In situ biodegradation studies of cellophane using indigenously developed bacterial consortia

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Abstract

Cellophane, a bio-based fibrous film is considered sustainable, biodegradable and potential substitute for petrochemical based plastic films. However, addition of extra coating agents are making them recalcitrant in nature and therefore, their persistence after disposal is becoming a serious environmental concern. In this study, previously defined bacterial consortia *viz.* C-I, C-II and C-III were used to evaluate the efficacy under *in situ* conditions for the possible remediation of cellophane film waste. Comparative analyses of recovered biodegraded films have shown extensive damage on the surface of the films. Fourier transform infrared (FTIR) spectroscopy analysis revealed that the consortium C-II and C-III have significant effect on the spectral absorptions of the degraded cellophane films. The simultaneous thermogravimetric-derivative thermogravimetry-differential thermal analysis (TG-DTG-DTA) of biodegraded samples treated with C-II and C-III also exhibited tremendous weight losses at 457°C (85.7% and 95.7%, respectively) as compared with respective control (85%). Furthermore, the progressive degradation of cellophane films was examined through scanning electron microscopy (SEM), revealing the effect of bacterial mixed cultures on surface topography. Conclusively, present study signifies the cellophane degrading potential of previously tested bacterial consortia under natural conditions which will be a step forward to develop as an eco-friendly tool for plastic waste disposal.

Introduction

The over-growing burden of synthetic plastic waste in natural ecosystems is the matter of environmental concern. The overall plastic waste generated by 2019 was 353 million tonnes, of which only about 9 and 19% waste was recycled and incinerated, respectively while other 50% of waste is dumped in landfills (OECD Report 2022). It was predicted that humankind will produce 26 billion metric tons of plastic waste by 2050 (Geyer et al. 2017). Over the past few decades, it was realized that biodegradable polymers synthesized from natural sources can be a substitute to synthetic petro-based plastics. In this context, cellophane was thought to be beneficial over conventional plastics due to its higher mechanical strength, chemical stability, biocompatibility and biodegradability (Hata et al. 2017). These suitable attributes of cellophane over conventional plastics are therefore implausible, serving enhanced functionality and generating additional benefits. However, to make moisture-proof and heat sealed, cellophanes are generally coated with polyvinyl chloride (PVC), carbon disulfide (CS_2), polyvinyl acetate (PVAc) or polyvinylidene chloride (PVdC), nitrocellulose (Hamedi et al. 2016; Rich and Patel 2015). These coatings restrict the cellulolytic activity of microorganisms and thus, biodegradability of the cellophane films gets reduced (Farajollahi et al. 2010; Robertson 2013). Therefore, a cost-effective, eco-friendly and sustainable approach is urgently needed before emerging the additional burden of cellophane waste.

In this scenario, microbial degradation of polymeric wastes appears to be an excellent tool, as incineration, land filling and recycling has several side-effects on environment. Further, managing different type of plastic wastes with single bioformulation is always be feasible and preferred. Therefore, three different predefined bacterial consortia *viz.* C-I, C-II and C-III were explored to evaluate the *in situ* biodegradation potential against cellophane film under soil ecosystem. The bacterial strains used to develop the consortia were formerly isolated from geographically different waste dumping grounds and from experimental soil pits (Satlewal et al. 2008; Negi et al. 2009; Kapri et al. 2009, **2010 a, b;** Sah et al. 2010; Raghuwanshi et al. 2016). Previous studies by author group have revealed their biodegrading ability to different polymers *viz.* lower density polyethylene (LDPE) (Satlewal et al. 2008; Soni et al. 2009; Kapri et al. 2009, **2010 a, b;** Sah et al. 2010), higher density polyethylene (HDPE) (Satlewal et al. 2008), PVC (Sah et al. 2011), epoxies (Negi et al. 2009; Raghuwanshi et al. 2015) and polyhydroxybutyrate (PHB) (Raghuwanshi et al. 2016; Debbarma et al. 2017). However, the biodegradation study of cellophane by means of predefined bacterial consortia is meager in literature. Therefore, present investigation is a step forward to develop an effective, economic and sustainable way to deal the plastic pollution including cellophane waste in the future.

Materials And Methods

Cellophane film

Commercially available transparent cellophane film (Segolike, Pengzhan Wanguo E-commerce (Shenzhen) Co., Ltd.) of 15m x 38 cm (L x W) dimension and 0.022 mm thickness was purchased from local market. To study *in situ* biodegradation, cellophane film was cut into 4×4 cm^2 sized coupon chips using blade and thereafter, surface of the film chips were sterilized with 70% ethanol for 10 min. and subsequently dried under vacuum at ambient temperature (Debbarma et al. 2017).

Bacterial Strains

The bacterial strains used in this study were collected from the department of microbiology, CBSH, GBPUAT, Pantnagar, India **(Supplementary Table 1)**. To revive the bacterial strains, glycerol stock cultures were inoculated into 5.0 mL nutrient broth (HiMedia, India) test tubes at their optimum pH (7 ± 0.2) and temperature (35 ± 1°C) and further, maintained on nutrient agar (HiMedia, India) for subsequent studies (Raghuwanshi et al. 2015, 2016; Debbarma et al. 2017).

Preparation Of Pre-defined Bacterial Consortia

For this, 500 µL of overnight grown cultures was withdrawn by using 1000 µL micropipette and inoculated into 10 mL nutrient broth test tubes aseptically for each respective bacterial strain. To achieve mid-log phase active culture, inoculated test tubes were subjected to incubate under incubator shaker (120 rpm) for 4 h at ambient growth conditions. The calculated quantity of each bacteria preparation (CFU mL^{-1}) from active culture (Table 1) was combined together to obtain bacterial consortium (Goel et al. 2011; Raghuwanshi et al. 2015).

Table 1
Calculated amount of bacteria preparation (CFU mL^{-1}) for active consortium preparation. Each value is the mean of three replicates. Values in parentheses indicate standard error.

Strains	Dilution factor	CFU mL^{-1}
MK3	10^7	64 (± 2)
PN12	10^7	160 (± 3)
MK4	10^7	211(± 2)
PW1	10^6	110 (± 3)
PS1	10^7	152 (± 1)
C1	10^6	98 (± 3)
Rb10	10^6	84 (± 2)
Rb11	10^6	175 (± 2)
Rb18	10^5	250 (± 3)
Rb1	10^5	100 (± 2)
Rb13	10^6	90 (± 1)
Rb19	10^6	180 (± 2)

Preparation Of Talc Based Bioformulations

Bioformulation was prepared through the collection of live bacterial cells in the form of pellets from 200 mL of ready mid-log phase active consortium by centrifuging (Sigma 3-16K) them in four parts using 50 mL sterile tubes at 500 rpm for 10 min. Subsequently, the collected live bacterial cells were mixed with 5 mL of sterilized distilled water and vortexed for making homogenous suspension of each tube. Later, the suspension were added with 10 g of talc (talcum powder; steatite; talc, fine powder; hydrous magnesium silicate purchased from HiMedia, India) aseptically and mixed thoroughly for the uniformity. Finally, the bioformulations were dried and stored at room temperature **(Kapri et al. 2010 a;** Debbarma et al. 2017).

Determination Of Average Shelf-life Of Bioformulation

Serial dilution plating method using nutrient agar medium was used to ascertain the average viability of bacterial strains in the talc based bioformulation **(**Goel et al. 2011; Debbarma et al. 2017). To confirm the bacterial viability, 1.0 g of bioformulation was added in 1.0 mL of sterilized water. Further, the bioformulation suspension was poured into 9.0 mL test tube of autoclaved water and vortexed vigorously to make the dilution of 10^{-1}. Likewise, the serial dilution was made upto 10^{-9}. Then, the CFU mL^{-1} counts were evaluated after the incubation period of 2 and 4 days at 35 ± 1°C. The same procedure was followed to confirm the viability of bioformulation at 7 days interval following up to 21 days and later

trailed by 15 days gap up to subsequent 70 days. This study was assessed to verify the alteration of bioformulation through the calculation of CFU mL^{-1} at the time of storage under ambient room temperature (Anwar et al. 2013).

In situ **biodegradation of cellophane film**

Experimental soil pit and treatment design

The comparative bio-deterioration of the cellophane film was studied and carried out into four experimental pits under ambient natural conditions (Fig. 1). The size of each pit was 60 cm x 30 cm x 60 cm (length x width x depth), which were half filled with top-soil taken from crop research centre (CRC), GBPUAT, Pantnagar, India. The soil then brought back to the net house and mashed up manually for proper aeration during the study. Thereafter, ~ 15–20 cut cellophane film coupon chips were placed randomly below the surface soil. Both cellophane pure (cellophane-P) and untreated cellophane in the soil pit (cellophane-UN) were served as negative and positive control, respectively. The remaining three soil pits inoculated with bioformulations *viz.* C-I, C-II and C-III, respectively **(Supplementary Table 1)** were regarded as treatments. The whole study was conducted for the period of 12 months. During this trial, practices *viz.* pit cleaning, moisture condition and aeration in the pits were checked after each 5 days of time gap (Anwar et al. 2013; Debbarma et al. 2017).

Recovery and collection of degraded sample

The biodegraded samples were recovered and collected in sampling bags after the incubation period with the help of trowel. Later, these samples were washed thoroughly with 70% ethanol for 10 min. to remove adhered soil particles and further, dried under vacuum for 24 h using vacuum desiccator. The collected samples as well as the controls were then subjected to structural, thermal and morphological analyses to comprehend the biodegradation nature of cellophane film after the soil incubation with active bioformulations (Anwar et al. 2016).

Analysis And Characterization Of Biodegraded Cellophane Film Samples

Effect of bacterial mixed cultures on the structural and morphological degradation of cellophane films was investigated through diversified analytical methods. Structural degradation of cellophane film was investigated through fourier transform infrared spectroscopy (FTIR) and simultaneous thermogravimetric-derivative thermogravimetry-differential thermal analysis (TG-DTG-DTA). Whereas, the morphological changes in cellophane film was investigated through scanning electron microscopy (SEM).

Fourier transform infrared spectroscopy (FTIR)

The alterations in the structural composition of the left over cellophane polymer over 12 months of soil incubation were analyzed by Perkin Elmer FTIR spectrophotometer (version 10.03.06). The profiles of FTIR peaks are represented in terms of wave numbers (cm^{-1}) ranging from 4000 to 450 cm^{-1}. Potassium bromide (KBr) was used as a beam splitter to record the spectra (Debbarma et al. 2017).

Simultaneous thermogravimetric-derivative thermogravimetry-differential thermal analysis (TG-DTG-DTA)

Simultaneous TG-DTG-DTA was performed after the analysis of FTIR spectra and compared the thermal characteristics of treated samples with that of positive control (cellophane-UN). This experiment was carried out on EXSTAR (SII 6300 EXSTAR) thermal analyzer. During the analysis, atmosphere condition was maintained under nitrogen at 200 mL min^{-1}.

Further, the samples were subjected to a thermal treatment keeping the temperature range between 35°C to 815°C accompanying a programmed heating rate (5°C min^{-1}) on platinum sample pan.

Scanning electron microscopy (SEM)

The surface aberrations on the biodegraded cellophane films were characterized by comparing the SEM micrographs. The samples were prepared through 10 min. washing with 70% ethanol followed by proper drying using desiccator. Further, these are gold coated and observed under SEM (JEOL JSM-6610 LV) at 8.00 kV EHT with magnification of 0.15 KX performed at CVASC, GBPAUT, Pantnagar, India.

Results And Discussion

Bacterial cultures, preparation of active consortia and viability and shelf-life of bioformulations

A total of 12 bacterial strains were selected for the cellophane film biodegradation studies which were already tested against variety of polymers *viz*. LDPE, HDPE, epoxy and epoxy silicone blends etc. **(Supplementary Table 1)**. Microorganisms can be applied individually for the biodegradation of different polymers (Hadad et al. 2005; Goel et al. 2008), however, they are found more efficient when employed as consortia (Raghuwanshi et al. 2015; Anwar et al. 2016; Debbarma et al. 2017). Therefore, mutual compatibility and colony forming units (CFU) mL^{-1} were calculated from mid-log phase cultures of each strain to prepare the active bacterial consortia (Table 1) (Goel et al. 2011; Raghuwanshi et al. 2015).

The synergistic effect of bacterial consortium would be only efficient when the carrier based bioformulation will be preserved in viable state for certain period of time (Shanmugam et al. 2011; **Saharan and Verma. 2015;** Namsena et al. 2016). Moreover, the viability of bioformulation for a definite time period is one of the advantageous aspects for commercialization purpose (Bazilah et al. 2011; Malusá et al. 2012). Nevertheless, the preparation of carrier based bioformulation provides congenial transport and effortless application of consortium in the target sites. In the above context, bioformulations prepared using talc as a carrier material was remains viable subsequent to 70 days at ambient room temperature (Table 2). Each consortium have shown varied growth in terms of CFU mL^{-1} after 2 days initial storage of bioformulation which was then taken as a 100% survival rate of the respective consortium. After the successive 70 days of storage, these consortia (C-I, C-II and C-III) have shown prolonged shelf-life with % survival decrease rate of 3.87%, 4.16% and 6.47%, respectively. This result proposed that the bacterial consortia remain active and viable in recently prepared bioformulations. Therefore, these consortia were recommended as suitable candidates for the *in situ* biodegradation studies of cellophane film (Debbarma et al. 2017).

Table 2
Shelf life of bioformulations under ambient storage temperature. Each value is the mean of three replicates. Values in parentheses indicate standard error.

Consortium	Dilution Factor	CFU mL^{-1} at subsequent time intervals (days)							
		2nd	**4th**	**11th**	**18th**	**25th**	**40th**	**55th**	**70th**
C-I	10^7	284(± 2)	283(± 2)	279(± 2)	280(± 2)	278(± 2)	275(± 2)	274(± 2)	273(± 2)
% survival decrease		0%	0.36%	1.76%	1.40%	2.11%	3.17%	3.52%	3.87%
C-II	10^7	288(± 2)	286(± 2)	283(± 2)	281(± 2)	280(± 2)	278(± 2)	276(± 2)	276(± 2)
% survival decrease		0%	0.70%	1.73%	2.43%	2.77%	3.47%	4.16%	4.16%
C-III	10^7	278(± 2)	276(± 2)	271(± 2)	269(± 2)	267(± 2)	265(± 2)	264(± 2)	260(± 2)
% survival decrease		0%	0.71%	2.51%	3.23%	3.95%	4.67%	5.03%	6.47%

Comparative Analysis Of Biodegraded Samples Through Ftir Spectroscopy

The biodegraded samples from each experimental pit were recovered and subjected to further analysis **(Supplementary Fig. 1)**. The changes in the polymeric structure of consortia treated cellophane were monitored *via* FTIR spectroscopy with reference to pure and untreated control. Analysis of biodegraded sample has shown variable peaks corresponding to CH_2 deformation *viz.* CH_2 stretching (asymmetrical), CH_2 rocking (asymmetrical) and C-C-C bond stretching, C-C bond bending, C-H bond stretching, CH_3 bending (symmetrical), C = O bond bending, OH bending and stretching. The changes in the structure were contrasted with pure cellophane film spectrum (cellophane-P) as a control. We have used δ, v, and ρ to represent the bending, stretching, and rocking vibrations, respectively. Moreover, the symmetrical and asymmetrical absorptions were represented by "$_{asym}$" and "$_{sym}$", respectively.

Pure cellophane (cellophane-P) has elucidated FTIR absorptions, distinctive wave numbers (cm^{-1}) corresponding to 3369.55 (v OH), 2923.69 (v_{asym} CH_2), 1646.95 (δ OH), 1385.27 (δ_{sym} CH_3), 1116.05 (δ C-C), 771.87 (ρ_{asym} CH_2) and 618.42 (v C-C-C), respectively **[**Fig. 2 **(a)] (**Table 3**)**. Untreated cellophane (cellophane-UN) samples recovered from soil bed after 12 months illustrated the wave numbers (cm^{-1}) at 3392.06 (v OH), 1645.23 (δ OH), 1385.28 (δ_{sym} CH_3), 1116.36 (δ C-C), and 618.53 (v C-C-C) **[**Fig. 2 **(b)]**. The absolute removal of v_{sym} CH_2 and ρ_{sym} CH_2 group from the polymeric backbone suggested that these changes are clearly attributed to the effect of environmental factors since cellophane-UN samples were not treated with bacterial consortium which confirms the hygroscopic nature of cellophane film. Furthermore, cellophane samples treated with C-I (Cellophane–T + C-I) were also showing similar spectrum with that of cellophane-UN which was corresponding to wave numbers (cm^{-1}) at 3391.95 (v OH), 1647.9 (δ OH), 1385.17 (δ_{sym} CH_3), 1116.22 (δ C-C), and 618.64 (v C-C-C) **[**Fig. 3 **(c)]**. Comparative result revealed that C-I has further no remarkable change in the absorptions. This indicates that the C-I does not work well in soil medium. However, samples treated with C-II (Cellophane–T + C-II) have shown significant shift in the absorption frequencies corresponding to wave numbers (cm^{-1}) at 3390.85 (v OH), 2921.03 (v_{sym} CH_2), 1739.07 (δ C = O), 1648.22 (δ OH), 1384.35 (δ_{sym} CH_3), 1116.6 (δ C-C), 973.85 (v C-H), 719.01 (ρ_{asym} CH_2) and

617.66 (v C-C-C) [Fig. 3 **(d)]**. The additional peaks of δ C = O and v C-H bond in the spectrum have indicated the effect of consortium C-II after the treatment. In addition, consortium C-III also contributed significant degradation on the samples (Cellophane–T + C-III) which was reflected in the FTIR absorptions corresponding to wave numbers (cm^{-1}) at 3400.77 (v OH), 1737.57 (δ C = O), 1647.93 (δ OH), 1384.98 (δ_{sym} CH_3), 1116.83 (δ C-C), and 758.95 (ρ_{asym} CH_2) [Fig. 3 **(e)]**. Exposure of C-III has induce remarkable changes in the spectra reflecting the complete degradation of v C-C-C and inclusion of δ C = O bond and the samples physically found to be opaque **(Supplementary Fig. 1).** The deviation in ρ_{asym} CH_2 absorption frequencies which depicted the lowered wave numbers (cm^{-1}) both in the case of cellophane–T + C-II and cellophane–T + C-III in comparison with cellophane-P, have also confirmed the influence of bacterial consortia. This indicates the potential of C-II and C-III towards the surface biodegradation of cellophane with reference to C-I and Control. The results of FTIR analysis have suggested the progressive biodegradation of cellophane film under natural conditions which was enhanced when treated with bacterial consortia. Similar result was found while studying the biodegradation of different polymers by using indigenous bacterial consortia *viz.* synthetic polymers (HDPE, LDPE and epoxies) and biopolymer (PHB) by our research group **(**Satlewal et al. 2008; Soni et al. 2008, 2009; **Kapri et al. 2010 a, b;** Debbarma et al. 2017). The FTIR spectrum showing absorptions peaks between 3369.55 and 3400.77 ranging from cellophane-P to cellophane–T + C-III was found to be similar with that of un-grafted membranes of cellophane which represents OH groups **(**Eldin et al. 2014). Further, the biodegradation of polycarbonate was carried out by *Arthrobacter* sp. and *Enterobacter* sp. revealing the alterations in FTIR spectra correspond to CH and C = O stretching absorptions, which shows the clear evident of carbon utilization by these bacteria **(**Goel et al. 2008; Soni et al. 2009). In case of LDPE biodegradation by thermophilic bacterium *Brevibacillus borstelensis* and *Lysinibacillus* sp, FTIR spectra have shown deviated frequencies of CH_2 and decreased absorption of carbonyl group **(**Hadad et al. 2005; Mukherjee et al. 2017). Conclusively, comparative results of FTIR spectra analysis have clearly revealed that the bacterial consortia C-II and C-III have potential to accelerate the natural degradation of the cellophane film. In order to have further insight into the relative potential of these two consortia towards the biodegradation of cellophane, the samples were analyzed through simultaneous TG-DTG-DTA.

Table 3
Comparative FT-IR spectral analysis of cellophane films treated with C-I, C-II and C-III under *in situ* conditions with reference to untreated control after 12 months.

Samples	**FT-IR cm^{-1}**								
	v OH	**v CH_2 (asym)**	**δ C = O**	**δ OH**	**δ CH_3(sym)**	**δ C-C**	**v C-H**	**ρ CH_2(asym)**	**v C-C-C**
Cellophane-P	3369.55	2923.69	-	1646.95	1385.27	1116.05	-	771.87	618.42
Cellophane –UN	3392.06	-	-	1645.23	1385.28	1116.36	-	-	618.53
Cellophane –T + C-I	3391.95	-	-	1647.9	1385.17	1116.22	-	-	618.64
Cellophane –T + C-II	3390.85	2921.03	1739.04	1648.22	1384.35	1116.6	973.85	719.01	617.66
Cellophane –T + C-III	3400.77	-	1737.57	1647.93	1384.98	1116.83	-	758.95	-

v-Stretching, **δ**-Bending, **ρ**-Rocking, **asym**-Asymmetric, **sym**-Symmetric, **P**-Pure, **UN**-Untreated, **T**-Treated, **C**-Consortium

Simultaneous Tg-dtg-dta Analysis Of Biodegraded Samples

The modifications in the sample composition and thermal stability of biodegraded samples were assessed by using thermogravimetric measures that precisely analyze the variations in weight of samples in relation to changes in temperature and time intervals. The characteristic thermographs were obtained due to endothermic or exothermic processes, when substances undergo chemical changes on heating at definite rate. A derivative thermogravimetry was used to tell the point or peak at which weight loss is most apparent. Thermal properties of consortia treated cellophane–T + C-II and cellophane–T + C-III degraded film in comparison with cellophane-UN have shown in Table 4 and the comparative thermograms of these samples are depicted in Fig. 3. It was observed that all the three samples started degrading at 101°C and the entire sample burnt down with 99.1%, 99.5% and 96.5% weight loss at 812°C for cellophane-UN, cellophane–T + C-II and cellophane–T + C-III, respectively. However, variable thermal degradation as well as progressive weight loss could be observed with increasing temperature in all the samples. In the present study, the temperature at 457°C was taken as standard to compare the thermal stability and weight loss of cellophane-UN with their consortium treated counterparts because the significant weight loss was observed for all the respective samples at this temperature (Soni et al. 2009) (Table 4). It was observed that cellophane-UN sample showed only 85% weight loss at 457°C. However, biodegraded cellophane–T + C-II and cellophane–T + C-III samples have shown advanced increment in weight loss *i.e.* 85.7% and 95.7%, respectively (Negi et al. 2009). The loss of weight of this polymer and the reduction rate during programmed thermal treatment is relative to the strength of the structural composition of the polymer (Satlewal et al. 2008).

Table 4
Comparative thermal analysis of degraded cellophane films under *in situ* conditions by the C-II and C-III consortia with reference to untreated cellophane control.

Treatments	DTG Peak Temperature		DTA Exotherm		DTA Endotherm		TG weight loss % at 457°C
	°C	Decomposition Rate (µg/min)	°C	ΔH (µV.s/mg)	°C	ΔH (µV.s/mg)	
Cellophane –UN	428	368.9033	-	-	427	-558	85
	453	375.3242	-	-	455	9.25	
Cellophane –T + C-II	432	412.2246	-	-	431	-827	85.7
	457	441.6191	-	-	466	2.50	
Cellophane –T + C-III	247	1125.188	256	476	438	-630	95.7
	255	1438.024	-	-	-	-	
	295	337.7871	-	-	-	-	

Thermal degradation of cellophane-UN has revealed DTA endotherm peaks at 427°C (75.4 µV) and 455°C (87.0 µV) with the heat of decomposition values ΔH -558 µV.s/mg and ΔH 9.25 µV.s/mg, respectively. Contrary to it, double DTG peaks at 428°C and 453°C with the decomposition rate 368.9033 µg/min and 375.3242 µg/min, respectively were also observed [Fig. 3 **(a)**]. Comparatively, the treated cellophane films exposed several DTG peaks showing the responsible temperatures along with higher decomposition rates (Table 4). The cellophane–T + C-II sample have shown DTA endotherm peaks shifted at 431°C (93.1 µV) and 466°C (112.1 µV) with ΔH -827 µV.s/mg and ΔH 2.50 µV.s/mg, respectively. This observation was supported by double DTG peak at 432°C and 457°C with higher decomposition rate as 412.2246 µg/min and 441.6191 µg/min, respectively; while, cellophane–T + C-III sample revealed three considerably lowered temperatures at DTG peaks than the cellophane-UN as well as cellophane–T + C-II [Fig. 3 **(b)**]. The DTG peaks were observed at 247°C, 255°C and 295°C with decomposition rate as 1125.188 µg/min, 1438.024 µg/min and 337.7871 µg/min, respectively. The DTA endotherm shifted to 438°C (62.5 µV) with heat of reactions of ΔH -630 µV.s/mg. Biodegraded cellophane–T + C-III

also exhibited DTA exotherm at much lower temperature at 256°C (10.2 μV) with heat of reactions of ΔH 476 μV.s/mg. Further, decomposition of cellophane–T + C-III was observed in multiple step with a steep weight loss in temperature ranging from 208°C (99% weight residue) to 457°C (4.3% weight residue) [Fig. 3 **(c)]**. Whereas, cellophane–T + C-II have shown multiple step decomposition with a steep weight loss in temperature ranging from 225°C (98.4% weight residue) to 545°C (0.7% weight residue) in comparison with cellophane–UN temperature ranging from 222°C (98.5% weight residue) to 543°C (0.8% weight residue) **(Kapri et al. 2010 a, b)**.

The thermal analysis has clearly revealed the action of bacterial consortia which determinately responsible for the progressive decomposition of biodegraded samples after the long period of incubation. Study also reveals that the C-III has the remarkable efficacy on cellophane film biodegradation than the other consortia with much higher decomposition rate and increased weight loss at comparatively lower temperatures. Nevertheless, cellophane–T + C-III sample also displayed both exotherm and endotherm peaks with variable ΔH. These results strongly showed the evidence on the effect of bacterial consortium C-III over cellophane polymer backbone. On the other hand, C-II treated biodegraded sample also demonstrated considerable changes in the endotherms, DTG peaks and the heat reactions ΔH comparing cellophane-UN. These changes in the thermal profiles of treated cellophane samples might be due to the reaction of bacterial enzymes with the functional groups present in the polymer, consequently, causes the alterations in chemical structure of the polymeric backbone as the result supported the FTIR spectra. Thus, it was clear that the bacterial consortia could utilize cellophane polymer as their energy source when treated. Further, the development of various DTG and DTA peaks was previously found and documented in the case of HDPE and LDPE, polycarbonate, nonporonized and poronized LDPE **(Kapri et al. 2010 a, b)**, epoxies and their silicone blends, epoxy and CME (Raghuwanshi et al. 2015) during the biodegradation studies.

Scanning Electron Microscopy (Sem) Micrographs

Based on the comparative results obtained from FTIR and thermal analysis, it was confirmed that the cellophane film was evidently degraded by consortium C-III. Therefore, further SEM micrographs were taken for the conclusive evident for biodegradation of cellophane film treated with C-III. As mentioned earlier, the whole *in situ* study for the cellophane biodegradation was conducted for 12 months. During this incubation period, changes in surface morphology by the bacterial consortium C-III were analyzed taking cellophane-P and cellophane-UN as references. The cellophane-P SEM micrograph was shown smooth and homogenous image [Fig. 4 **(a)]**. However, the cellophane-UN samples morphology was found to be heterogeneous and scrape like marks were observed [Fig. 4 **(b)]**. These surface aberrations of cellophane-UN could be totally due to the natural reasons *viz.* pressure of soil burial, soil temperature, slow initiation of natural degradation, etc. however, samples from C-III treated have shown more extensive surface deterioration than the control (Anwar et al. 2013). The SEM image obtained from treated (C-III) cellophane was distinguishable from pure (P) and untreated (UN) film as the fissures and crumbles on the film surface were extensive showing major attributes *viz.* well resolved distortions, fissures, formation of tiny cavities, etc. [Fig. 4 **(c)]** (Debbarma et al. 2017). This heterogeneous surface morphology on the surface of treated cellophane was obviously imparted after the exposure of bacterial consortium C-III which further substantiates the results of FTIR and thermal analyses. Thus, the SEM micrographs revealed the intensive surface deterioration of treated cellophane film after 12 months incubation under natural conditions.

Conclusions

This study revealed that the previously developed indigenous bacterial consortia for the degradation of various polymers could also significantly degrade commercially available cellophane film under natural conditions. We have found that bacterial consortium C-III was more efficient in cellophane biodegradation. Comparative studies clearly suggested that the consortium C-III was better competent than the consortium C-I and C-II. However, all used bacterial consortia have shown

the ability to utilize cellophane polymer as their carbon source and subsequently enhanced the biodeterioration of cellophane film. The suggestive reasons behind this could be the natural adaptation of bacterial consortia when applied in the experimental pit with the cellophane and later, the bacteria might have colonized on the surface of the cellophane. The talc based bioformulations have shown their viability for longer duration of storage. This was obviously very helpful during the biodegradation study for longer period of incubation under ambient natural conditions. Thus, these findings could be useful perspective in management of plastic waste as cost effective green technology without producing carcinogenic gases. Conclusively, this study provides the insight scenario of physicochemical aspects of the cellophane biodegradation under soil ecosystems.

Declarations

Availability of data and material

Supplementary material is available and included in the text.

Competing interests

The authors confirm that this article content has no conflict of interest.

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Authors' contributions

Study conceptualization and design was contributed by Reeta Goel. All data collection, compilation and first draft of the manuscript was written by Prasenjit Debbarma. FTIR, TG and SEM data analysis was done by Mohammad Ghulam Haider Zaidi and Prasenjit Debbarma. Deep Chandra Suyal, Amit Yadav and Yogesh Shouche commented and corrected the previous version of the manuscript. All authors read and approved the final manuscript.

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Figures

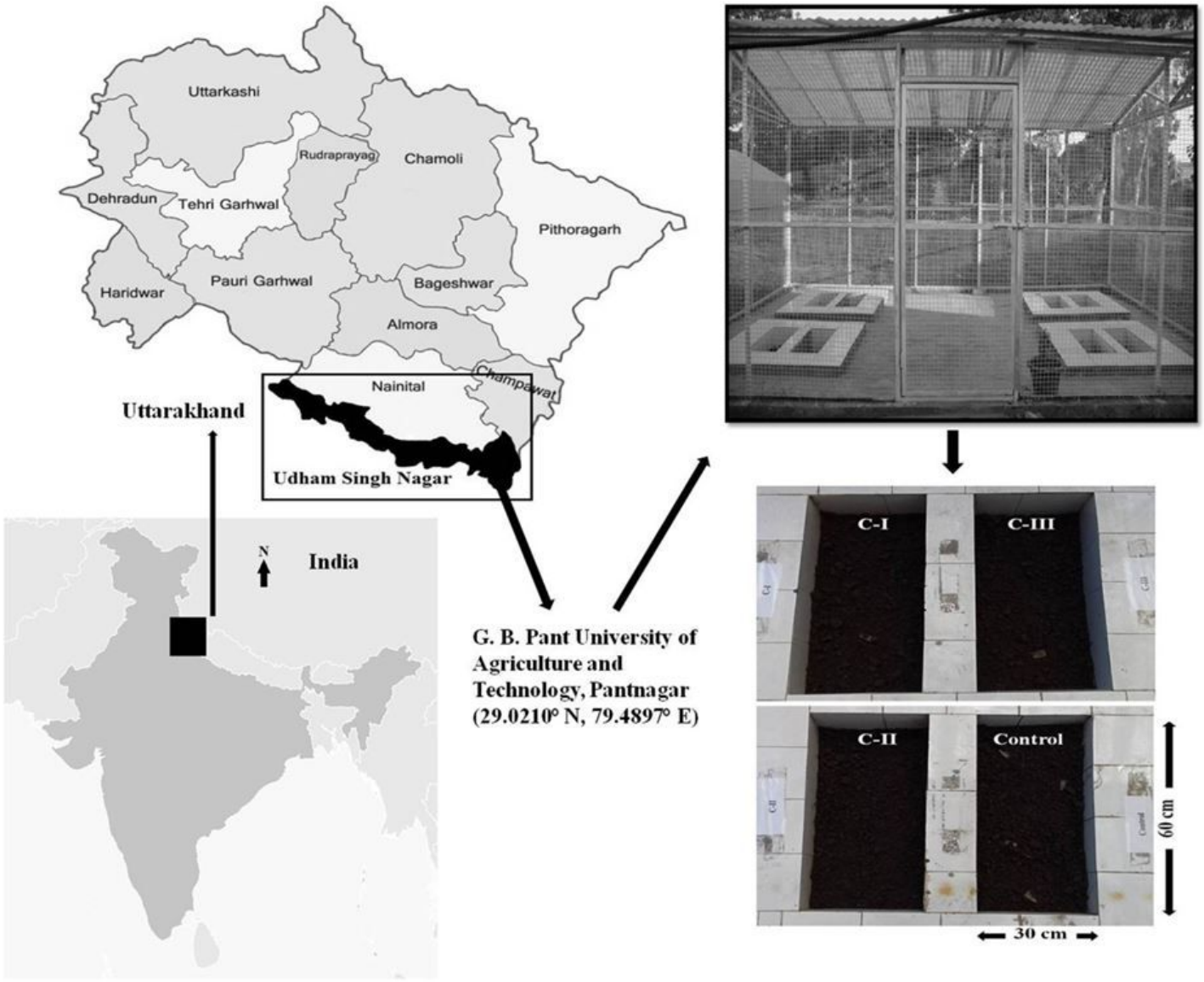

Figure 1

Experimental design for *in situ* biodegradation studies of cellophane film.

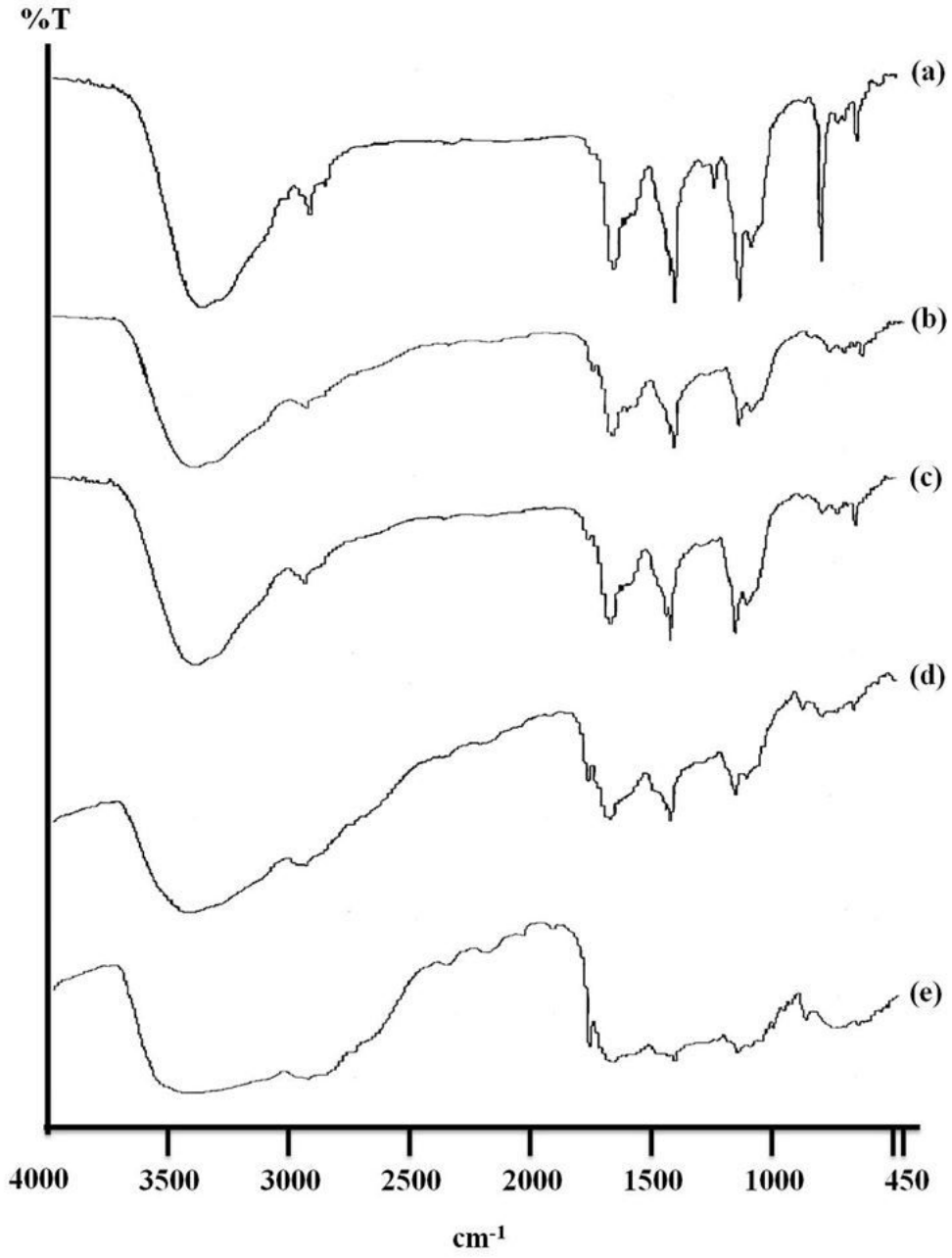

Figure 2

Comparative FTIR spectra of cellophane-P (a), cellophane-UN (b), cellophane-T+C-I (c), cellophane-T+C-II (d) and cellophane-T+C-III (e) over 12 months of incubation, respectively.

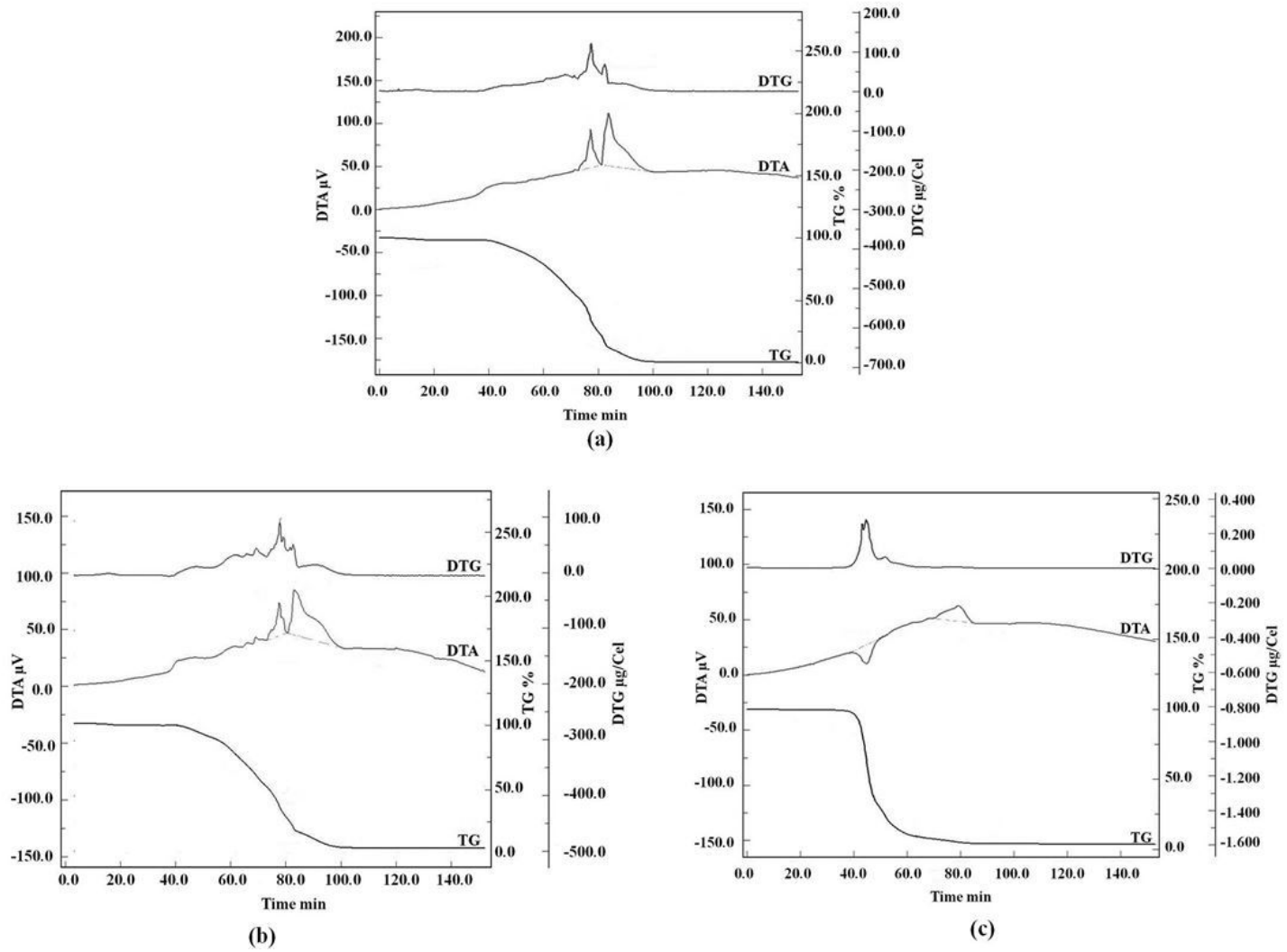

Figure 3

Simultaneous TG-DTG-DTA of cellophane degraded samples after 12 months of incubation under *in situ* conditions treated with C-II and C-III (b and c, respectively) with reference to cellophane-UN (a) as control.

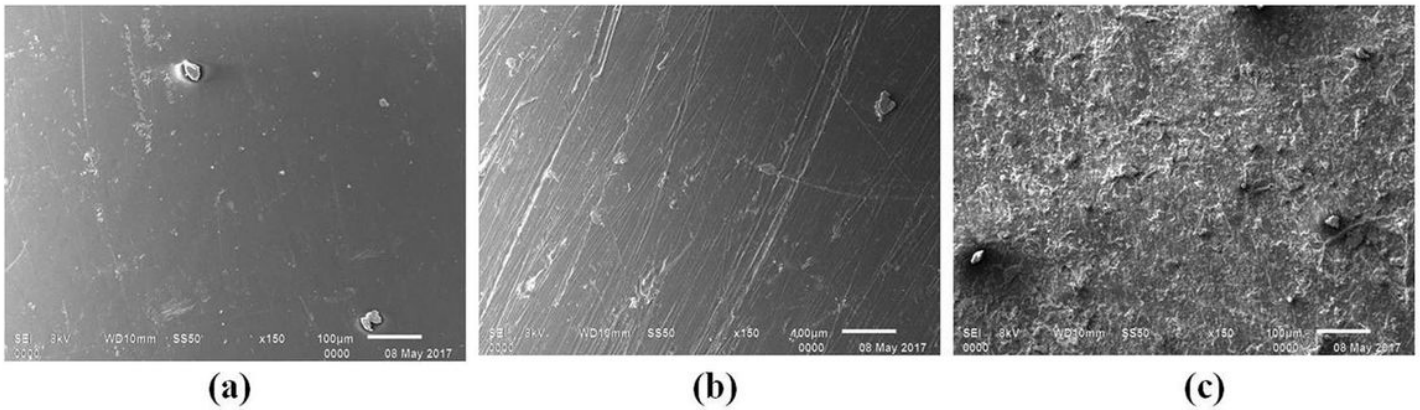

Figure 4

Comparative SEM micrographs of cellophane-T+C-III (c) with reference to cellophane-P (a) and cellophane-UN (b)

Supplementary Files

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