for next 10 days and the samples were analyzed by GC for the extent of HCH degradation. For the estimation of residual HCH in soil slurry experiments, the reaction mixture was extracted with 100 mL acetone:hexane (1:1). This was repeated twice and the extracted solvents were pooled and passed through anhydrous sodium sulphate. A suitable aliquot was then analyzed by GC.

HCH Degradation in soil slurry

Soil samples were collected from the garden (CSIR-National Botanical Research Institute, Lucknow, India) and passed through a 1.0 mm wire mesh sieve to obtain a uniform particle size. The soil used was alluvium with sandy loam and had no history of chlorinated pesticide contamination. The soil characteristics were 7.0 ± 0.3 (pH), 8.13 ± 1.0% carbon, 1.00 ± 0.04% nitrogen, and 3.82 ± 0.5 cmdc/kg of cation exchange capacity. The soil was sterilized for further experimental work.

For the amendment of soils with HCH, 500 gm of soil was spiked with 20 mL of pesticide solution (100 mg HCH isomers) in acetone. The mixture was stirred vigorously to get homogeneous distribution of the pesticide in the soil. This was transferred in a wide rectangular pan and left under the fume hood (28°C) for 2 days to evaporate any traces of acetone. The final samples were stored in glass vials under refrigeration. Twenty-five gram of this amended soil was added to 50 mL mineral medium (soil/medium = 1:2 w/v) and slurry was incubated with strain NM05 for 30 days. For relative comparison with liquid culture experiments, 0.1 OD_{600} inoculum size of NM05 and 40 µg/mL of each rhamnolipid, sophorolipid and trehalose-containing lipid were used. Flasks containing medium, and HCH but no inoculum or no surfactant served as controls. All the experimental flasks and controls were incubated at 30°C on a gyratory shaker at 240 rpm. In order to quantify the degradation of HCH isomers, triplicate set of flasks were withdrawn and harvested for analysis after 30 days of incubation.

Statistical analysis

The aqueous phase extractions were done in triplicates for each surfactant concentration for HCH isomers. Similarly, soil slurry extractions were performed in triplicates for at 15 days interval over a period of 90 days. Solubility of HCH isomer in presence of the test surfactants and the residual HCH concentration as a measure of degradation were analyzed statistically with the help of prism insta version 3·0 (Graphpad, San Diego, CA, USA, http://www.graphpad.com) by using one-way analyses of variance (ANOVA) followed by Dunnett's Multiple Comparison Test ($p < 0.05$). A value of $p < 0.05$ was considered statistically significant.

Results

Effect of biosurfactants on solubility of HCH isomers

An enhanced solubility of all four HCH isomers at different concentration of biosurfactants rhanmnolipid, sopohorolipid and trehalose-containing lipid was observed (Table 1). HCH isomers at 200 ppm (0.68 µM), were several fold (α-HCH, 20; β-HCH, 40; γ-HCH, 11; and δ-HCH, 20folds) higher than the reported aqueous solubility (10, 5, 17, 10 ppm of α-, β-, γ-, and δ-HCH, respectively) (www.atsdr.cdc.gov). The solubilization capacity was calculated (Table 1) based on the data obtained from GC quantification of remaining HCH. The increase in the solubilization of HCH isomers in presence of surfactants peaked in the range of 40–60 µg/ml concentration of surfactants and the higher concentration range of 80–100 µg/ml of surfactant was actually less solubilizing. While rhamnolipid and sophorolipid showed maximum solubility of HCH at 40 µg/mL, the trehalose-containing lipid exhibited maximum solubility at 60 µg/mL. The critical micelle concentration (CMC) of all three test surfactants varied slightly for the each isomer. Among the three, sophorolipid appeared to provide higher solubility for α- and δ-HCH. However, for more persistent β-isomer, rhamnolipid could solubilize it two-fold more than the other two surfactants (Table 1). These solubility concentration values suggest ~3–9fold increase in solubilization of HCH isomers, particularly for the sophorolipid which showed 9folds increase in solubility for α-HCH which was 40.4 and 35.6% higher than rhamnolipid and trehalose-containing lipid, respectively (Table 1). The effect of biosurfactants on the solubility of HCH isomers was

Table 1 Solubilization of hexachlorocyclohexane-isomers by rhamnolipid, sophorolipid and trehalose tetraester

Surfactant	Concentration of surfactant in μg/ml	α-HCH (μg/ml)	β-HCH (μg/ml)	γ-HCH (μg/ml)	δ-HCH (μg/ml)
Rhamnolipid-JBR	Control[a]	38.6	13.4	54.9	20
	20	51	13.4	49	32
	40	53.7	30.5	76.8	32
	60	48.6	13.7	47.4	27.7
	80	40.1	17	45.8	24
	100	24.2	18.6	41	22.5
Sophorolipid	Control[a]	37	13.6	56	25
	20	81	17.6	58.4	49
	40	90	19.2	61.5	49.4
	60	76	16.1	59.8	48.7
	80	65	12.1	55.1	42.9
	100	63	12.2	33.7	41.4
Trehalose tertraester	Control[a]	40	13.2	52	22
	20	42	11.79	55	31
	40	44	11.46	58	29.7
	60	58.1	12.07	61	37
	80	56.5	11.75	56	33.95
	100	57.9	11.74	54	22.55

Initial concentration of HCH isomers were 200 μg/ml (0.68 μM)

[a] No surfactant

further statistically analyzed by comparing the control group (without surfactant) with test samples having biosurfactant. One-way analysis of variance result showed the p value $p < 0.0001$ for each concentration of biosurfactant for each isomer of HCH, which infers mean is significantly different.

Effect of biosurfactants on biodegradation of HCH isomers in liquid culture by strain NM05

Figure 1 shows the effect of surfactants on the biodegradation of HCH isomers by strain NM05 at the optimum CMC value of surfactant concentration (40 μg/mL), based on the solubilization experiments. After 10 days of incubation, around 60% degradation of all the HCH isomers was achieved in the absence of surfactant. However, in the presence of surfactant there was an enhanced (~95%) degradation for most of the HCH isomers (Fig. 1). The degradation rate of HCH isomers was slow, as expected for a substrate with low water solubility in the absence of biosurfactant, however, a 10–20fold increase was observed in the presence of biosurfactant. An increase in the growth of strain NM05 was observed following the addition of surfactants, indicating the higher substrate availability and utilization with respect to control setup which did not receive any surfactant. For all the isomers, nearly 70% degradation was achieved in just 2–4 days in the presence of surfactant, in contrast to only 60% biodegradation in 10 days in absence of surfactant. And more than 95% degradation of all the isomers was observed in 8–10 days.

Biodegradation of HCH isomers in presence of surfactants under soil conditions

Soil slurry spiked with HCH isomers and strain NM05 as inoculum showed enhanced degradation of HCH isomers in presence of test biosurfactants (Fig. 2). The rate of degradation estimated in the soil slurry was found to be slower than liquid cultures, as degradation was observed only after 30 days. In the absence of surfactant, HCH degradation by *Sphingomonas* sp. NM05 was comparable in all the flasks (Fig. 2. A2, B2, C2). After 30 days of incubation without surfactant, the residual HCH isomers were α-57–63, β-88–100, γ-28–34, and δ-44–52 μg/mL as analyzed by GC (Fig. 2). The degradation efficiency in the presence of strain NM05 was around 80–95% as compared to the controls where bacteria and surfactant

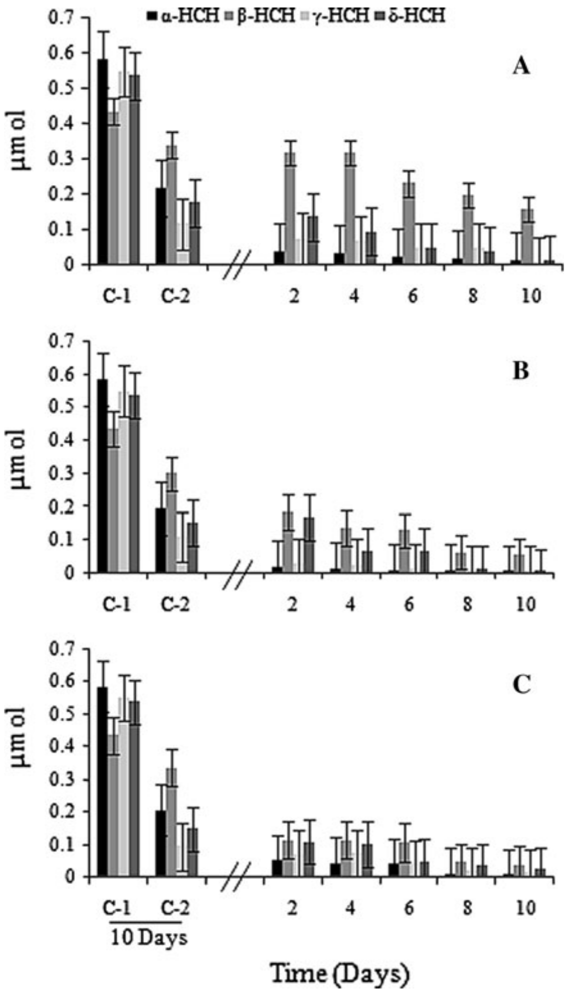

Fig. 1 Effect of surfactants on the biodegradation of hexachlorocyclohexane (HCH) isomers by *Sphingomonas* sp. NM05 **a** Rhamnolipid, **b** Sophorolipid, **c** Trehalose. The CMC of 40 µg/mL was used for all the surfactants. Both the controls without inoculum (C-1) and without surfactant (C-2) were extracted after 10 days of incubation. Each *bar* represents the HCH Each bar represents the concentration (µg/mL) of each isomer at the respective days of incubation with standard error

were not added. One-way ANOVA followed by Dunnett's Multiple Comparison test ($p < 0.05$) for the degradation of pesticide after treatment with biosurfactant over a time period of 90 days revealed significant difference in the mean for residual concentration of HCH isomers. However, the results from surfactant amended medium showed significant enhancement in degradation of HCH by all the three biosurfactant studied. Among these, rhamnolipid and sophorolipid showed 85–95% degradation for all the isomers. Though there was a variation among the percentage degradation by each biosurfactant for HCH isomers, exhibited better efficiency over other two surfactants. Figure 2 as also observed under liquid culture condition. Under test the conditions, the degradation efficiency of the biosurfactants on HCH isomers were in the order of sophorolipid > rhamnolipid > trehalose-containing lipid. The higher degradation rate may be attributed to enhanced accessibility of HCH isomers due to partitioning of molecules in the microenvironment or by enhanced bioavailability of the HCH isomers to the strain NM05 which could utilize it as a substrate for its growth.

Discussion

This study demonstrates the effect of three structurally different biosurfactants on HCH biodegradation. A rhamnolipid reported to exhibit relatively high surface activity (Mata-Sandoval et al. 2000, 2002; Zhang et al. 1997; Desai and Banat 1997), a sophorolipid with moderate activity (Gobbert et al. 1984; Hommel et al. 1987) and a trehalose-containing lipid with mild activity (Rapp et al. 1979; Sifour et al. 2007) were employed to study solubilization and consequent enhancement of biodegradation of a highly persistent chlorinated pesticide. The CMC was found to be in the range of 40–60 µg of biosurfactant rendering 3–9fold enhanced solubilization of HCH pesticide in liquid medium. The CMC values were derived based on previous studies on other hydrophobic compounds (Mata-Sandoval et al. 1999). It was observed that sopohorolipid dissolved 90 µg/mL of α-HCH which is nearly two times higher than rhamnolipid (53.7 µg/mL) and trehalose-containing lipid (58.1 µg/mL) surfactants. Similarly, rhamnolipid showed increased solubility for *β*- and *δ*-HCH unlike the other two surfactants. These results also suggest that each surfactant exhibited a specific affinity to HCH isomers, possibly due to partitioning of substrate for micelles formation. Despite the difference in solubilization capacity among the surfactants, ~3–9fold (Table 1) increase in dissolution of the HCH isomers was obtained for all the surfactants. However, it should be noted that no biosurfactant under investigation clearly showed higher solubility (Table 1) for all the HCH isomers. This may be due to their structural properties, aqueous phase thermodynamics and spatial arrangement of the chloride molecules in HCH isomers.

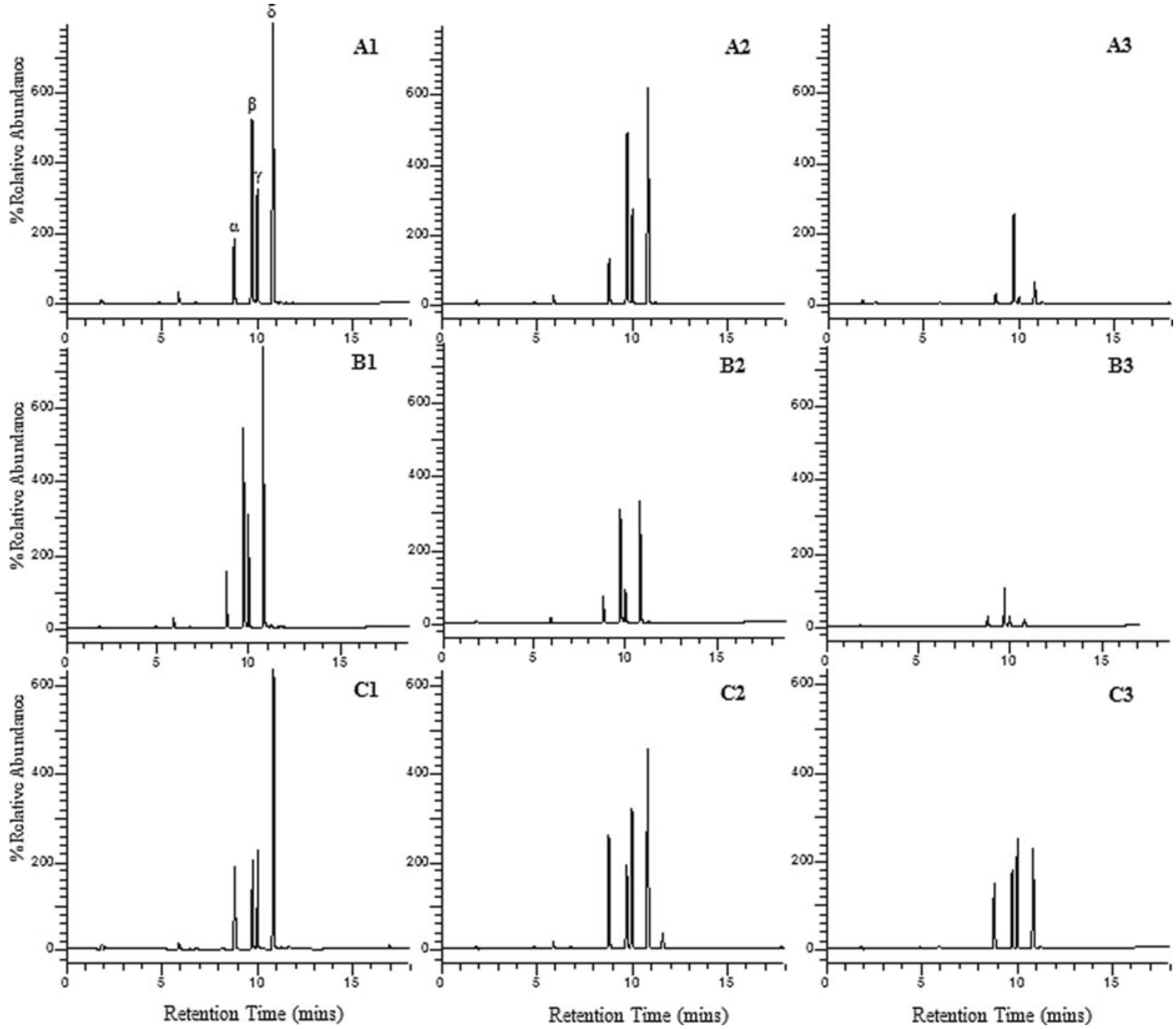

Fig. 2 Effect of surfactant on biodegradation of spiked HCH in soil in presence of 40 μg/mL surfactant. Initial HCH concentration was 200 μg/ml (0.68 μM) and biodegradation was initiated by adding 0.1 OD_{600} cells of *Sphingomonas* sp. NM05. All the experimental reaction was extracted with solvent mixture after 30 days and residual HCH was analyzed on GC. *A1*, *A2* and *A3* setup represent without inoculum, without surfactant and with inoculum and rhamnolipid surfactant, respectively. *B1*, *B2* and *B3* represent setup without inoculum, without surfactant and with inoculum and sophorolipid-surfactant, respectively. *C1*, *C2* and *C3* represent setup without inoculum, without surfactant and with inoculum and trehalose-surfactant, respectively

Increased solubilization of isomers, in presence of surfactant concurrently enhanced (30–50%) biodegradation by *Sphingomonas* sp. strain NM05. The rate of biodegradation also increased as the time reduces from 10 to 2 days for the same extent of degradation. However, it is important to note that this enhancement was achieved in 2 days of incubation in contrast to the time frame of 10 days in the absence of surfactant. The degradation of all the isomers showed enhancement, however, α-, *β*-and *δ*-HCH degradation had initial lag phase till 6 days in presence of rhamnolipid and sophorolipid, respectively. In contrast, a uniform degradation pattern by the strain NM05 was observed for all four isomers in presence of trehalose surfactant (Fig. 1).

These results suggest that among all the three surfactants used, sophorolipid displayed higher solubilization and degradation capability for all the HCH isomers. It is also noteworthy that during the batch culture studies using strain NM05, there was an increase of about 0.17 OD_{600} in total bacterial biomass when sophorolipid was added than either of the other surfactant. This response was due to the enhanced growth in presence of surfactant and consequently enhanced degradation in 8 days (Fig. 1). The presence of surfactant may either facilitate the bioavailability of the pesticide or there is a possibility of stimulating the metabolic machinery whereby both HCH and biosurfactant served as a substrate for the growth of the bacteria or it may affect the bioaccessibility by increasing dissolution of HCH (Semple et al. 2004). However, with the data obtained it is not possible to conclude as to which biosurfactant, under culture conditions, may be preferentially beneficial to the strain NM05 for its degradation capabilities. Though, recently HCH degradation has been extensively studied, from different parts of the world (Frister 2006; Thomas et al. 1996; Nagata et al. 1999; Kumari et al. 2002), the role of biosurfactant in its degradation kinetics has not been evaluated.

Enhanced biodegradation of HCH-spiked soil slurry incubated with surfactant is also attributable to the accessibility of the HCH and the efficiency of surfactant in the decreasing order of sophorolipid, rhamnolipid and trehalose-containing lipid. Three distinct observations were made after the addition of surfactant, (1) enhanced degradation of HCH isomers in equal proportion in trehalose biosurfactant amended culture medium, (2) in presence of rhamnolipid, 30% of residual β-isomer was detected indicating the inability of surfactant to affect its solubilization to the same extent of three isomers, (3). Presence of sopohorolipid exhibited preferential degradation of α- and γ- over β- and δ-isomers, however, it showed maximum enhancement in degradation of HCH isomers among all the three surfactants after 8–10 days of incubation. This pattern of degradation may be attributed to the differential solubilization properties of the individual surfactants toward HCH isomers in mineral medium. Interestingly, the biodegradation behavior by strain NM05 in presence of all of the surfactants was enhanced, indicating that the solubilization and availability (Table 1) of HCH isomers was significantly increased by the use of biosurfactant (Fig. 1). It is noteworthy that the calculated increase of about 30–40% in degradation of all the isomers in 2–4 days is significantly higher with any of the biosurfactant added. All these results suggest that sophorolipid was more efficient in solubilization of all the HCH-isomers except β-HCH. Thus, in presence of sophorolipid the strain NM05 could efficiently utilize HCH as sole source of carbon and energy.

Organochlorine pesticides are usually inaccessible to the microbes due to their hydrophobic nature. In aqueous phase, the accessibility of the pesticide is more as compared to the pesticide under soil conditions due to other organic and inorganic matters which may hinder with the micelle formation. Usually biosurfactants enhance the micelles formation; however, under soil conditions various intrinsic factors may affect the interaction of the biosurfactant, target pesticide and the microorganism. Considering the above factors, in the present study, efforts were made to use a soil which has low high humic contents or other organic matters that may affect surfactant and chemical interactions. Also to minimize such effects uniformly sieved and autoclaved soil was used for the degradation studies.

Chlorinated pesticides are poorly soluble in water, and hence their availability is highly limited to microorganisms. Particularly, aqueous solubility of HCH (10–20 mg/L), DDT (0.025 mg/L) and endosulfan (0.33 mg/L) are too less, thus making them more persistent and recalcitrant towards microbial assimilation. This has led to increase in their residues in polluted sites, especially in respect to HCH in countries like Germany, Spain, Poland, India and China (Frister 2006). With ageing, there is a decrease in bioaccessibility and bioavailability of toxic pollutants at the contaminated sites due to their adsorption in soil matrix, which can be overcome by application of biosurfactants. These surfactants desorb and dissolve hydrophobic compounds, like pesticides, alkanes or chloroaromatic compounds. To the best of our knowledge this report is perhaps the first attempt to explore enhanced degradation of HCH isomers in the presence of three biosurfactants to develop protocols for bioremediation of chlorinated toxic pollutants in the environment. In conclusion, the results obtained in this study reveal that biosurfactants enhances HCH biodegradation using *Sphingomonas* sp. NM05 possibly by desorption and dissolution of the contaminant.

Acknowledgments We thank Dr. Stefan Kaschabek, Department of Environmental Microbiology, Technical University-Bergakademie Freiberg, Germany, for his generous gift of biosurfactants. Dr. Deepak Agarwal, Former Scientist at Indian Institute of Toxicology Research, Lucknow, India is appreciated for his valuable suggestions and critical reading of the manuscript. This manuscript carries IITR communication number 2956.

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