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Fouling and Degradation of Polycarbonate in Seawater: Field and Lab Studies

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Abstract Biofouling and ensuing microbial mediated degradation of Bisphenol A polycarbonate was studied by immersing the samples in sea water of Bay of Bengal (Chennai, India) for 3 months and also under controlled laboratory conditions with marine mixed microbial consortia for 12 months. A 9% weight loss in the sample was observed after 1 year of incubation under in vitro laboratory conditions. A 5% reduction in number average molecular weight and an additional oligomer with a molecular weight of 930 was observed in the same sample. Contact angle decreased by 11% indicating an increase in the surface hydrophilicity. The specific heat decreased by 44% and glass transition temperature decreased by 3 °C with respect to the control indicating chain scission. Formation of new hydroxyl groups and cleavage of carbonate bonds in polycarbonate suggested biodegradation. About 9 $\mu g\ mL^{-1}$ of Bisphenol A, a monomer of polycarbonate, as well as its oxidized products were detected in the supernatant. The nature of degradation in field and in vitro was different. It was predominantly oxidation in the former and hydrolysis in the later environment. A strain exhibiting hydrolase activity was isolated at the end of the 12 months from the in vitro mixed consortia and was identified, based on biochemical and 16S rDNA tests, as *Pseudomonas* sp. BP2 (GenBank accession no. EU920674).

Keywords *Pseudomonas* sp. BP2 · Biodegradation · GPC · FTIR · Polycarbonate · Field studies

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Introduction

Poly (bisphenol-A-carbonate) (PC) finds extensive applications, ranging from windshields on boats to biomonitoring units in water analysis due to its durability, optical clarity, toughness and its ability to be blended with other polymers. About 2.7 million tons of PC is produced annually [1]. The steep rise in its production in the recent past and its durability has lead to increasing problems of littering and challenges in waste management. When this polymer is exposed to seawater there is a possibility of Bisphenol A (BPA), one of the monomers, leaching out [2]. BPA has been classified as an endocrine disruptor due to its estrogenic action and has an acute toxicity in the range of 1–10 $\mu g\ mL^{-1}$ for freshwater and marine species [3]. Hence it is essential to establish the stability and durability of this polymer in the environment through systematic studies.

In the past decades, a great deal of attention has been focused on evaluating the various methods for the biodegradation of polymers, ranging from immersing them in seawater to evaluating them in the controlled laboratory conditions [4]. The field test represents the ideal environmental conditions to study the fate of the polymer in real time, but it carries its own disadvantages, such as the environmental parameters are not controllable and the contribution by abiotic and biotic factors towards degradation cannot be exactly established. On the other hand, controlled laboratory conditions overcome several of these problems.

Polycarbonate has been used as a substrate for biofilm growth in estuarine water [5], in lakes [6, 7] and in drinking water distribution system, but these authors do not report the fate of the polymer. To date there is very limited research on biodegradation of Bisphenol A polycarbonates.

Sivalingam and Madras studied kinetic aspects of enzyme mediated degradation of polycarbonate [8] while Artham and Doble [9] suggested various strategies to degrade it [9]. Goel et al. [10] based on UV–Visible spectroscopic studies has concluded that *Arthrobacter* species was able to utilize polycarbonate.

The objectives of the present study are to access the stability and biodegradability of PC under mesophilic conditions by characterizing the biofilm, isolate and identify potential microorganism which may use this polymer as sole carbon source, understand the mechanism of degradation and verify the release of BPA under controlled laboratory conditions.

Materials and Methods

Materials

Poly (bisphenol-A-carbonate), a Lexan® grade resin, with weight average molecular weight of 57,800 was a gift from GE plastics, India. A 3% solution of the polycarbonate resin in chloroform (HPLC grade, Merck Limited, Mumbai, India) was prepared and films of 0.125 mm thickness were cast by pouring the solution on a glass plate and allowing the solvent to evaporate. The cast films were then dried overnight in a vacuum oven at 50 °C. The resulting films were cut into 150 × 20 mm strips for field study and 40 × 20 mm for in vitro study.

Field Studies

Sampling Site

The polycarbonate samples were vertically immersed in the ocean waters of Bay of Bengal at a depth of 3 m near Fisheries survey of India (FSI), Chennai, India (Lat: 13° 7′ 34″ N, Lon: 80° 17′ 49″ E) using fiber reinforced racks from August to October 2006 (3 months). The racks were fixed to plastic buoy which was suspended in the sea. A set of three polymer samples were removed every month from the sea and were analyzed.

Biofilm Characterization

These films were transported to the lab in sterile sample bottles containing filtered (0.2 µm, Millipore®) and autoclaved (120 °C for 20 min at a pressure of 15 psi) sea water. The biofilm formed was scraped from the polymer surface in the laboratory using a nylon brush as suggested by Sharma et al. [11] and was dispersed in 125 mL of sterile sea water. Parameters such as total suspended solids, organic carbon and chlorophyll *a* were estimated as a function of time to assess the development of biofilm on the polymer surface. It was observed by other researchers that these parameters play a key role in the biofouling [12, 13].

Total suspended solids were measured gravimetrically as suggested by Parsons et al. [14]. The procedure consisted of filtering a 20 mL sample through a pre-ignited (400 °C, 4 h) and pre-weighed Whatman® GF/C filter paper, then dried at 100 °C for 1 h and reweighed to get the dry weight of the residual biomass. Samples for photosynthetic pigment chlorophyll *a*, which represents the algal growth, were stored at −20 °C until analysis. Around 15 mL of water sample containing scraped biofilm suspension was filtered through a 0.22 µm Millipore® filter paper and the filtrate was dissolved in 90% acetone for extracting the chlorophyll *a* [13]. The samples were incubated for 12 h at 4 °C in the dark after which the absorbance at 630, 645, 665 and 700 nm was measured using a UV spectrophotometer (Perkin Elmer, Lambda 35).

Around 0.1 mL of sea water sample consisting of scraped biofilm suspension was used to estimate total protein and carbohydrates in the biofilm. The former was estimated according to Bradford's method while phenol–sulfuric acid method was employed to estimate the later.

After removing the soft biofilm the polymer was cleaned with 10% hydrochloric acid to remove the macrofoulants and then it is dried in a hot air oven at 40 °C for 4 h. This film was further characterized using several techniques.

In Vitro Stability Studies of the Polymer

The biofilm scarped into the sterile seawater was vortexed for 10 min to obtain cell suspensions of mixed consortia. An aliquot of 1 mL was drawn from this suspension and was inoculated into a 250 mL erlenmeyer flask containing 100 mL of sterile sea water and polycarbonate films in duplicate. Ammonium nitrate (1 g L^{-1}) was added as the inorganic nitrogen source while polycarbonate served as the sole carbon source. The flasks were then incubated at 30 °C at 200 rpm on an orbital shaker (Scigenics Pvt. Ltd., India) for 12 months. The loss in water due to evaporation during the course of the study was compensated by adding fresh seawater so as to make up the volume to 100 mL.

Bacterial Viability Test

An epifluorescence staining method using LIVE/DEAD® bacterial viability kit (*Bac*Light™, Invitrogen, Germany) was used to qualitatively assess the viable growth on the polycarbonate samples. The kit contains two nucleic acid-binding stains namely, SYTO 9™ and propidium iodide. These stains differ in their ability to penetrate through healthy bacterial cells. The former stains all the cells green,

while propidium iodide penetrates through damaged cells membranes only. Hence live cells fluoresce green while dead cells appear red [15].

After 12 months of incubation, the samples were removed aseptically and washed with sterile water to remove loosely attached bacteria. The films were air dried and were stained with *Bac*Light^{TM}, incubated for 10–15 min in the dark and then the images were captured using a fluorescence microscope (Leica DM5000, Germany) with a blue filter at an excitation of 475 nm.

Total Protein and Carbohydrates

After removing the films from the media, the total protein concentration in the supernatant was determined according to the method reported by Bradford [16]. Bovine albumin (Himedia Limited, Mumbai, India) solutions were used as standards. The absorbance was evaluated spectrophotometrically (JASCO 550, Japan) at 595 nm. The total carbohydrates were analyzed according to the method suggested by Dubois et al. [17]. Glucose was used as the standard (Himedia Limited, Mumbai, India) and the absorbance was measured at 495 nm. All the experiments were done in triplicate and the mean and standard deviation are reported here. Uninoculated flasks with only the polycarbonate films served as the positive control and, flasks with the mixed inocula but without the polymer films served as the negative control.

Isolation and Characterization of PC Adaptive Bacteria

The polycarbonate film from the 12 month lab study was removed from the medium, and gently washed with sterile seawater to remove loosely attached bacteria. It was placed in 10 mL of sterile seawater and vortexed for 1 min to obtain cell suspensions. These suspensions were serially diluted and plated on Zobell's marine agar (Himedia Laboratories Pvt. Ltd., India). Plates were then incubated at 30 °C for 2 days and were successively restreaked for purification. The highest abundant strain (BP2) with hydrolase activity, based on *p*-nitro phenyl palmitate assay, was isolated and standard biochemical tests such as gram staining; catalase, oxidase, sulphide and indole production; citarte utilization; nitrate reduction; lipid and starch hydrolysis and MRVP were performed on it [18]. Working cultures were maintained on marine agar slants at 4 °C.

The 16S rDNA sequencing of the strain was carried out through an external agency (Genie Sanmar Groups, Bangalore, India). The nucleotide sequence of the 16S rDNA gene (1499 nucleotides) was searched for pair wise alignment using BLASTN program version 2.2.18+ [19]. This sequence is deposited with GenBank under accession no. EU920674. Reference sequences were obtained from GenBank database [20]. Phylogenetic inference was obtained by using neighbor joining method [21].

The metabolites of degradation such as Bisphenol A were analyzed using HPLC and GC-MS and the details of the protocols are discussed in more detail elsewhere.

Polymer Characterization

The changes in the chemical and surface properties of the polymer were determined based on the following methods.

Surface Wetting Properties

Contact angle is an indication of the hydrophobicity of the surface, and higher is its value higher is the hydrophobicity. It was measured using the sessile drop method with an Easy Drop Contact Angle Measuring System (Kruss, Germany). The polymer film was supported on a glass slide and a drop of Millipore® grade distilled water was placed on it using a syringe. The image of the drop was captured by a camera and was processed by DSA2 software which calculated both the left and right contact angles the drop made with the surface to an accuracy of ±0.1°. Contact angle was measured on five different locations on the polymer and the average values were reported here.

Surface Changes

Surface morphology of the polymer was investigated with a Scanning electron microscope (SEM) (FEI Quanta 200). A 10 × 10 mm piece was cut from the polymer sample and placed on the sample holder and was scanned within an area of 100 μm^2 at a magnification of 500×. The surface topography and roughness of the polymer films were determined with an Atomic force microscope (AFM) (Nanoscope III AFM microscope provided with an ADCS controller). The sample was mounted on a piezoelectric scanner and the surface was scanned with a silicon nitride tip in contact mode within an area of 10 μm^2. The surface roughness was calculated in terms of root mean square roughness (rms).

Thermal Analysis—Differential Scanning Calorimetry

The glass transition temperature (T_g) and the change in heat capacity (ΔCp) of the polymer were estimated with a NETZCH Phoenix DSC-7 differential scanning calorimeter, calibrated with indium standard. About 5–7 mg of sample was used in the study. The scanning was performed over a temperature range of 50–300 °C at a heating rate of 10 °C min^{-1} under nitrogen atmosphere.

FTIR Analysis

A Jasco N4200 (Japan) fourier transform infrared spectrophotometer(FTIR), was used for detecting the formation of new functional groups or changes in the amount of existing functional groups. The spectra were recorded at a resolution of 4 cm^{-1} in the frequency range of 4,000–400 cm^{-1}, calibrated with polystyrene standards. The analysis was performed using Horizontal Attenuated Total Reflectance (HATR) mode by accumulating 32 scans. The readings were taken in triplicates.

^{1}H and ^{13}C NMR Analysis of the Polymer

^{1}H and ^{13}C NMR spectra of the polymer were measured at room temperature with a Brucker Avance III spectrometer (Massachusetts, USA) operating at 500 MHz, using $CDCl_3$ as the solvent and tetramethylsilane as an internal standard.

Gel Permeation Chromatography

A GPC (model Shimadzu 20A, Japan) was used to measure the molecular weight distribution of the polycarbonate films. The films were dissolved in HPLC grade tetrahydrofuran (Merck Pvt. Ltd, India) at a concentration of 1 mg mL^{-1}. A Styragel guard column (4.6 mm × 30 mm) and two PLgel columns (PL mixed B and PL 10E4 with effective molecular weight ranges of 200–2,000,000, and 400–4,00,000 g mol^{-1}, respectively, 300 × 7.6 mm, Polymer Laboratories, UK) were used for the analysis. The samples were run in the GPC with the following settings: injection volume of 20.0 μL, flow rate of 1 mL min^{-1}, and a temperature of 35 °C. The GPC was calibrated using narrow molecular weight distribution polystyrene standards (Easical, Polymer Laboratories, UK). All molecular measurements were measured relative to these standards. The UV variable wavelength detector at 254 nm (model SPD 20A, Japan) was used as the aromatic groups in polycarbonate absorb strongly near this wavelength and the refractive index (RI) detector (model RID10A) was also used for the measurements.

CHN Analysis of In Vitro Samples

The dried biomass and the control and in vitro polymer samples were subjected to elemental analysis using a Perkin Elmer 2400 Series II CHN analyzer (USA) for estimating the carbon content. Based on the results from elemental analysis a mass-balance for carbon during the degradation process is estimated.

Results and Discussion

Field Studies—Fouling and Polymer Stability

Figure 1 shows the picture of the polycarbonate samples retrieved from the field after one and 3 months. The films were torn and were in a dilapidated state due to ocean currents and fouling and hence it was difficult to measure the weight loss. The average temperature, pH and salinity of the sea water during the 3 months of field study at FSI were, 29 ± 1.5 °C, 8 ± 0.2 and 32 ± 1.5 (ppt), respectively. The biofilm settlement, in terms of total suspended solids (TSS), total protein, total carbohydrates and chlorophyll *a,* are listed in Table 1. The TSS are highest in the month of August 2006, probably due to the termination of southwest monsoon and commencement of north east winds leading to changes in ocean currents in Bay of Bengal in this region, which helps in the deposition of foulers onto the polymer surface. Similar findings were reported earlier [22]. The total protein and

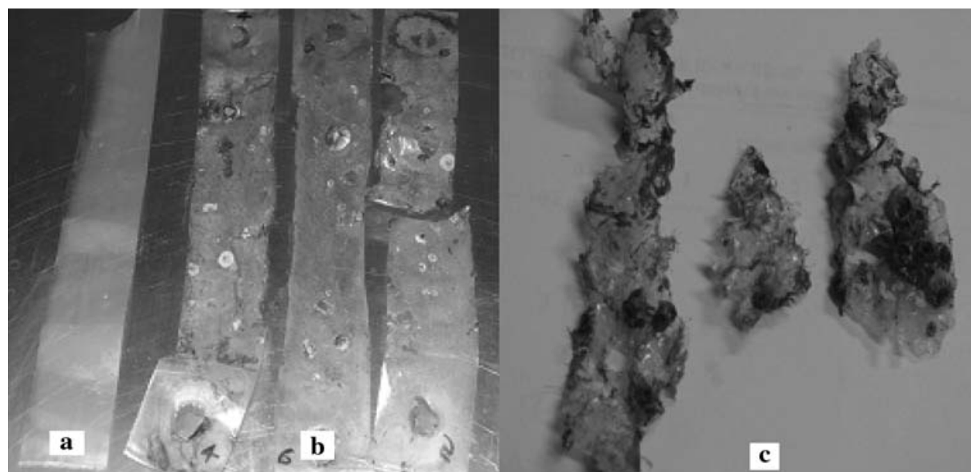

Fig. 1 Polycarbonate films **a** control sample, **b** after the slime was scraped from 1 month sample and **c** removed from sea water after 3 months

Table 1 Analysis of the biofilm on the polycarbonate samples for 3 months at FSI

Samples removed in the month of	Biofilm solids (TSS) (mg cm^{-2}) × 10^{-2}	Total protein (mg cm^{-2})	Total carbohydrates (mg cm^{-2})	Chlorophyll *a* (μg cm^{-2})
August	0.62 ± 0.11	0.080 ± 0.028	0.19 ± 0.10	0.57 ± 0.11
September	0.37 ± 0.22	0.092 ± 0.066	0.52 ± 0.25	0.52 ± 0.20
October	0.21 ± 0.16	0.033 ± 0.005	0.20 ± 0.06	0.06 ± 0.03

carbohydrates are highest in the samples removed in the month of September 2006 when compared to other months. It is well known that the mature biofilm, which consists of both micro and macrofoulers, takes about few weeks to develop. Compere et al. [23] suggested that the proteins are the first compounds to be adsorbed on the substratum followed by polysaccharides, which is in line with the current observations. Microbes in the ocean do not adhere to substrate directly as they have to overcome the repulsive charges on the surface. Hence they secrete exopolymeric substances rich in carbohydrates. These can also be a source of energy for the microorganisms. Biofilms formed on the surface of the polymers are complex communities in which phototrophs and heterotrophs are embedded in a highly hydrated matrix of extracellular polymeric substances (EPS) [24]. Apart from carbohydrates the microbes also excrete extracellular enzymes for their metabolism. These enzymes present in biofilm act on the polymer surface leading to its degradation. SEM photographs of field samples (Fig. 2) indicate surface deterioration and pitting while the surface roughness (Table 2) increased from 14 nm for the control to 23 nm for the field samples (3 months exposure in sea). It is reported that generally polymer biodegradation proceeds via surface attack by the bacteria [25–27] present in the biofilm. It is also observed that the disastrous effects of biofilms on polymers vary from discoloration to complete degradation

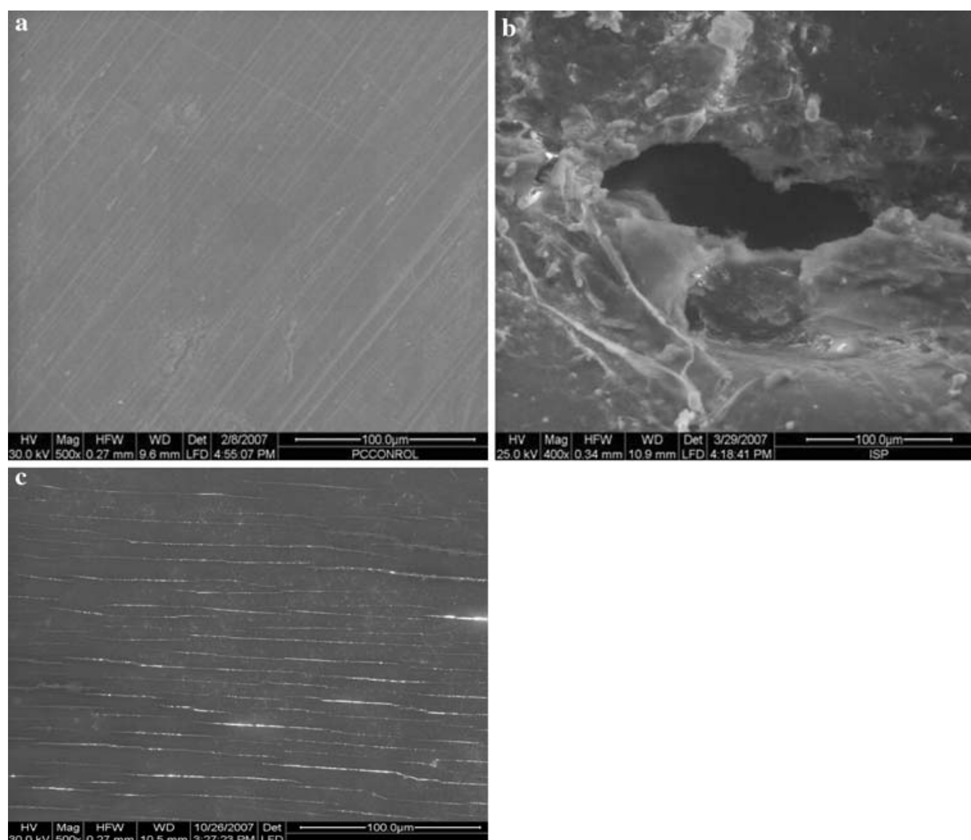

Fig. 2 Scanning electron micrograph pictures of the polymer samples **a** control, **b** removed after 3 months from sea (FSI) and **c** after 12 months in vitro studies

Table 2 Physiochemical analysis of the polycarbonate samples after 3 months at FSI (PCFS) and 12 months in vitro studies (PCIV)

Sample code	DSC		GPC			Contact angle	Surface roughness
	T_g (°C)	ΔC_p J $(g \times K)^{-1}$	M_w (g mol^{-1})	M_n (g mol^{-1})	PD		Rms (nm)
Control	149.2	0.212	57,693	37,087	1.55	78.48 ± 1.24°	14
PCFS	146.2	0.174	55,139	34,465	1.59	71.30 ± 0.92°	23
PCIV	148.6	0.118	57,528	35,092	1.60	70.51 ± 1.85°	20

[28–30]. Although similar long term studies with polycarbonate in other oceans are not reported to compare the present study, bacterial adhesion studies on PC for shorter duration (4 days) are reported in estuarine sea water [5]. The authors observed that biofilm community changes with time irrespective of the surface they are growing on.

In Vitro Studies—Fouling and Polymer Stability

In vitro studies were performed with the microbial consortium that was isolated from the scraped biofilm from field studies. The surface roughness of the in vitro sample (as determined by AFM) which was exposed for 12 months to microbial consortia, in sea water under controlled laboratory conditions increased from 14 nm to 20 nm. The biofilm on the PC (Fig. 3), removed after 12 months of incubation in the lab was visually observed using bacterial viability kit (See "Bacterial Viability Test"). The presence of green and red fluorescence indicates the presence of live and viable bacteria and non-viable bacteria, respectively on the polymer film. These results suggest that the bacteria are viable and able to survive for 12 months with polycarbonate as the sole carbon source. About 1.53 ± 0.15 mg mL^{-1} of protein was found in the supernatant which was much higher than that of the negative control (0.19 ± 0.02 mg mL^{-1}) indicating a higher biomass in the flask with polymer. The hydrolase activity was 0.25 U mL^{-1} whereas, no activity was detected for the control without polycarbonate (negative control).

The biodegradation of polymers normally refers to an attack by microorganisms and the process is heterogeneous. Microorganisms are unable to transport the polymeric material directly into the cells due to lack of its solubility in water and its size. The microorganisms excrete extracellular enzymes which depolmerize the polymers outside the cells [27]. These enzymes constitute the total protein in the supernatant, and it can be hypothesized that due to lack of readily available carbon source, the viable bacteria for its survival, has excreted higher amount of enzymes for degrading the polymer when compared to control with no polymer.

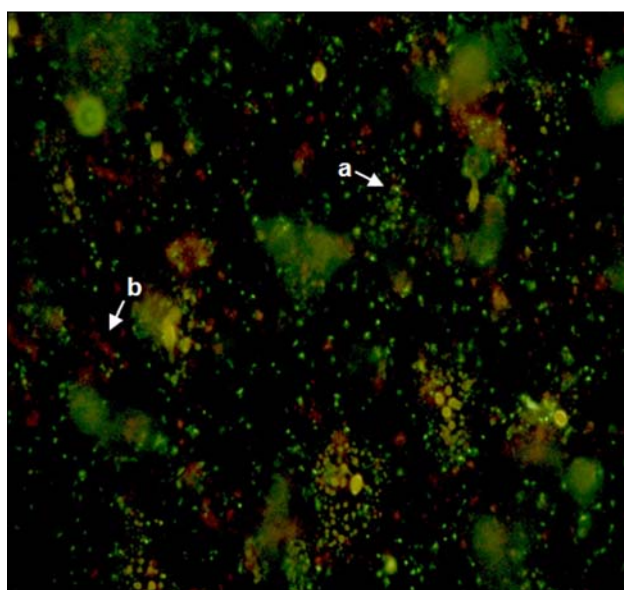

Fig. 3 *BacLight*® picture of biofilm on polycarbonate surface after 12 months of in vitro study. **a** Green fluorescence—live cells; **b** Red fluorescence—dead cells (color figure online)

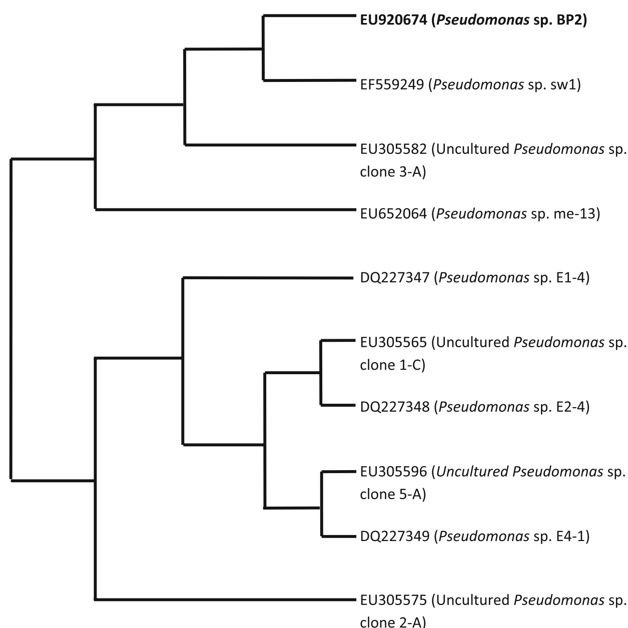

Fig. 4 Phylogenetic tree of the isolated strain *Pseudomonas* sp. BP2 (neighbor joining method)

Isolation of PC Adaptive Microorganisms

The most abundant strain (BP2), isolated after 12 months of lab studies, was identified by standard biochemical tests and by 16S rDNA analysis. The analysis reveals that this strain, named as *Pseudomonas* sp. BP2 (GenBank accession no. EU920674) has a 99% homology with *Pseudomonas* sp. sw1 (GenBank accession no. EF559249) (Fig. 4). The later strain is a sulfonylurea herbicide degrading bacterium. *Pseudomonas* sp. BP2 formed light yellow colonies and is a gram-negative, rod shaped aerobic bacterium. It also showed to be catalase, oxidase and citrate positive and has hydrolyzed lipid and starch indicating the presence of hydrolases such as lipases and cellulases, respectively.

Analysis of Degraded Polycarbonate

The physiochemical analyses of the polymer sample removed after 3 months from the sea and after 12 months of the in vitro study are presented in the Table 2.

DSC thermograms (Fig. 5) for field and laboratory samples clearly indicate a decrease in glass transition temperature (measured at its inflection point) which could be due to chain scission in the polymer leading to an increase in its mobility [31]. A considerable decrease in the

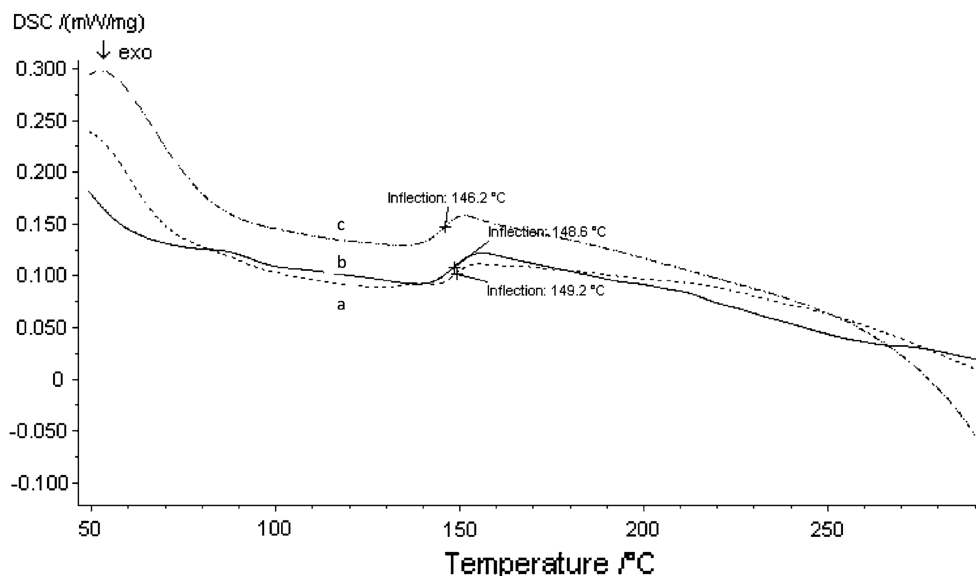

Fig. 5 DSC thermograms of the polycarbonate samples (*a*) control, (*b*) from field after 3 months (*c*) and from in vitro study after 12 months

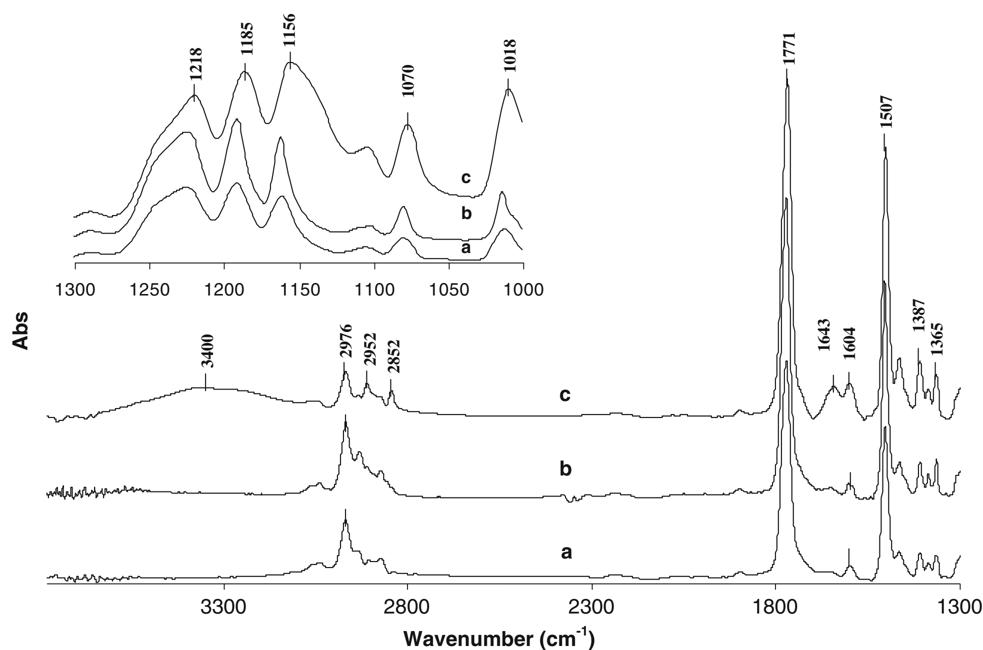

Fig. 6 FTIR spectra of (*a*) pristine polycarbonate and (*b*) polycarbonate exposed to seawater for 3 months (in field) and (*c*) for 12 months (controlled laboratory conditions), inset: expanded region from 1,000 to 1,300 cm^{-1}

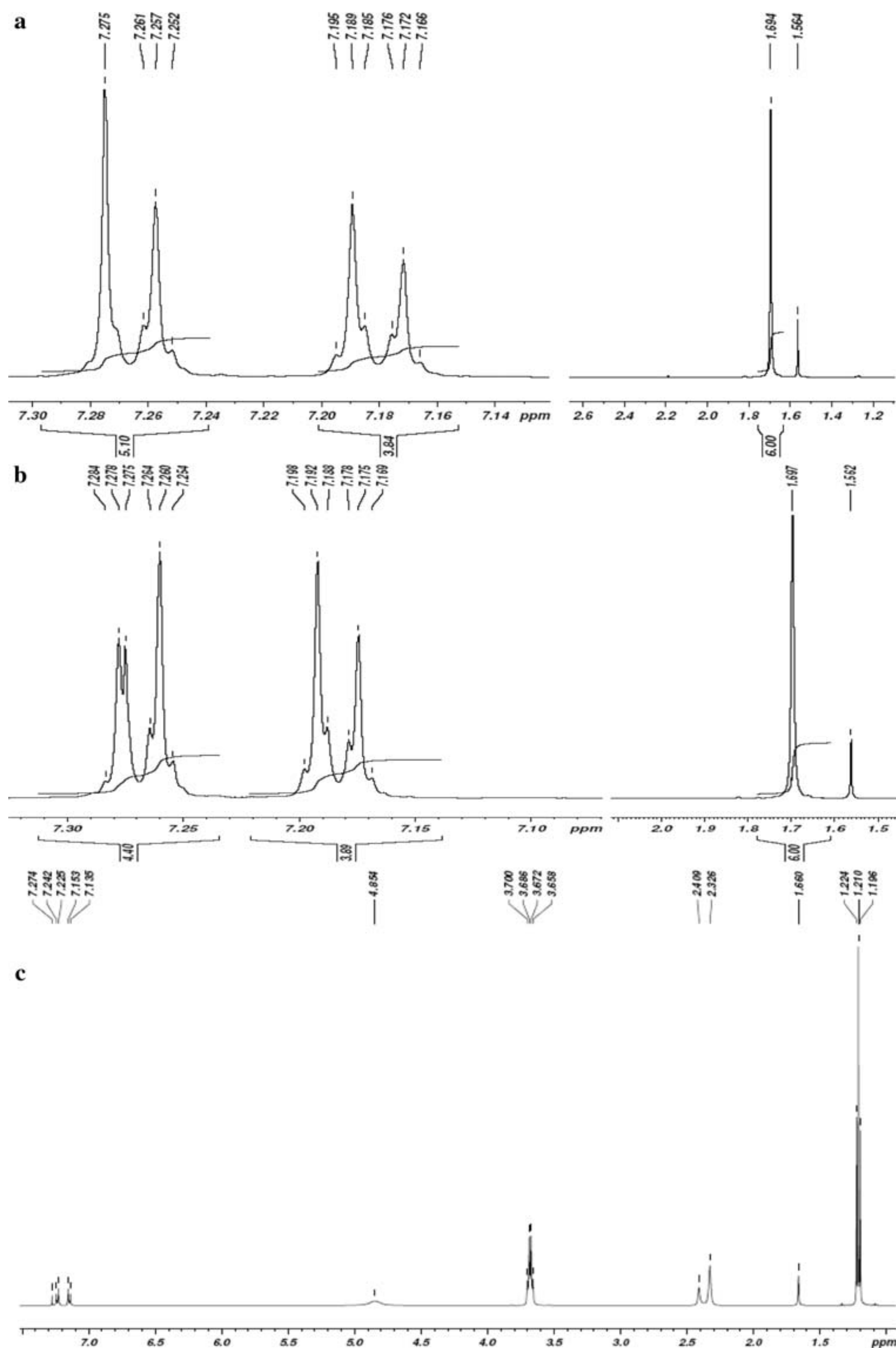

Fig. 7 ^{1}H NMR spectra (expanded) of the polycarbonate samples **a** control, **b** from field after 3 months **c** and from in vitro study after 12 months

heat capacities is also observed in the treated sample when compared to control. When there is a chain scission in the polymer molecule due to degradation, the heat capacity of the polymer decreases.

Figure 6 shows the FTIR spectra of polycarbonate exposed to seawater for 3 months (in field) and 12 months (in laboratory conditions) along with the control sample. The pristine polycarbonate has major vibrational frequencies at 2,800–3,200 cm^{-1} corresponding to CH stretching. Absorption peaks at 1,771 and 1,507 cm^{-1} correspond to carbonate carbonyl and aromatic C=C stretching vibrations, respectively. The CH symmetrical deformation vibration of the methyl in isopropyl group results in two absorption bands located at 1,365 and 1,387 cm^{-1} [32]. An

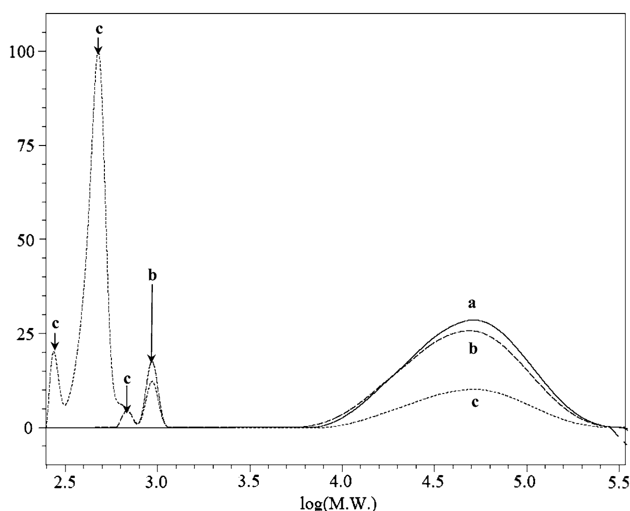

Fig. 8 Gel permeation chromatogram of the polycarbonate samples (*a*) control, (*b*) from field after 3 months (*c*) and from in vitro study after 12 months. Arrows indicate the formation of smaller molecules

intense triplet observed in the range of 1,220–1,150 cm^{-1} corresponds to the C–O stretching frequencies of the carbonate linkage, and the peak at 1,174 cm^{-1} is the carbon-hydroxy stretching band. It was observed that the sp3 C–H peaks for the field samples were more prominent than that of the control implying formation of substituted alkyl phenols during the degradation of polycarbonate [33]. This was also conformed from the increase in intensity of 1,365 cm^{-1} peak for the field and in vitro samples [34]. A broad peak at 3,400 cm^{-1} was observed in the case of in vitro sample due to the formation of hydroxyl groups suggesting the hydrolysis of carbonate bonds. The peak at 1,643 cm^{-1} corresponds to an aliphatic C=C stretching formed during the C–C bond cleavage from isopropyl group. These results suggest that even though the polycarbonate undergoes degradation when exposed to seawater, the type of degradation were different for field and under laboratory conditions.

Manzur et al. [35] observed that during microbial degradation of polyethylene an increase in relative crystallinity was observed at the expense of loss of amorphous region in the polymer. Polycarbonate being predominantly amorphous in nature the crystalline content was not detected during the thermal analysis (DSC), while an increase in band intensities at 1,156, 1,185 and 1,218 cm^{-1} corresponding to the crystalline content [36] was observed in the FTIR spectra (Fig. 6 inset) of both the field and in vitro samples suggesting degradation of the amorphous region.

The proton NMR spectra (Fig. 7) of the control polycarbonate sample displayed an aromatic A_2B_2 pattern at 7.16 and 7.24 ppm, and absorption for the gem-dimethyl at 1.68 ppm, relative to tetramethylsilane $(CH_3)_4Si$. An unresolved multiplet from protons corresponding to aromatic carbon was observed in the field sample (Fig. 7b). A new peak in ^{13}C spectra (supporting information) resulting from the methylene carbon next to the aromatic group (sp^2 carbon; about 128 ppm) in the degraded Bisphenol A polycarbonate was observed indicating the possible cleavage of C–C bond.

A change in molecular weight distribution is a key parameter in evaluating degradation of the polymer. The number average molecular weight of field and in vitro samples reduced by 5%, and a metabolite peak with a molecular weight of 934, probably of an oligomer (Fig. 8) was observed (Supporting information). Decrease in number average molecular weight (M_n) of the treated samples also indicates degradation. About 9 µg mL^{-1} Bisphenol A, which is one of the monomers for polycarbonate as well as its oxidized products namely 4-Hydroxyacetophenone, 4-Hydroxybenzaldehyde and 4-Hydroxybenzoic acid were detected (Supporting information). These metabolites were

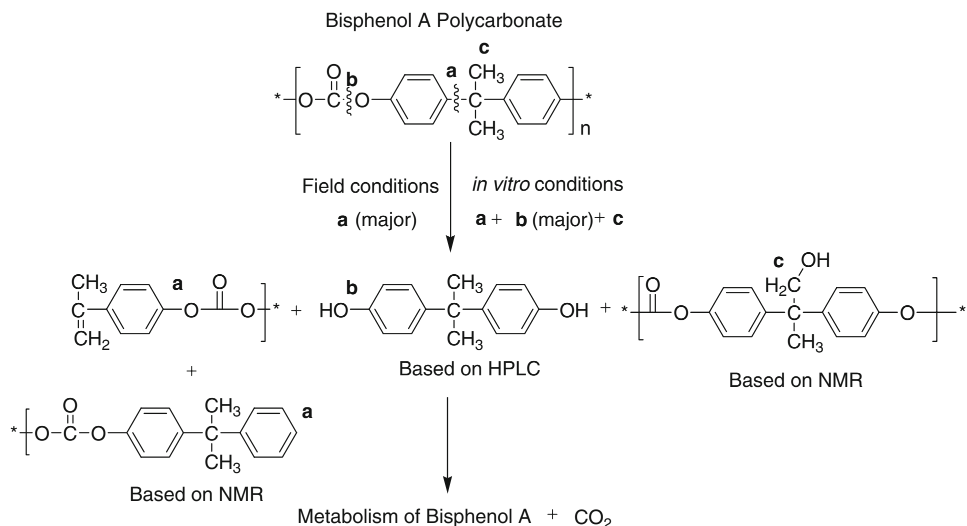

Fig. 9 Probable mechanism of Polycarbonate degradation in field and in laboratory conditions

Table 3 Elemental analysis of polycarbonate from the 12 months in vitro samples and the biomass obtained during the degradation

Element	PC ref % (w/w)	PC-IV-12 M % (w/w)	Biomass % (w/w)	Negative control % (w/w)
C	75.88	70.86	10.92	1.19
H	4.97	4.51	3.73	0.97
N	0.06	0.09	0.65	0.50

detected from the supernatant of the 12-month culture medium. The small reduction in the molecular weight of the polycarbonate (Table 2) indicates that only short segments of macromolecules are removed, and hence is not able to alter the weight average molecular weight considerably, but relatively decreased the number average molecular weight. An increase in the polydispersity index is observed which could be due to the extracellular enzymes acting upon the polymer by an exo-type, which is end-chain scission, leading to fragmentation of higher molecules.

Contact angle of the field and in vitro samples when compared to the control sample has reduced by 10% (Table 2), indicating an increase in the hydrophilicity of the surface. This could be due to chemical changes on the surface of the polymer which corroborate with the FTIR spectral findings. Artham et al. [22] also observed, under similar environmental conditions, that deposition of biofilm solids led to a decrease in contact angle after 6 months of exposure to sea water.

CHN analysis (Table 3) indicated that the PC charged initially and recovered after 12 months of in vitro studies contained 75.88 and 70.86% of elemental carbon, respectively. Nearly 4.55 mg of dry biomass was collected at the end of the study period which contained 10.99% of elemental carbon. Only 8.16% of carbon is not accounted for which could have gone as water soluble metabolites and hence not detected.

Based on different analysis done on degraded polycarbonate a probable mechanism for the biodegradation has been proposed (Fig. 9). In the field studies oxidative type of cleavage was observed, while in laboratory conditions both hydrolytic as well as oxidative type of degradation was observed. Bisphenol A release confirms the hydrolysis of carbonate bond while a broad peak at 4.2 ppm in ^{1}H NMR and a broad peak at 3,500 cm^{-1} in FTIR for in vitro sample confirms oxidation on methyl group. This kind of functional group was observed during thermal degradation of polycarbonate [33] even though thermal degradation cannot be compared to the biodegradation.

Conclusions

The above reported results, based on biochemical analysis, suggest that bacteria were viable after 12 months of incubation with polycarbonate as a sole carbon source and have initiated the degradation (9% weight loss) of polycarbonate into smaller oligomers for their metabolism and growth. A detailed chemical analysis of the degraded polymer clearly indicates several chemical changes are initiated by the bacteria. The nature of degradation in the field and in laboratory conditions was different. Oxidative type of degradation was major in the field while hydrolytic type of degradation was observed in laboratory conditions. The Mn decreased by 5% and a metabolite with a molecular weight of 930 was observed in both the places. Considerable increase in surface roughness and surface turning more hydrophilic indicated that the microbial attack is initiated on the polymer surface. The metabolite BPA, identified in this study, has recently evoked large concern from scientific community worldwide due to its disastrous effects. Release of Bisphenol A (9 µg mL^{-1}) and its oxidized products into the sea water could be a consequence during this process. Therefore this study proves beyond any doubt that polycarbonate disposed in marine environment can degrade slowly.

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