academicJournals

Vol. 9(24), pp. 1558-1572, 17 June, 2015
DOI: 10.5897/AJMR2015.7462
Article Number: 978814854029
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Comparative biodegradation studies of cow dung modified epoxy with epoxy using an indigenously developed bacterial consortium

Shikha Raghuwanshi[1]*, Harshita Negi[1], Tithi Aggarwal[2], MGH Zaidi[2] and Reeta Goel[1]

[1]Department of Microbiology, C.B.S.H., G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India.
[2]Department of Chemistry, C.B.S.H., G. B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttarakhand, India.

Received 2 March, 2015; Accepted 15 June, 2015

Thermoplastic-based polymers and their blends are recalcitrant in nature. Based on their extensive use, huge amount of polymeric waste is being produced annually, which impart serious threat on the natural ecosystem. Considering this scenario, it is needed to take some immediate actions to keep the ecosystem dynamic and secure. Therefore, this study was carried out in order to evaluate an indigenously developed bacterial consortium for the biodegradation of epoxy and a blend of epoxy with cow dung that is cow dung modified epoxy (CME). These polymers were preliminary screened against the used bacteria individually for determination of optimum concentration to utilize them as carbon source. For this purpose, the comparative *in vitro* biodegradation studies were carried out using the bacterial consortium. Relatively, better biodegradation potential of developed consortium was observed for epoxy in comparison to CME, as higher biomass and more sustained growth of consortium was obtained in the presence of epoxy. Further, the progressive *in situ* degradation of epoxy and CME films was also conducted in the presence and absence of consortium for three and six months under natural conditions. For this purpose, bioformulation of bacteria was used to inoculate the soil. The treated samples were analyzed for their comparative spectral, thermal and morphological changes. Thus, the present study reveals that the used bacterial strains have the potential to act upon epoxy and CME polymers and capable to impart changes in the surface morphology during incubation in soil.

Key words: Epoxy, cow dung modified epoxy, biodegradation, bacterial consortium, Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), thermogravimetry-differential thermal analysis (TG–DTG–DTA).

INTRODUCTION

The immense applications of synthetic polymers and their blends in different fields viz. packing, agriculture, marine, medicine, scientific, technological and household etc. has raised the issue of socio-environmental concern. Among

*Corresponding author. E-mail: shikha.singhkul9@mail.com. Tel: 05944-233341.

Table 1. Bacteria potential for the biodegradation of Epoxy and its blends.

Polymer treated	Bacteria used	Reference
Stressed carbon-reinforced epoxy composite	Sulfate Reducing Bacteria (SRB)	Wanger et al., 1996
Epoxy and Epoxy Silicone Blends (ESBs)	*Psedomonas aeruginosa, Microbacterium sp, Bacterium Te68R and Psedomonas putida*	Negi et al., 2009
Epoxy and MF-modified polyurethane films	*Psedomonas aeruginosa strains*	Dutta et al., 2010
Organic epoxy	*Bacillus* and *Virgibacillus*	Wang et al., 2013
Epoxy resin	*Bacillus* and *Pseudomonas*	Pangallo et al., 2014
Epoxy-PU composites filled with ferrocene	Biological medium 199 (BM199)	Rudenchyk et al., 2015

Table 2. Isolation profile of the bacterial cultures used in the study.

Isolation site	Description of strain(s)	Reference
Artificial soil bed, Pantnagar	PN12	Satlewal (2008)
Soil sample, Pantnagar	MK3, MK4	Satlewal (2008)

these polymers, epoxy resins and its blends are of great scientific and technological interest due to their ease of processability and durability. Therefore, with the increase in production and exploitation of these polymers, the amount of waste has been raised enormously and accelerating the problem of solid waste disposal. Poor solid waste management in the developing countries causes major threat to public health and environment, and reduces the quality of life, in both urban and rural areas (Wang et al., 2011). Thus, need of this hour is to protect the environment by proper management of the polymeric waste using an ecofriendly biological means. The literature survey revealed that a variety of microbes are capable of carrying out biodegradation of epoxy and its bends (Table 1). Thus, microbial degradation may be one way to deal with the epoxy polymeric waste (Pangallo et al., 2014).

Furthermore, in a previous study, conducted by our research group, the rate of weight loss of epoxy and its silicon blend after 15 days of incubation under *in vitro* biodegradation using indigenously developed bacterial consortia was found to be 34.17 and 36.9%, respectively (Negi et al., 2009). Considering the fact, epoxy and a synthesized blend of Epoxy that is Cow Dung Modified Epoxy (CME) was tested in the present study for the biodegradation using the potential bacterial consortium. However, the ultimate aim of the study was to screen a cost-effective and eco-friendly additive (cow dung) to synthesize a blend with epoxy resin, which would be increasing the commercial applicability of the compound, after finding it's biodegradability. In addition, the growth profiling and potential of this used and other reported consortium was proved to be higher in comparison to a single strain (Sah et al., 2010; Pandey et al., 2015), thus, a bacterial consortium was used for comparative biodegradation assay.

MATERIALS AND METHODS

Starting materials

The epoxy and corresponding CME blend (with cow dung as additive) were synthesized in the Department of Chemistry, CBSH, Pantnagar, India. However, the exact constituting concentration of blend is not disclosed (under patent process). The compounds (in form of powder and films of size 1 × 1 cm^2) were investigated as the primary carbon source for tested bacteria. Nutrient broth (Hi Media, Mumbai, India) was taken as medium for the bacterial growth. For *in vitro* studies, Minimal Broth Davis without dextrose (Minimal Broth) (containing grams per litre: 7.0 K_2HPO_4; 2.0 KH_2PO_4; 0.5 $Na_3C_6H_5O_7$; 0.1 $MgSO_4.7H_2O$ and 1.0 $(NH_4)_2SO_4$) (Hi Media, Mumbai, India) medium was used which is deprived of carbon source.

Bacterial consortium

The selected bacterial cultures were retrieved from the departmental culture collection of Microbiology, CBSH, G. B. Pant University of Agriculture and Technology, Pantnagar, India (Table 2). These were originally isolated from different artificial soil beds and further characterized by 16S rDNA sequencing and the sequences were submitted to GenBank, NCBI. These cultures were selected based on their pre-identified potential to degrade a variety of polymers like LDPE (Kapri et al., 2010), HDPE (Satlewal et al., 2008), Epoxy, and Epoxy silicone blends (Negi et al., 2009) and PVC (Anwar et al, 2013). These bacterial strains *viz*. *Bacterium*Te68R strain PN12 (DQ423487), *Microbacterium* sp. strain MK3 (DQ318884) and *Pseudomonas putida* strain MK4 (DQ318885) were characterized and maintained as glycerol stocks. Further, an aliquot of 200 µL was withdrawn from glycerol stocks and the cultures were revived by inoculating into 10 mL of nutrient

broth (HiMedia, India) test tube at their optimum conditions and maintained on nutrient agar (HiMedia, India) at optimum pH (7.0 ± 0.2) and growth temperatures. Based on the preliminary nutritional screening, the bacterial consortium was developed. For this purpose, a single colony from each culture was inoculated into 20 mL flask containing 10 mL of nutrient broth (pH 7.0 ± 0.2) and the flasks were incubated at 37°C for 16 h with continuous shaking at 120 rpm to prepare active cultures which should be in mid-log phase of their growth. Further, the equal calculated amount of colony forming unit per mL (CFU/mL) of each strain was mixed for the development of consortium as described by Goel et al., 2011. The compatibility of the each used strains was also tested for the preparation of consortium and reported earlier by our research group (Sah et al., 2011).

Determination of optimum tolerance level of Epoxy and CME

The optimum concentration of Epoxy and CME, tolerated by the consortium was determined. For this purpose, the bacteria inoculated in the Nutrient Broth to prepare active culture, as mentioned previously in section of bacterial consortium. The active culture (an aliquot of 20 µL, OD=0.40) was inoculated into 96-well cell culture plate containing 200 µL Minimal Broth Davis without Dextrose per well. The crushed Epoxy and CME were then added at increasing concentrations from 0 to 8 mg/mL. Cell culture plate was incubated at optimum growth temperature (37±1°C) with continuous shaking (120 rpm) for overnight. The optimum tolerance level was further confirmed by inoculation of respective bacteria with 5 mL minimal broth into test tubes. And the similar concentrations of tested compounds were used in each tube and then incubated at optimum growth temperature (37±1°C) with continuous shaking (120 rpm) for overnight. The absorbance was recorded for all the treatments at 600 nm wavelength with ELISA plate reader and spectrophotometer after filtration (using whatman filter paper of 2.5 µm particle retention size) of non-biodegraded compound for 96-well plate and test tubes, respectively.

***In vitro* biodegradation studies of Epoxy and CME using consortium**

For this purpose, crushed Epoxy and CME samples were surface-sterilized with 70% ethanol for 10 min and subsequently dried under vacuum. The dried samples were added to 200 mL autoclaved minimal broth in 500 mL Erlenmeyer flasks. A total volume of 300 µL of active culture was then added to the minimal broth. Minimal broth with Epoxy and CME (5 mg/mL of medium i.e. 1.0 gm) were served as the negative control for their respective treatments. The flask having bacterial culture served as the positive control. And the flask containing Minimal Broth Davis with culture and crushed Epoxy/CME (5 mg/mL of medium) served as treatment. Flasks were incubated at optimum growth temperature (37±1°C) with continuous shaking at 120 rpm.

Statistical analysis

The *in vitro* experiments parameters viz. OD600, CFU/mL and λ max were performed with three replicates per treatment. Data were analyzed by ANOVA. Mean difference of the treatments was considered to be significant at the 5 % level.

Recovery of compounds after biodegradation and analysis

The treated polymer samples were recovered from the broth after four days of incubation. Then, the broth was filtered (using whatman filter paper of 2.5 µm particle retention size) and the bulk surface sample (residue) was recovered from filtrate. Filtrate was centrifuged at 8000 rpm (Sigma) for 10 min at 4°C. Pellet was discarded and supernatant was kept in oven at 60°C for overnight to evaporate the water from the dissolved compound. After evaporation, the dried biodegraded sample along with the remaining residue was used for spectral and thermal analysis through Fourier transform infrared spectroscopy (FT-IR) and simultaneous thermogravimetric-differential thermogravimetry-differential thermal analysis (TG–DTG–DTA), respectively.

Fourier transform infrared (FT-IR) analysis

Treated epoxy and CME compounds were analyzed by FT-IR (Perkin Elmer spectrum version 10.03.06) and different peaks relative to CH_2 deformation, CH_2 bending (symmetrical), CH_2 bending (asymmetrical), CH_2 stretching, CH stretching and C-O bond formation were compared taking untreated Epoxy and CME as the reference.

Simultaneous differential thermal analysis (TG–DTG–DTA)

TG-DTG-DTA analysis was done on a TG analyzer (EXSTAR TG/DTA 6300) from 30 to 800°C under a nitrogen atmosphere (200 mL/min) using a heating ramp of 10^0C/min in an Alumina pan.

***In situ* biodegradation studies**

Preparation of bioformulation

The rationale of using bioformulation was to maintain bacterial cell viability under adverse environmental conditions (sunlight-UV exposure, temperature fluctuation due to seasonal changes and inhibitory action of indigenous population etc.), so that they can persist longer in the soil under the *in situ* experiment. In addition, it also facilitates the easy transport; direct application; proper mixing in the soil and accessibility anytime at the site of action. For this purpose, Talc (composed of talcum steatite, talc fine powder and hydrous magnesium silicate) was purchased from Himedia Lab Pvt Ltd, Mumbai, India. The active consortium (600 mL) was prepared as discussed in section of bacterial consortium and the culture was then centrifuged at 15000 rpm for 10 min to remove the bacterial cells. Later, the supernatant was decanted and the pellets were added to 30 g of talc under sterile conditions and mixed properly. The mixture was then emptied into glass petri-plates, and kept at room temperature (28±1°C) aseptically for air-drying. For storage the bioformulation were kept at cool and dry place and subsequently checked for viability.

Shelf life of bioformulation

The viability of bacterial isolates in the formulation was ascertained by serial dilution plating method. For this purpose, one gm of talc-based formulation was dissolved in 10 mL of sterile distilled water and then subsequent dilutions were prepared up to 10^{-7} dilution. The dilution plating was done in nutrient agar medium. The plates were incubated at 37±1°C and the viability was checked initially after 2 and 4 days and then after regular interval of 7 days for subsequent 3 weeks followed by 15-days interval up to 180th day of storage of bioformulation as performed earlier by Sah et al., 2011. The above pattern was followed keeping in view the rapidity of

Table Sd1. Optimum tolerance level of polymer observed against used bacteria in the study.

Bacterial strain		*CFU/mL at the increasing concentration of Epoxy and CME (mg/mL)								
		0 mg/mL	1 mg/mL	2 mg/mL	3 mg/mL	4 mg/mL	5 mg/mL	6 mg/mL	7 mg/mL	8 mg/mL
MK3	Epoxy	36×10^{1}	33×10^{2}	30×10^{3}	43×10^{4}	33×10^{6}	64×10^{7}	50×10^{5}	33×10^{4}	66×10^{3}
	CME	50	33×10^{1}	38×10^{2}	70×10^{3}	31×10^{4}	50×10^{6}	64×10^{4}	30×10^{3}	60×10^{2}
MK4	Epoxy	39×10^{1}	44×10^{2}	34×10^{3}	63×10^{4}	33×10^{6}	33×10^{7}	40×10^{5}	36×10^{4}	55×10^{3}
	CME	45	33×10^{1}	34×10^{2}	39×10^{3}	40×10^{4}	64×10^{6}	60×10^{4}	37×10^{3}	62×10^{2}
PN12	Epoxy	45×10^{1}	30×10^{2}	346×10^{3}	60×10^{4}	33×10^{6}	160×107	33×10^{5}	56×10^{4}	78×10^{3}
	CME	40	33×10^{1}	37×10^{2}	32×10^{3}	43×10^{4}	70×10^{6}	68×10^{4}	34×10^{3}	54×10^{2}

*The data are average of triplicate experimental values**.**

changes in viable counts during storage. The plate counts were carried out in triplicates and the CFU/mL was calculated as the average of three readings.

Biodegradation assay

For *in situ* biodegradation assay, the top soil was dug from a barren land at Pantnagar, India and filled into 45×34 cm^2 sinks. The Epoxy and CME film coupons of dimensions 1×1 cm^2 were surface sterilized with 70% ethanol for 10 min and dried in vacuum. The dried films were then placed at various depths below the soil surface in respective treatment. The prepared bioformulation were added to the soil and then incubated in natural condition. Further, autoclaved distilled water was sprinkled at regular intervals of two weeks to maintain the moisture content of the soil. Aeration conditions were maintained by shoveling the soil at regular intervals. Moreover, the untreated pure Epoxy and CME coupons were taken as control for both the soil incubated polymer samples either in presence and absence of consortium.

Recovery of compounds from soil bed and analysis

The treated polymer film samples were carefully recovered from the soil after a period of subsequent three and six months. The recovered films were washed with sterile water to remove soil and contaminant and then subsequently dried under vacuum. The dried biodegraded samples were used for spectral and morphological analysis through FT-IR and Scanning Electron Microscopy (SEM), respectively.

Fourier transform infrared (FT-IR) analysis

The Epoxy and CME film samples were analyzed by FT-IR as described previously for *in vitro* biodegradation studies.

Scanning electron microscopy (SEM) analysis

For this analysis, the film samples were metallized with gold particles and analyzed by SEM (JEOL JSM-6610 LV) at 15.00 kV EHT under two successive magnifications (1.00 and 3.00 KX).

RESULTS

Determination of optimum tolerance level of epoxy and CME

The preliminary screening of epoxy and CME for their toxicity against used bacteria was observed that the optimum tolerance level of these polymers for respective bacterial strains was found to be 5 mg/mL (Table Sd1). Thus, the given concentration of polymers was used for the further *in vitro* biodegradation studies.

Comparative growth profiling of consortium in presence and absence of epoxy and CME

The growth profile of consortium was compared in the presence and absence of epoxy and CME (Figure 1). It was shown that epoxy did not affect the growth of bacteria, as the stationary phase was achieved within 3 days itself and bacterial biomass as depicted by the OD_{600} (Figure 1a and Table Sd2) and CFU/mL (Figure 1b and Table Sd3) was comparatively higher in presence of Epoxy (5 mg/mL). However, it was found that CME interfered the growth pattern of the bacteria as the stationary phase was achieved earlier i.e. within 48 h of the incubation (Figure 1a) and CFU count was lower in comparison to the controls (Figure 1b). This effect may be due to the organic matter and antibacterial property of cow dung (Waziri and Suleiman, 2013). Moreover, the significant increase in the OD_{600} was observed in the presence of CME, as the additive of the blend i.e. cow dung tended to dissolve in water and imparted a yellowish color to the medium resulted to increase in OD_{600} (Figure 1a). Thus, the comparative account reflected that the bacterial growth was better in the presence of Epoxy, as compared to CME.

In vitro biodegradation assay of Epoxy and CME using consortium

In the absence of consortium, Epoxy has shown the constant λ max during the incubation period (Figure 2 and Table Sd4). However, in presence of consortium, a shift in λ max of Epoxy from 218 to 222 nm within 2 days of incubation period and further to 216 nm within 4 days has illustrated the changes in the backbone of the

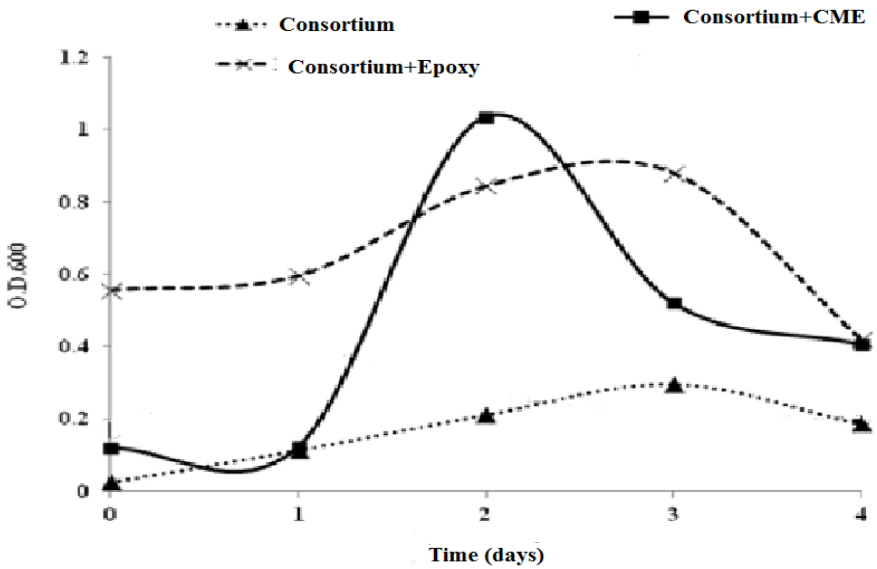

Figure 1a. Growth profiling of bacterial consortium in presence and absence of Epoxy and CME

Table Sd2. Two-way ANOVA of growth profiling of bacterial consortium in presence and absence of Epoxy and CME (using STPR2 software).

Day	Consortium OD600[a]	Epoxy+Consortium OD600[a]	CME+Consortium OD600[a]
0	0.023± 0.0011547	0.556 ±0.0008819	0.12±0.011547
1	0.112±0.001	0.595±0.0020817	0.123±0.0017321
2	0.21±0.0152753	0.843±0.002	1.035±0.0015275
3	0.296±0.0028868	0.88±0.011547	0.52±0.0173205
4	0.185±0.0008819	0.421±0.0034641	0.407±0.0017321

SEM: A (Treatment): 0.00322; B (Days): 0.00417; AXB (Interaction): 0.00722. CD (5%): A (Treatment): 0.00935; B (Days): 0.00120; AXB (Interaction): 0.00209. a, mean of three replicates. Data were analyzed statistically at the 5% ($p>0.05$) level of significance.

polymer. Moreover, CME has shown similar pattern of change in λ max either in presence (275 to 215 nm) or absence (280 to 220 nm) of the consortium during the incubation period of 4 days (Figure 2). It may be due to the organic content of CD, tends to dissolves in to the broth during the incubation period. Thus, these results demonstrated that the bacterial consortium has not imparted any significant change in the polymeric backbone of CME in the liquid medium. This may be due to the anonymous properties of cow dung which hampering the bacteria to act upon the CME.

Analysis of *in vitro* treated compounds

FT-IR spectra

FT-IR absorptions (cm^{-1}) of epoxy and CME are shown in Figure 3. Untreated Epoxy (Epoxy-U) has shown peaks corresponding to vOH (3435.58), $vasCH_3$ (3019.52), $vasCH_2$ (2966.43- 2400.38), vC=O (1720.87), δOH (1606.35), δNH (1509.77), $\delta asCH_2$ (1470.84), δCH (1384.52), γCH (1290.39, 1216.82), δC-C (1123.67), vC-O-C (1075.55, 1039.80), ρCH_2 (772.03) and vC-C-C (669.3, 626.85) (Figure 3a). The untreated CME (CME-U) has shown peaks corresponding to vOH (3435.97), δOH (1634.95), γCH (1219.47), ρCH_2 (772.21) and vC-C-C (685.31, 673.82) (Figure 3c). Such characteristic absorptions in the IR spectra of Epoxy and CME clearly indicated their synthesis. And the shift in the absorption frequency in CME to higher wave number clearly indicated the presence of CD in the Epoxy matrix. The consortium treated epoxy (Epoxy-T) has shown peaks corresponding to vOH (3798.52-3443.89), $vasCH_2$ (2342.75, 2143.06), δOH (1635.91), γCH (1218.91), vC-O-C (1074.06), ρCH_2 (771.96) and vC-C-C (685.27, 673.34) (Figure

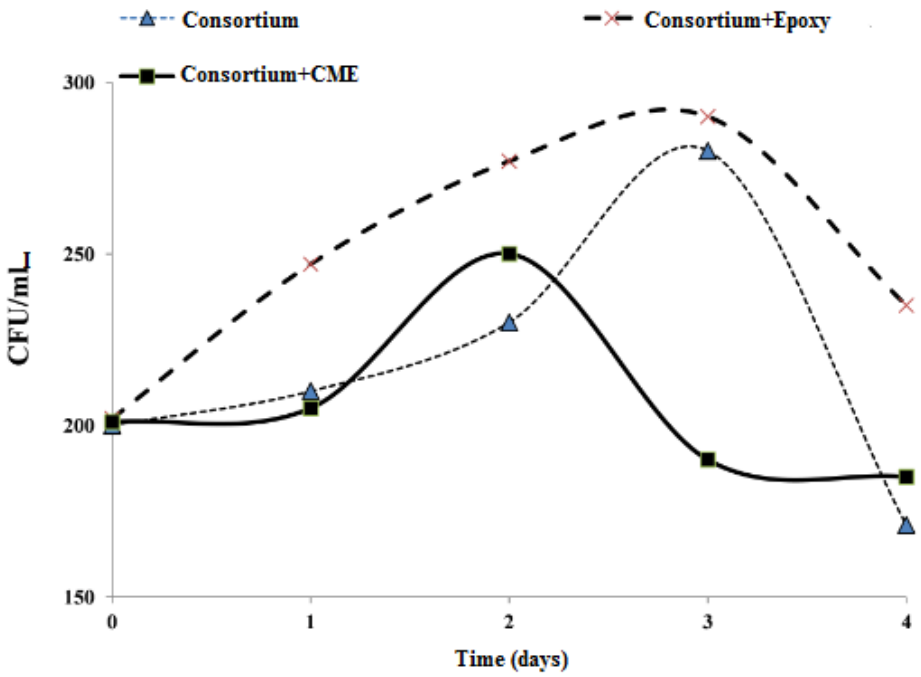

Figure 1b. Comparative analysis of colony forming units per ml (CFU/mL) in presence of Epoxy and CME with respect to control.

Table Sd3. Two-way ANOVA of comparative analysis of colony forming units per ml (CFU/mL) in presence of Epoxy and CME with respect to control (STPR2 software).

Days	Consortium CFU/mL[a]	Epoxy+Consortium CFU/mL[a]	CME+Consortium CFU/mL[a]
0	200±0.5773503	200±1.1547005	200±1.1547005
1	212±1.1547005	242±1.1547005	205±1.1547005
2	225±2.081666	275±2.8867513	250±2.8867513
3	270±1.1547005	285±1.4529663	170±0.5773503
4	175±0.8819171	230±1.7320508	185±2.081666

SEM: A (Treatment): 0.718; B (Days): 0.927; AXB (Interaction): 1.606. CD (5%): A (Treatment): 2.080; B (Days): 2.686; AXB (Interaction): 4.652. a: Mean of three replicates. Data were analyzed statistically at the 5% ($p>0.05$) level of significance.

3b). The disappearance of peaks at wave numbers 3019.52 (vas CH_3), 1720.87 (vC=O), 1509.77 (δNH), 1470.84 (δCH, asymt), 1384.52 (δCH) and 1123.67 (δC-C) with simultaneous increase in the wave number of vOH from 3435.58 to 3798.52 for Epoxy-T (Figure 3b) indicated its degradation.

The consortium treated CME (CME-T) has shown peaks corresponding to vOH (3435.82), vasCH_2 (2400.05, 2143.87), δOH (1637.38), γCH (1219.34), vC-O-C (1049.34), ρCH_2 (772.27) and vC-C-C (672.97) (Figure **3**d). The comparative study of the IR spectra revealed that the presence of consortium has enhanced the hygroscopic character of CME-T due to the appearance of absorption frequency corresponding to water molecule ranging 1634.95-1637.38 cm^{-1}. However, simultaneous increase in the wave number of vOH was not found for treated CME as compared to Epoxy-T, illustrating no structural change in CME during the incubation period of 4 days into the liquid medium. Further, all the samples were hygroscopic with banding vibration of H_2O from 1606.35 to 1639.28.

Simultaneous TG–DTG–DTA

The changes in the weight of treated compounds at

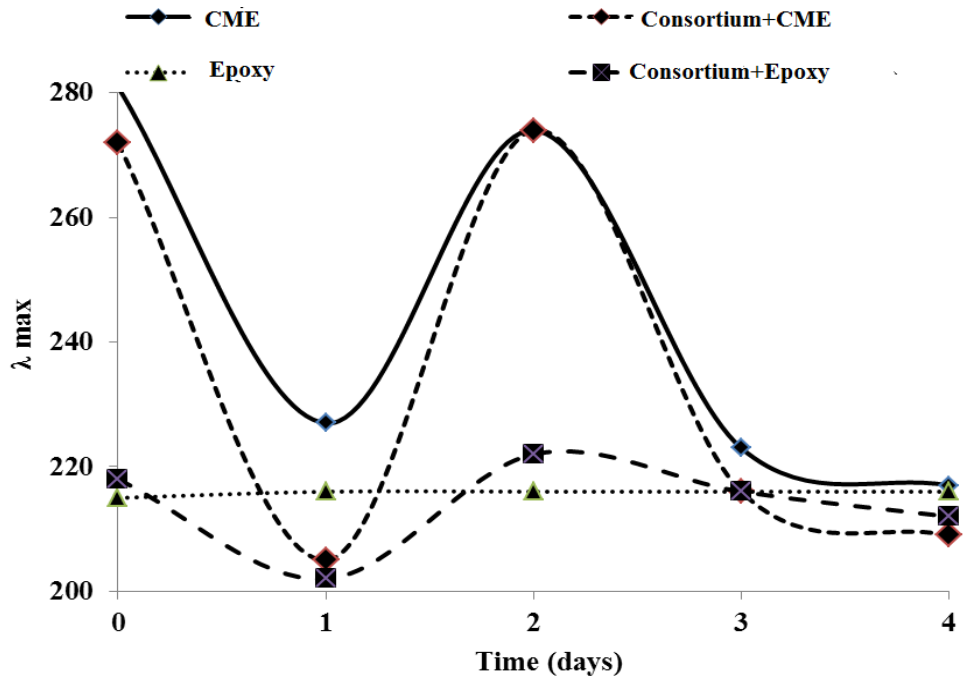

Figure 2. Comparative *in vitro* biodegradation assay of Epoxy and CME using bacterial consortium

Table Sd4. Two-way ANOVA of comparative *in vitro* biodegradation assay of Epoxy and CME using bacterial consortium (using STPR2 software).

Day	Epoxy λmax[a]	CME λmax[a]	Epoxy+Consortium λmax[a]	CME+Consortium λmax[a]
0	212± 1.1547005	281±0.5773503	221±0.8819171	272±1.1547005
1	212±1.7320508	227±1.1547005	202±1.1547005	205±1.7320508
2	213±2	274±1.5275252	225±1.1547005	274±0.5773503
3	213±2.081666	223±1.1547005	215±2.3094011	216±1.7320508
4	213±0.5773503	217±0.5773503	211±1.1547005	209±0.5773503

SEM: A (treatment): 0.595; B (days): 0.665; AXB (interaction): 1.33. CD (5%): A (Treatment): 1.704; B (Days): 1.905; AXB (interaction): 3.811. a: mean of three replicates. Data were analyzed statistically at the 5% (p>0.05) level of significance.

defined temperatures and time intervals and their thermal stability was observed through thermal gravimetric analysis. The simultaneous DTA-DTG-TG and thermal data of the consortia treated Epoxy and CME samples with reference to untreated Epoxy and CME as controls has been summarized in Figure 4 and Table 3, respectively. Epoxy-U has shown two-step decomposition reactions (Figure 4a). First step decomposition of Epoxy-U started at 200°C with 94.41% residue. This was supported with a DTG peak temperature of 230°C with rate of decomposition 0.27 mg/min. The Second step decomposition started at 300°C with 79.49% residue. This was supported with a broad DTG peak at 349°C with rate of decomposition with 0.67 mg/min. A combined DTA peak was appeared at 329°C with signal voltage of 19.7 µV that shows heat of decomposition of Epoxy-U (-) 391 mJ/mg. A weight loss of 0.03% at 100°C was found due to moisture content. A rapid weight loss was observed in the temperature range 300-400°C leaving weight residue 38.71%. This may be assigned to the oligomeric Epoxy content formed during the process of curing. The weight loss from 400°C onwards was very slow. The decomposition of Epoxy-U was terminated at 700°C leaving char residue 3.13%.

Relatively, the Epoxy-T has shown reduced thermal stability over cured Epoxy resin (Figure 4b). A rapid

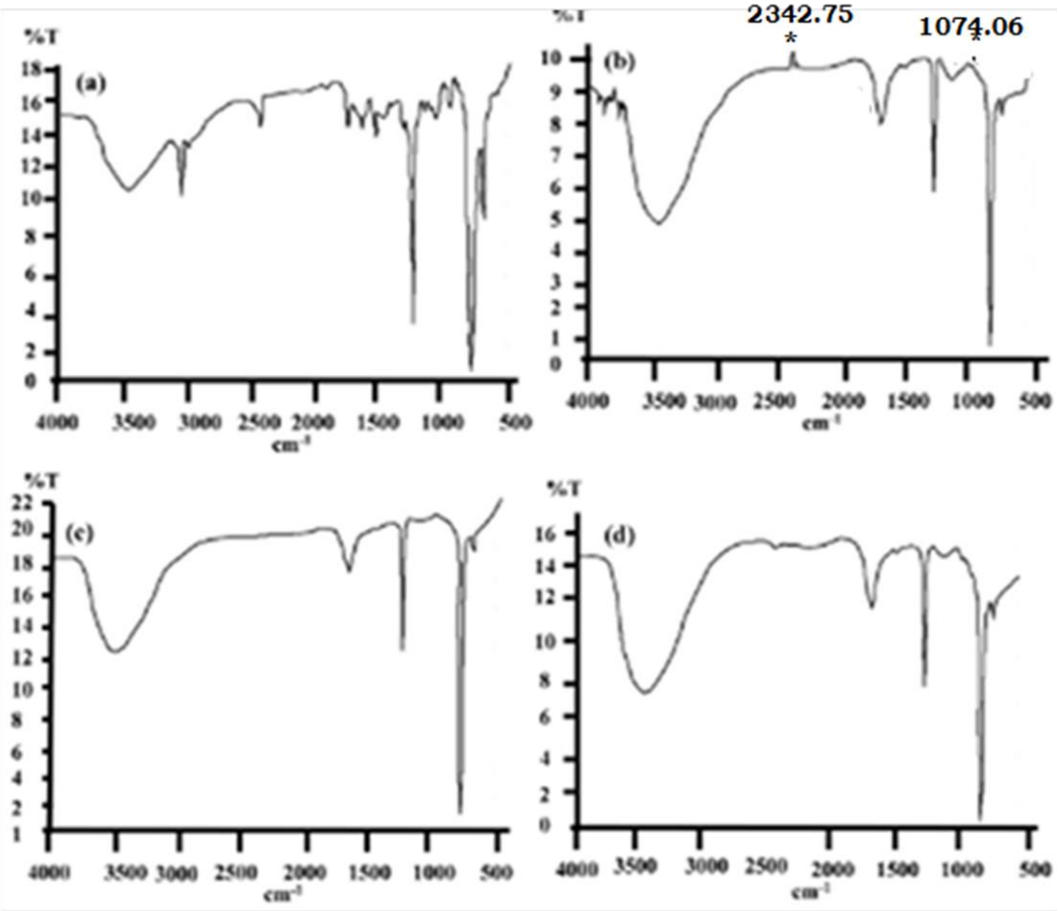

Figure 3. Effect of percentage transmittance on the wave number of (a) untreated and (b) treated Epoxy, (c) untreated and (d) treated CME, respectively during *in vitro* biodegradation studies.

weight loss was observed by Epoxy-T consisting of five-step degradation process. The rate of weight loss under defined conditions is inversely proportional to the size of the polymeric chains. And the 4.90% weight loss at 100°C was due to moisture content. The release of the moisture from the composite has been started at 62°C at the rate of 0.110 mg/min. The first step decomposition was appeared at 142°C leaving weight residue 90.9% with a sharp DTG peak at 151°C with rate of decomposition 0.274 mg/min. This has shown a DTA endotherm at 154°C with heat of fusion 56 mJ/mg. The second step decomposition was appeared at 200°C leaving weight residue 85.22% with heat of fusion 152 mJ/mg at 226°C. This was supported with a DTG at 213°C with rate of decomposition 0.141 mg/min. The decomposition of Epoxy-T further progressed leaving two minor profiles of degradation, which were appeared at 362°C with 0.149 mg/min and 637^{o}C with 0.59 mg/min. Further, the decomposition was ended with 57.20% char residue at 819°C.

The modification of Epoxy by cow dung has drastically reduced its thermal stability. But this has contributed a remarkable increase in the moisture content. The first step of decomposition of CME-U started at 200°C with 86.35% weight residue with a weak DTG peak temperature of 207°C with rate of decomposition 0.131 mg/min (Figure 4c). This was supported with a DTA at 214°C with heat of decomposition 334 mJ/mg at signal voltage (-) 14.8 µV. The second step decomposition reaction was started at 300°C leaving weight residue 79.33%. This was supported with a normal DTG at 320°C with rate of decomposition 0.164 mg/min. Due to hygroscopic contribution of cow dung to CME, moisture content of 8.77% was found. This was supported with a DTG at 72°C with elimination of water molecule at 0.243 mg/min rate of decomposition. The corresponding heat of dehydration of CME-U was appeared at 75°C with heat of decomposition 169 mJ/mg at signal voltage -15.2 µV. Weight loss at 300°C onwards was found to be very slow. The decomposition was terminated at 700°C leaving char

Table 3. Thermal analysis of treated and untreated Epoxy and CME.

Sample	DTG peak temperature		DTA	
			Endotherm	
	°C	Rate (mg/min)	°C	ΔH (mJ/mg)
Epoxy -U	230	0.27	329	-391
	349	0.67	-	-
Epoxy -T	62	0.110	154	56
	151	0.274	226	152
	213	0.141	-	-
	362	0.149	-	-
	637	0.59	-	-
CME-U	72	0.243	75	169
	207	0.131	214	334
	320	0.164	-	-
CME-T	77	0.284	79	201
	205	0.135	216	153
	332	0.174	-	-

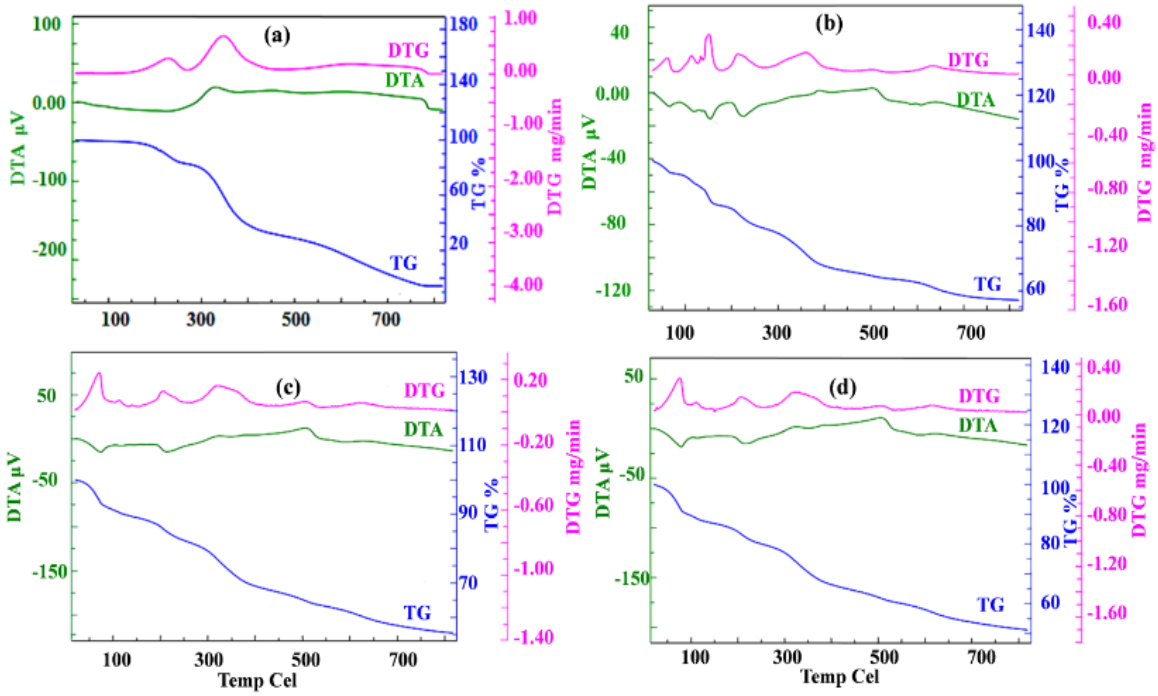

Figure 4. Thermal analysis of Epoxy-T (b) and CME-T (d) with reference to Epoxy-U (a) and CME-U (c), as their control, respectively.

residue 57.59%. The CME in the presence of consortium has shown similar degradation pattern as CME-U (Figure 4d). A comparative account of the thermogram of both the samples reveals that the presence of bacterial consortium has contributed to the reduced heat of fusion and greater moisture content over CME-U.

Shelf life of bioformulations

The viability of consortium was tested in talc-based formulation for a storage period up to 70 days. With the progression of the storage, consortium showed a sustained viability, whereby the counts dropped marginally from 279×10^6 to 253×10^6 after 70 days of storage. This consortium was found to be stable and viable as bioformulation. For commercialization, the viability of bioinoculants in a prescribed formulation for a certain period of storage is desirable (Bazilah et al., 2011). Similar studies of viability counts were also conducted on PGPR bioinoculants using sawdust as carrier (Arora et al., 2008). Talc based formulation has also been reported for PGPR strains for the storage and management of various plant pathogens (Shanmugam et al., 2011).

Analysis of *in situ* biodegraded Epoxy and CME

The comparative biodegradation of Epoxy and CME film was observed over 3 and 6 months of incubation using bioformulation in soil bed under natural conditions.

FT-IR spectra

FT-IR spectra of Epoxy (Figure 5) and CME (Figure 6) was conducted from 4000 to 500 cm^{-1}. Untreated pure Epoxy (Epoxy-P) has shown peaks corresponding to vOH (3435.58), vasCH_3 (3019.52), vasCH_2 (2966.43- 2400.38), vC=O (1720.87), δOH (1606.35), δNH (1509.77), δasCH_2 (1470.84), δCH (1384.52), γCH (1290.39), δC-C (1123.67), vC-O-C (1075.55), ρCH_2 (772.03) and vC-C-C (669.3, 626.85) (Figure 5a).

The epoxy treated in uninoculated soil (Epoxy-UN) for 3 months has shown peaks corresponding to vOH (3370.35), vasCH_2 (2920.40), δCH (1384.2), vC-O-C (1072.27) and ρCH2 (772.03) (Figure 5b). However, after 6 months of incubation the Epoxy-UN has shown peaks corresponding to vOH (3430.48), vasCH_2 (2958.89), vC=O (1733.26), δOH (1630.71), δasCH_2 (1455.29), δCH (1390.10), γCH (1250.01), δC-C (1164.13), vC-O-C (1066.52) and ρCH_2 (754.1) (Figure 5c). The spectral changes i.e. appearance of new peaks in Epoxy-UN spectra may have been occurred due to the inhabitant bacteria present in the soil and the prolonged incubation under soil bed. Environmental factors may also be assigned for these changes that is light, temperature, rain etc.

The epoxy treated in inoculated soil (Epoxy-T) for 3 months has shown peaks corresponding to vOH (3682.21), vas CH_3 (3019.02), vasCH_2 (2975.88, 2399.94), δOH (1601.53), δNH (1522.49), δasCH_2 (1476.19, 1422.55), γCH (1215.89), vC-O-C (1045.96), ρCH_2 (757.01) and vC-C-C (669.16, 627.14) (Figure 5d). However, after 6 months of incubation Epoxy-T has shown peaks corresponding to vOH (3694.48, 36.59.45) vasCH_3 (3019.97), δOH (1626.87), δasCH_2 (1402.81), γCH (1215.91), vC-O-C (1069.04), ρCH_2 (759.01) and vC-C-C (669.29) (Figure 5e).

The spectral changes had occurred in both the cases (Figure 5a, d and 5a, b). However, comparing 5 (a, d) to 5 (a, b) it was found that the later observed more changes. Conclusively, it was observed that spectral changes are due to the inhabitant bacteria present in the soil and the prolonged incubation under soil bed. Environmental factors may also be assigned for these changes. If, the consortium have had imparted the negative effect, it may be depicted more pronounced in case of 6 months result (Figure 5c, e) but it was not observed. The biodegradation has been shown clearly in Figure 5e as the loss of peaks at vasCH_2 (2958.89), vC=O (1733.26), δCH (1390.10) and δC-C (1164.13) as compared to peaks shown in Figure 5c even though the same consortium has worked for prolonged time period. So, we can conclude that bacterial consortium has not imparted any negative effect on the indigenous population.

The untreated pure CME (CME-P) has shown peaks corresponding to vOH (3435.97), δOH (1634.95), γCH (1219.47), ρCH_2 (772.21) and vC-C-C (685.31, 673.82) (Figure 6a). The CME kept in uninoculated soil (CME-UN) for three months, has shown peaks corresponding to vOH (3683.07, 3620.45, 3437.19), vas CH_3 (3019.43), vasCH_2 (2974.67, 2400.2), vC=O (1724.61), δOH (1602.05), δNH (1518.13), δasCH_2 (1475.82, 1422.49) and γCH (1215.83), vC-O-C (1045.90), ρCH_2 (758.07) and vC-C-C (669.13, 627.29) (Figure 6b). However, after 6 months, the CME-UN has shown peaks corresponding to vOH (3788.11, 3401.62), vas CH_3 (3019.94), δOH (1616.91), δCH (1385.05) and γCH (1216.16), vC-O-C (1068.86), ρCH_2 (770.97) and vC-C-C (669.23) (Figure 6c).

The 3 months bacteria treated CME (CME-T) sample has shown peaks corresponding to vOH (3436.44), vas CH_3 (3019.38, 2400.15), vC=O (1727.31), δCH (1384.3), γCH (1215.63), vC-O-C (1046.08), ρCH_2 (757.27) and vC-C-C (669.16) (Figure 6d). However, subsequently after 6 months, CME-T has shown peaks corresponding to vOH (3788.09, 3403.30), vCH_2 (2927.51), vC=O (1727.43), δOH (1627.13), δCH (1385.03), vC-O-C (1068.68) and ρCH_2 (770.45) (Figure 6e).

The peak corresponding to vOH has a different appearance in Figure 6 (a) (b) and (d)). In case of pure CME (a) it is at vOH (3435.97), untreated CME (b) has vOH (3683.07, 3620.45, 3437.19) and CME-T (d) has vOH (3436.44). It means that lower OH frequency represents the hygroscopic nature. By the treatment of microbial consortia, it has retained its hydroscopic nature rather in case of CME-UN (b), it has loosed the same.

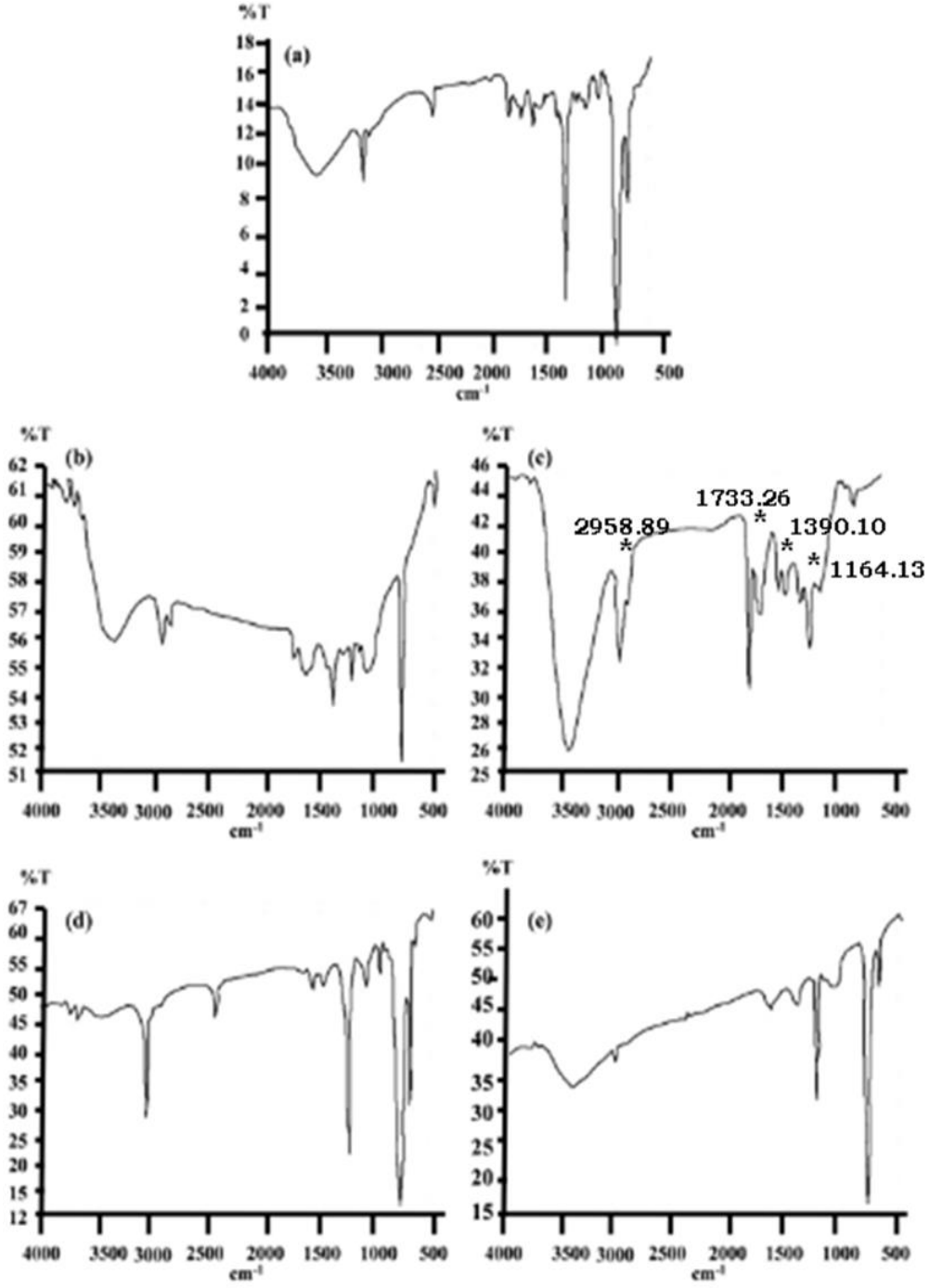

Figure 5. Effect of percentage transmittance on the wave number of pure Epoxy (a) Epoxy-UN (b), Epoxy-T (d) after 3 months and Epoxy-UN (c), Epoxy-T (e) after 6 months, respectively during *in situ* biodegradation studies.

There was not much significant change in the IR spectra of untreated and treated CME (Figure 6c and 6e) (500-1000). As only one peak corresponding to vC-C-C (669.23) has completely decomposed. Whereas, vas CH_3 (3019.94) and γCH (1216.16) has also vanished and vCH_2 (2927.51), vC=O (1727.43) are newly formed during treatment of microbial consortium. Conclusively, there was no significant change observed.

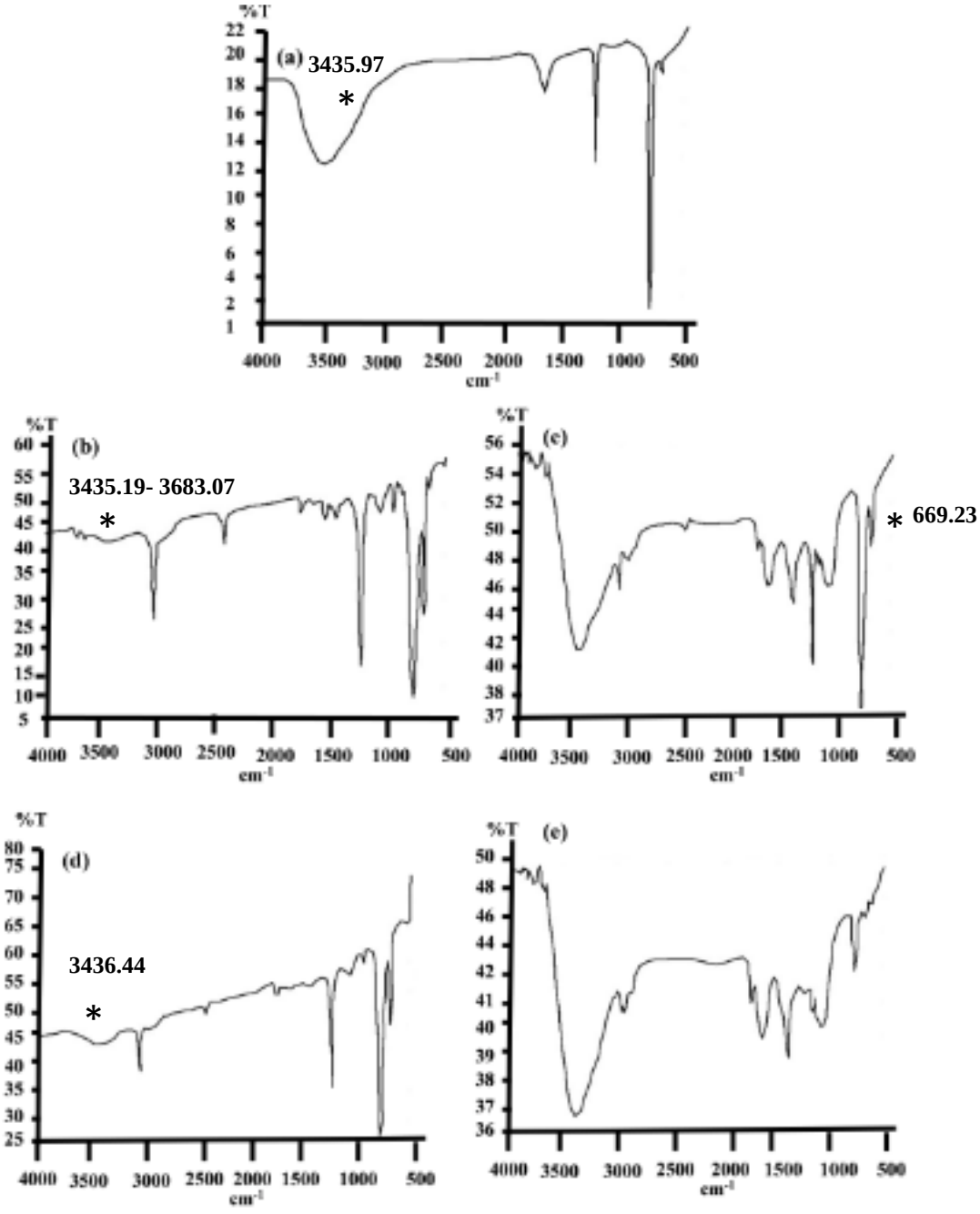

Figure 6. Effect of percentage transmittance on the wave number of pure CME (a) CME-UN (b), CME-T (d) after 3 months and CME-UN (c), CME-T (e) after 6 months, respectively during *in situ* biodegradation studies.

Scanning electron microscopy

The morphological changes on the surface of Epoxy (Figure 7) and CME films (Figure 8) due to the action of consortium were analyzed after incubation in soil for the period of 3 and subsequent 6 months. The untreated pure Epoxy film, which was taken as a reference, revealed smooth and homogenous morphology (Figure 7a). However, the Epoxy incubated in absence of consortium has been relatively changed after 3 months of incubation (Figure 7b). The abiotic factors like sunlight, rain, air and cooling under the soil bed may be responsible for the mechanical changes and heterogeneous surface of incubated film. Further, the occurrence of fissures, heterogeneous morphology and the surface resolutions and cracking of polymeric film were found to be

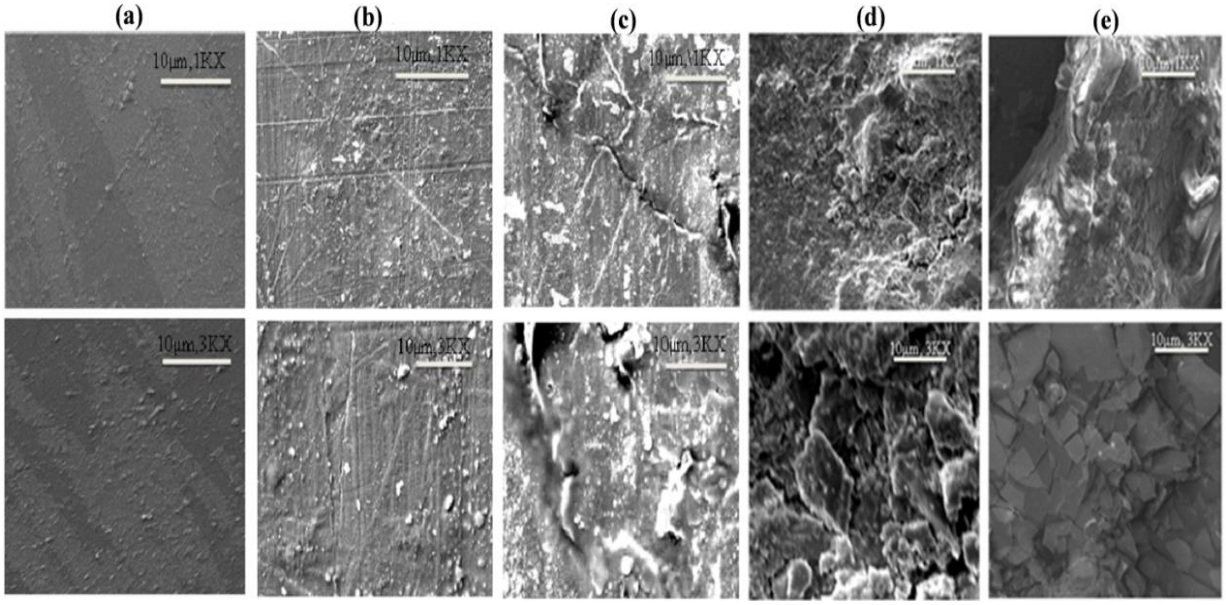

Figure 7. The morphology of the Epoxy film after incubation in soil; for 3 months in the absence (b) and presence (c) of consortia; for 6 months in the absence (d) and presence (e) of consortia, in reference to untreated pure Epoxy film as control (a). Scale bars=10 µm, 5 µm; Magnification=1.00, 3.00 KX, respectively.

remarkably increased in the epoxy film, incubated in consortium enriched soil (Figure 7c).

Subsequently, after 6 months of incubation, the well resolved worn-out areas with randomly distributed fissures may be either due to pressure of soil or the action of inhabitant microbes on the Epoxy film surface, incubated in absence of used bacteria (Figure 7d). Furthermore, in the bacteria enriched soil, the well resolved worn-out areas and cracks on the polymer surface were more pronounced (Figure 7e).

Similarly, the untreated CME film has also shown comparatively homogenous and smooth surface topography (Figure 8a). However, the changes in the CME films after incubation period of 3 months either without (Figure 8b) or with consortia (Figure 8c) observed as non-significant, may be due to the improved durability of CME film using cow dung as additive**.** Whereas, after 6 months of incubation period, the surfacial changes in CME film recovered from uninoculated soil, were observed (Figure 8d) and the surfacial changes were significant (as shown by well resolved fissures) in the film samples recovered from inoculated soil due to the action of used consortium on the polymer surface after the incubation period of 6 months (Figure 8e).

DISCUSSION

The present study was carried out with an aim of comparative *in vitro* and *in situ* biodegradation of Epoxy and Cow Dung Modified Epoxy (CME) using indigenously developed potential bacterial consortium. *In vitro* biodegradation assay revealed that the epoxy does not show any negative effect on the growth of bacterial strain during incubation period of 4 days in the liquid medium. In addition, it was observed that epoxy is degradable in the presence of used bacterial consortium which was apparent through changes in λ max, simultaneous shift in the wave numbers of various groups, reduced thermal stability, rapid weight loss and five steps degradation over untreated Epoxy resin as described by Negi et al (2009) as well.

However, the blend of Epoxy with cow dung that is CME interfered in the growth pattern and also rendered the growth of consortium within 2 days of incubation in liquid medium, as CFU/mL was found to reduce significantly. Further, no change in the λ max, structural and thermal property of CME, in the presence of bacterial consortium indicates that the used consortium was unable to cause any significant change in the polymeric backbone. It indicates the negative effect of used additive of blend that is cow dung on the bacterial growth (Waziri and Suleiman, 2013). Thus, the *in vitro* biodegradation studies revealed that the CME was not biodegraded by the used bacterial consortium during incubation period of 4 days. It may be due to the short period of incubation. In addition, the CME was used in crushed form and the interaction of bacteria to the compound was direct resulting to the

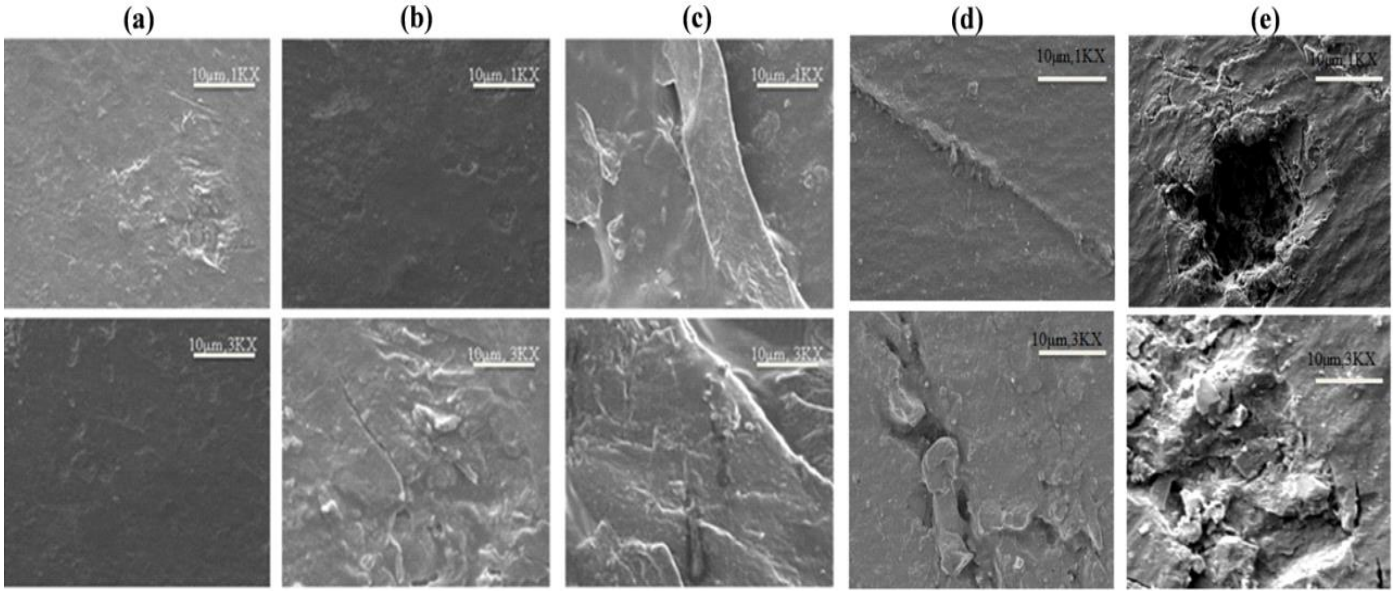

Figure 8. The morphology of the CME film after incubation in soil; for 3 months in the absence (b) and presence (c) of consortia; for 6 months in the absence (d) and presence (e) of consortia, in reference to untreated pure CME film as control (a). Scale bars= 10 µm, 5 µm; Magnification=1.00, 3.00 KX, respectively.

negative effect of cow dung on bacterial strains. However, the extent of biodegradation is also dependent on the physical form of the polymer. Thus, the *in situ* biodegradation study was conducted to observe the effect of natural conditions and natural microbial community with the indigenous bacterial consortium on both CME and epoxy films.

The Epoxy samples treated in natural condition for 3 months of incubation in presence of bacterial consortium showed occurrence of some new IR peaks and a remarkable decrease in vC-O-C with respect to its untreated control whereas, the spectral changes in the treated CME due to the consortium were non-significant upto 3 months of incubation. Thus, the comparative *in situ* biodegradation study indicated that the Epoxy surfacial degradation was achieved comparatively earlier and more pronounced in natural condition as the bacteria was able to act upon the epoxy polymeric backbone as depicted in the SEM micrographs. However, the additive cow dung interfere the bacteria to act upon the CME polymer backbone. Although, the morphological and spectral changes in CME film were achieved only after 6 months of incubation period.

Conclusively, the study found that the used bacterial consortium has the potential to utilize Epoxy under both *in vitro* and natural conditions as compared to its cow dung blend. This study also concludes that the CME samples could be degraded by potential bacterial consortium after keeping them in natural conditions for incubation period of more than five to six months.

ACKNOWLEDGEMENTS

This work was supported by Department of Biotechnology (DBT) grant to R.G. We are also thankful to CDRI (SAIF), Lucknow and Instrumentation Centre, IIT Roorkee and College of Veterinary and Animal Sciences, G.B.P.U.A.T., Pantnagar, Dr. Yogesh Souche, NCCS, Pune, for the FT-IR, TG–DTG–DTA, SEM and 16S rDNA sequencing, respectively.

Conflict of interest

Authors hereby declare no conflict of interest.

REFERENCES

Anwar MS, Negi H, Zaidi MGH, Gupta S, Goel R (2013). Biodegradation studies of thermoplastics in nature using indigenously developed microbial consortium. Braz. Arch. Biol. Technol. 56(3): 475-484.

Arora NK, Khare E, Naraian R, Maheshwari DK (2008). Sawdust as a superior carrier for production of multipurpose bioinoculant using plant growth promoting rhizobial and psedomonads strains and their impact on productivity of *Trifolium vapanse*. Curr. Sci. 95(1): 90-94.

Bazilah ABI, Sariah M, Abidin MAZ, Yasmeen S (2011). Effect of carrier and temperature on the viability of *Burkholderia* sp (UPMB3) and *Pseudomonas* (UPMP3) during storage. Int. J. Agric. Biol. 13(2):198-202.

Dutta S, Karak N, Saikia JP, Konwar BK (2010). Biodegradation of epoxy and MF modified polyurethane films derived from a sustainable resource. J. Polym. Environ. 18(3):167-176.

Goel R, Sah A, Kapri A, Negi H (2011). Process for the preparation of talc based formulation of LDPE biodegrading consortia for field application. DBT, 2010, India 213/DEL/2011 (Patent).

Kapri A, Zaidi MGH, Goel R (2010). Implication of SPION and NBT nanoparticles upon *in vitro* and *in situ* biodegradation of LDPE film. J. Microbiol. Biotechnol. 20(5):1032-1041.
Negi H, Kapri A, Zaidi MGH, Satlewal A, Goel R (2009). Comparative *in vitro* biodegradation studies of epoxy and its silicone blend by selected microbial consortia. Int. Biodeter. Biodegr. 63(5):553-558.
Pandey P, Swati P, H, M, S, Yadav M, Tiwari A (2015). Nanoparticles accelerated *in vitro* biodegradation of LDPE: A review. Adv. Appl. Sci. Res. 6(4):17-22.
Pangallo D, Buckova M, Krakova L, Puskarova A, Sakova N, Grivalsky T, Chovanova K, Zemankova M (2014). Biodegradation of epoxy resin: a microbial survey through culture-independent and culture-dependent approaches. Environ. Microbiol. 17(2):462-479.
Rudenchyk T, Rhozhnova R, Shtompel T, Galatenko N, Ostapuk S (2015). Research on biodegradation of epoxy-polyurethane composited filled with ferrocene in the model biological medium. Chem. Chemical Technol. 9(1):61-68.
Sah A, Kapri A, Negi H, Anwar S, Goel R (2011). Comparative shelf life and efficacy of LDPE and PVC degrading bacterial consortia under bioformulation. Ekologija. 57(2):55-61.
Sah A, Kapri A, Zaidi MGH, Negi H, Goel R (2010). Implications of fullerene-60 upon *in vitro* LDPE biodegradation. J. Microbiol. Biotechnol. 20(5):908-916.
Satlewal A, Soni R, Zaidi MGH, Shouche Y, Goel R (2008). Comparative biodegradation of LDPE and HDPE using an indigenously developed microbial consortium. J. Microbiol. Biotechnol. 18(3):477-482.
Shanmugan V, Kanoujia N, Singh M, Singh S, Prasad R (2011). Biocontrol of vascular wilt and corn rot of gladiolus caused by *Fusarium oxysporium f*. sp *gladioli* using plant growth promoting rhizobacterial mixture. Crop Prot. XXX(2011): 1-7.
Wagner PA, Little BJ, Hart KR, Ray RI (1996). Biodegradation of composite materials. Int. Biodeter. Biodegrad. 36(4):125-132.
Wang H, He J, Kim Y, Kamata T (2011). Municipal solid waste management in small towns: An economic analysis conducted in Yunna, China, Policy Research Working Paper. 5767:1-25.
Wang J, Xu Z, Peng SC, Xia MS, Yue ZB, Chen TH (2013). Screening of epoxy-degrading halophiles and their application in high-salt wastewater treatment. Environ. Sci. 4:1510-1516.
Waziri M, Suleiman, JS (2013). Physicochemical properties and antimicrobial activity of evaporated extract of cow dung against some pathogens. J. Sci. Res. 5(1):135-141.