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Biodegradable and biocompatible epoxidized vegetable oil modified thermostable poly(vinyl chloride): Thermal and performance characteristics post biodegradation with *Pseudomonas aeruginosa* and *Achromobacter* sp.

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ABSTRACT

The increased production of municipal solid waste by the disposal of plastic materials heightens the urgency to develop biodegradable materials for daily use. In vitro-biodegradation study on poly(vinyl chloride) (PVC) plasticized by epoxidized *Mesua ferrea* L. seed oil at three different weight percentages (PVC/ENO ratio of 75/25, 50/50 and 25/75) was conducted by using *Pseudomonas aeruginosa* and *Achromobacter* sp. bacteria. The test bacterial species were able to grow on the polymer matrix by using it as a source of energy; however the pristine PVC did not support the microbial growth. The PVC/ENO material of 25/75 ratio showed the highest percent (%) of biodegradation compared to other tested systems. The bacterial count and the dry biomass post 180 days of inoculation in 25/75 plasticized PVC suggested bacterial growth at the expense of degradation of the system. The tensile strength of 25/75 PVC/ENO system, post 180 days of inoculation by *Pseudomonas aeruginosa* and *Achromobacter* sp. decreased by about 53% and 43% respectively. Further, surface erosion phenomenon and structural change of the matrix after bacterial growth, as studied by FTIR and SEM analysis of PVC/ENO of 25/75 ratio exhibited noticeable deterioration post 180 days of inoculation.

1. Introduction

PVC captures a considerable share in the industry [1–3] with applications reported across a wide spectrum. However, its commercial feasibility is often confronted by the issues of non-biodegradability and eco-toxicity associated with the disposal of the single-time-use products. This calls for devising novel strategies to endow inherent biodegradability of PVC-based materials, standing as an answer to the growing concerns of landfill and incineration [4–9].

The limited literature reports on biodegradation of plasticized PVC by *Pseudomonas aeruginosa* and fungi [10,11] highlight more on the microbial degradation rather than providing a mechanistic explanation on the alterations in the structural aspects and its consequences on the performance characteristics. Herein we wish to report a comparative analysis of the degradation pattern of plasticized PVC by *Pseudomonas aeruginosa* and *Achromobacter* sp. isolated from soil samples of Assam (Northeastern state of India) and its effect on the various properties of the polymer.

A critical aspect of biodegradation is that the films need to be resistant to degradation both prior to and during use, yet undergo degradation if discarded into either terrestrial or aquatic environments [10]. These conflicting demands of resistance to microbial colonisation during use and degradability when discarded can sometimes be resolved by the inclusion of additives to the polymer. These additives enhance the rate of degradation of some highly resistant polymers, e.g., starch in polythene. Materials of organic origin, including the natural polymers, have an inherent tendency to decompose [12,13]. Consequently, the bio-resource based polymers play a significant role when modified or used in mixtures of synthetic polymers for their degradability.

Renewable resources like fats and oils can be chemically or enzymatically treated to fabricate materials that often act as a surrogate for petroleum-based products. Biodegradability, renewability, sustainability, aptitude to facile modification and non-toxicity make them the apt choice as starting raw material [14]. Epoxidized sunflower oil has been the most widely used plasticizer due to its renewability and environment benign nature [15]. In the present study therefore, *Mesea ferrea* L. seed oil has been used as plasticizer for the PVC. *Mesua ferrea* plant bears exceptionally high oil-containing (70%) seeds, and the favorable fatty acid composition of the oil enables its use for the preparation of epoxidized oil [16,17].

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This is the first report on the biodegradation of epoxidized *Mesua ferrea* L. seed oil plasticized PVC. Identification of *Pseudomonas aeruginosa* and *Achromobacter* sp. were used to study the biodegradation of plasticized PVC for a period of 180 days. Further the effect of biodegradation on the performance characteristics and thermal properties of PVC plasticized by epoxidized *Mesua ferrea* L. seed oil was also evaluated.

2. Experimental

2.1. Materials

Mesua ferrea L. seeds (collected from Jamugurihat, Assam) were utilized for the extraction of the oil. Hydrogen peroxide (50%, v/v), acetic acid (99%, v/v), sulfuric acid (Merck, Mumbai), and organically modified montmorillonite (OMMT, 20–25% modified with octadecylamine, Aldrich) were used as received. PVC (molecular weight, $M_n = 1.5 \times 10^3$ g/mol, density = 1.4 g/cc) was obtained from local market (Kumud Enterprise, India) and has been used as the base polymer. The chemicals used for biodegradation studies were purchased from Merck–Schuchardt, Germany.

2.2. Epoxidized Mesua ferrea L. seed oil

The purified oil was epoxidized through in situ peracid method [17]. Briefly, 40 g of the oil was treated with 4.4 g of CH_3COOH with a required amount of H_2SO_4 (2%, v/v of the H_2O_2–CH_3COOH mixture) and stirred for 30 min. Then 13.4 g of 50% (v/v) aqueous H_2O_2 was added into the mixture and stirred for another 30 min. The reaction was then continued for 8 h with constant stirring at 55–60 °C. The resultant product was washed with water and dried under vacuum. The product, i.e. the epoxidized *Mesua ferrea* L. seed oil was coded as ENO.

2.3. Modification of PVC by ENO and OMMT

The pristine PVC was modified by different loadings (0–75%, w/w) of ENO using THF as the solvent as reported earlier [18]. A desired amount of 10% (w/v) solution of PVC in THF was added to the required amount of ENO-swelled-OMMT (5 wt% with respect to the system) solution in the same solvent (5–6%, w/v) and the mixture were stirred vigorously on a magnetic stirrer. Different compositions of PVC/ENO (100/0, 75/25, 50/50 and 25/75, w/w) with 5 wt% OMMT (in each case) were prepared separately. The mixtures were thoroughly mixed on a magnetic stirrer for 5 h followed by sonication for 10 min (30% amplitude, half cycle of UP200S, Germany). The samples were then cast on glass plates to get thin films and dried at room temperature for 4–5 days to remove the traces of residual solvent. The systems were coded as PVCNC, PVCENC25, PVCENC50 and PVCENC75 for the compositions of PVC/ENO: 100/0, 75/25, 50/50 and 25/75 respectively with 5 wt% OMMT.

2.4. Microbial growth in presence of pristine PVC and PVC/ENO nanocomposite

The isolation, screening and subsequent selection of the potential strains capable of degrading PVC/ENO was based on the following three steps.

Step I: Enrichment culture technique was used to isolate bacteria from crude oil contaminated soil samples. Enrichment cultures were initially established by suspending crude oil contaminated soil samples (1.0 g) in 100 mL of M9 medium [19], to which added 1% (w/w) 25%, 50% and 75% (w/w) PVC/ENO systems as a sole source of carbon and energy.

The cultures were incubated at 37 °C with shaking at 200 rpm for 7 days. After 7 days, 1 mL inoculum was transferred to the fresh medium and incubated for 7 more days under the identical conditions. The process was repeated thrice and finally 1 mL inoculum from the third enrichment culture was serially diluted in sterile distilled water up to 10^{-7}.

Step II: 100 μL of 10^{-7} dilution culture was plated in the nutrient agar plates. The plates were incubated at 37 °C for 12 h. Plate count technique was used with the help of digital colony counter (Lapiz, India) for the determination of bacterial concentration in all samples. The process was repeated thrice. Subcultures were done by inoculating in fresh nutrient agar plates. Isolates exhibiting distinct colony morphology were isolated by repeated sub culturing in nutrient agar plates until purified strains were obtained (as confirmed by assessment of cellular and colony morphology). A total of 12 distinct isolates were obtained from enrichment culture.

Step III: All such cultures were supplemented with 25% (w/w) PVC/ENO as sole source of carbon and energy and incubated at 37 °C and 200 rpm for 7 days. Optical density was measured at 600 nm from 0 to 7 days of incubation. Those isolates able to grow on PVC ENO medium (OD value of 1.07 ± 1.0 and above) were selected further selected for PVC ENO degradation.

Growth Kinetics of bacterial strains used in this study viz., *P. aeruginosa* and *Achromobacter* sp. were grown on pristine PVC and PVC/ENO system was assessed by measuring the bacterial cell population (viable count), protein concentration and dry biomass with respect to time [19]. The flasks which have only PVC/ENO nanocomposites and no bacteria served as positive control and the flasks with only inoculums served as negative control system.

2.5. Identification of bacterial strains

Identification was done by studying the morphological and physiological-biochemical properties of bacteria and 16S rRNA gene sequencing [20].The primer sets were 5′-AAGGAGGTGATCCATCCAGCCGCA-3′ (forward primer) and 5′-AGAGTTTGATCCTGGCTCAG-3′ (reverse primer). The PCR conditions consisted of an initial denaturation at 96 °C for 5 min followed by 30 cycles of 92 °C for 1 min, annealing to primers at 55 °C for 2 min and extension at 72 °C for 5 min. PCR amplified product was separated using agarose gel electrophoresis.

2.6. Phylogenetic analysis

The 16S rRNA sequence of bacteria under study was aligned with reference sequences showing sequence homology from the NCBI database using the multiple sequence alignment programme of MEGA4 [21]. Phylogenetic trees were constructed by distance matrix-based cluster algorithms viz. unweighted pair group method with averages (UPGMA), neighbour joining [22], maximum-likelihood [23] and maximum-parsimony [24] analyses. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 638 positions in the final dataset. The trees were rooted using *Bacillus* sp. HSCC 1649T (accession no. AB045097) as out-group. The stability of the trees obtained from the above cluster analyses was assessed by using BOOTSTRAP program in sets of 1000 resamplings (MEGA 4).

2.7. Biodegradation of PVCENC75 in culture medium

Percent of biodegradation of PVCENC75 by individual bacterium was determined by quantifying the residual that remained in the flasks post inoculation at different time intervals [25]. The percent (%) of biodegradation and abiotic loss of PVCENC75 were evaluated by comparing the residual weight with the control sample as well as with initial weight of the same. The un-inoculated flasks served as control system (positive control). Residual of the samples in the

Table 1
Yield of bacterial dry biomass and protein content after 96 h of culture, values are mean ± S.D. of triplicate determinations.

Bacterial species	PVC/ENO	Cells/mL	Dry biomass (g/L)	Protein content (mg/mL)
Negative control	–	ND	ND	ND
P. aeruginosa	0	$0.5 \pm 0.01 \times 10^6$	0.22 ± 0.01	0.30 ± 0.01
	25	$0.8 \pm 0.02 \times 10^6$	0.36 ± 0.01	0.53 ± 0.02
	50	$1.6 \pm 0.01 \times 10^6$	2.17 ± 0.005	0.85 ± 0.008
	75	$2.3 \pm 0.007 \times 10^6$	2.24 ± 0.008	1.50 ± 0.01
Achromobacter sp.	0	$0.5 \pm 0.02 \times 10^6$	0.10 ± 0.005	0.25 ± 0.04
	25	$0.7 \pm 0.01 \times 10^6$	0.17 ± 0.04	0.35 ± 0.01
	50	$0.9 \pm 0.01 \times 10^6$	0.80 ± 0.01	0.68 ± 0.05
	75	$1.1 \pm 0.005 \times 10^6$	1.33 ± 0.01	0.93 ± 0.02

ND, not determined; S.D., standard deviation.

culture medium at the onset and after the end of the experiment was determined with standard gravimetric method.

3. Measurements

FTIR spectra of pristine PVCENC75 and biodegraded samples were recorded in FTIR spectrometer (Impact-410, Nicolet, USA) using KBr pellet. The surface morphology of the biodegraded samples was studied (post platinum coating) by a JEOL scanning electron microscope (JSM-6390LV SEM).

Universal testing machine (Zwick Z010, Germany) with 10 kN load cell was used to measure the tensile strength and elongation at break (as per the ASTM D 412-51T) of the samples at a jaw speed of 40 mm/min. The hardness of the films was measured using Shore-A hardness tester. The front impact strength test was carried out by falling ball method using an impact tester (S.C. Dey Co., Kolkata) with a maximum test height of 100 cm. In this test a weight of 850 g was allowed to fall on the film coated on a mild steel plate from minimum to maximum falling heights. The maximum height was taken as the impact resistance up to which the film was not damaged.

Thermogravimetric (TG) analysis was carried out by Shimadzu TG 50 using nitrogen flow rate of 30 mL/min and at heating rate of 10 °C/min.

The chlorine content of PVC, PVCENC75 and post biodegradation after 180 days was determined by Schoniger Oxygen Combustion method (S.C. Deys' & Co.). The chlorine content was calculated by the following equation.

$$N = \frac{[(C_1V_1 - C_2V_2) \times 0.0035 \times 100]}{m}$$

where C_1 and V_1 are the concentration and volume of $AgNO_3$ solution, C_2 and V_2 are the concentration and volume of ammonium thiocyanate solution and 'm' is the amount of polymer used in gram.

4. Results and discussion

4.1. Screening, isolation and pure culture of pristine PVC and PVC/ENO nanocomposite utilizing bacteria

Survival of microorganisms in the medium containing pristine PVC and PVC/ENO nanocomposite is a key deciding factor in the rate of biodegradation of these compounds in liquid culture medium. In the present study, a total of 12 pure bacterial isolates were obtained from the crude oil contaminated soil samples collected from oil well sites of Assam Asset, Assam, India. The isolates displayed differential abilities with respect to utilization of PVC/ENO as the sole source of carbon and energy. Out of the pure cultures, 02 bacterial isolates NBTU01 and NBTU02 showed better growth in M9 medium supplemented with 1% (w/w) of 75% (w/w) PVC/ENO systems as a sole source of carbon and energy. It was evident from the increase in bacterial growth rate with respect to time in nutrient agar plates (data not shown).

The bacterial strains utilized for biodegradation of pristine PVC and PVC/ENO nanocomposite were isolated from crude petroleum-oil contaminated soil samples collected from petroleum oil fields of North-East India. The bacteria were able to utilize PVC/ENO nanocomposite as the sole source of carbon and energy. This was vouched by the increase in bacterial dry biomass, protein content of culture supernatant and cell density post 96 h of incubation in different percentages of PVC/ENO system containing medium (Table 1). Bacterial cell density in presence of different percentages of PVC/ENO nanocomposite ranged from 1.1×10^6 to 2.3×10^6 mL^{-1} post 96 h of incubation. In sharp contrast, the growth of bacteria in absence of PVC/ENO nanocomposite (negative control) was negligible (Table 1).

4.2. Identification of bacteria strains

The strain NBTU01 was an aerobic Gram-negative rod, catalase and oxidase positive and did not hydrolyse casein. Colonies of the strains on Luria–Bertani (LB) agar medium plates were round, smooth, slightly mucoid, greenish in color, regular and opaque. Bacterial isolate NBTU01 was identified as *Pseudomonas species* on the basis of morphological and phenotypic characteristics. Sequence analysis of the 16S rRNA gene reveals 93–99% similarity to *Pseudomonas aeruginosa* strain. The phylogenetic tree constructed from the sequence data by neighbour joining method (Fig. 1) showed the detailed evolutionary relationships between strain NBTU01 and other closely related *Pseudomonas* species. 99% 16S rRNA sequence identity represented the closest phylogenetic neighbour of the strain NBTU01 as *Pseudomonas aeruginosa* (Gen Bank accession number HM103334) (Fig. 1). It is worthy to mention that

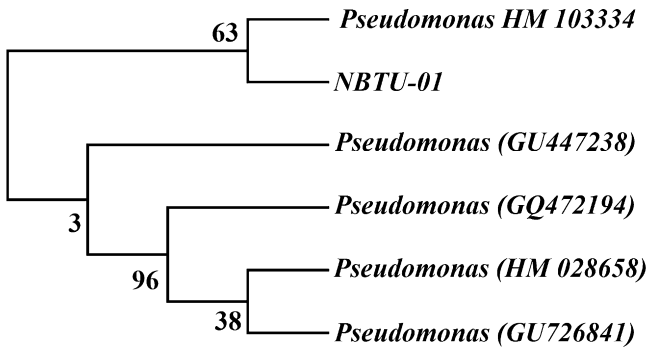

Fig. 1. Phylogenetic relationships of strain NBTU 01 and other closely related *Pseudomonas* species based on 16S rRNA sequencing. The tree was generated using the neighbour-joining method and the sequence from *Bacillus* sp. HSCC 1649T (accession no. AB045097) was considered as out-group. The data set was resampled 1000 times by using the bootstrap option, and percentage values are given at the nodes. Bar, 0.01 substitutions per site.

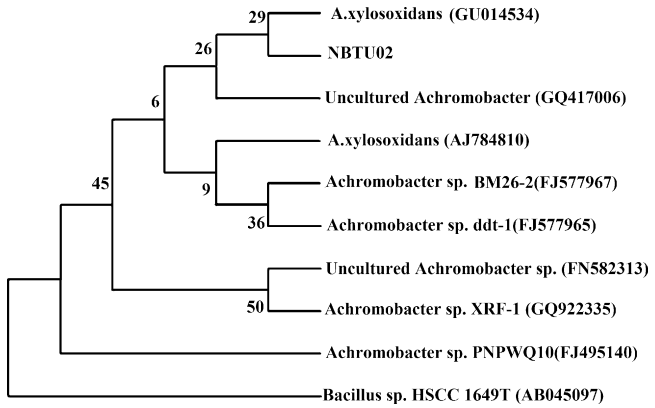

Fig. 2. Phylogenetic relationships of strain NBTU 02 and other closely related *Achromobacter* species based on 16S rRNA sequencing. The tree was generated using the neighbour-joining method and the sequence from *Bacillus* sp. HSCC 1649T (accession no. AB045097) was considered as out-group. The data set was resampled 1000 times by using the bootstrap option, and percentage values are given at the nodes. Bar, 0.01 substitutions per site.

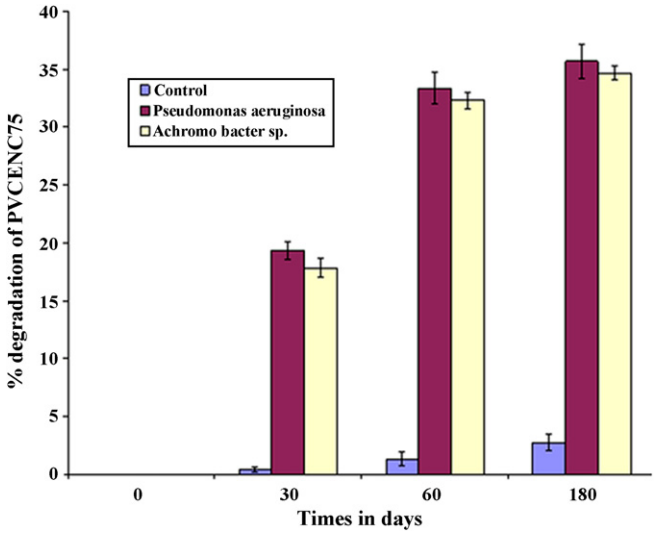

Fig. 3. Biodegradation of PVCENC75 by individual bacterial isolates, with standard deviation of ±0.07 for triplicate determinations.

topologies near NBTU01 (Gen Bank accession number JQ241432) estimated from the distance (neighbour-joining and UPGMA) and the maximum-likelihood and parsimony analyses were essentially consistent.

The strain NBTU02 was an aerobic Gram-negative rod-shaped strain. This strain was negative for the following tests: growth at 42 °C; acid production from glucose, xylose, mannitol, lactose, sucrose, and maltose. It showed positive tests for catalase, oxidase, urease and gelatin hydrolysis. Bacterial isolate NBTU02 was identified as *Achromobacter* sp. on the basis of morphological and phenotypic characteristics. Sequence analysis of the 16S rRNA gene of NBTU02 reveals 92–93% similarity to *Achromobacter* species. The 16S rRNA based phylogenetic analysis demonstrated 93.0% sequence similarity of NBTU02 with *Achromobacter xylosoxidans* strain J2 (Gen Bank accession number GU014534) reinforcing that NBTU02 belongs to the genus *Achromobacter*. Since the 16S rRNA resemblance was only 93% therefore, NBTU02 could not be placed under any recognized species of *Achromobacter*. The phylogenetic tree constructed from the sequence data by neighbour joining method (Fig. 2) showed the detailed evolutionary relationships between strains NBTU02 (Gen Bank accession number JN034907) and other closely related *Achromobacter* species and demonstrated a distinct phylogenetic position of this strain within the genus.

4.3. Biodegradation of PVC/ENO nanocomposite in culture

Biodegradation of PVC/ENO nanocomposite in the culture medium by bacteria of interest is an important criterion for successful bioremediation and waste management. The higher percentage (%) degradation of PVC/ENO nanocomposite by individual bacterial isolates was evident from the time course dependent decrease in PVC/ENO contents from culture medium (Fig. 3).

The percent (%) of biodegradation of PVCENC75 is shown in Fig. 3. It was demonstrated that *Pseudomonas aeruginosa* and *Achromobacter* sp. could degrade 35.65% and 34.62% PVCENC75 respectively, post 180 days of inoculation (Fig. 3). In contrast, the degradation of PVCENC75 in control flasks was only 2.28% under the identical conditions (Fig. 3). In the beginning of biodegradation, the rate of weight loss appeared very slow, owing to the fact that during this initial phase of biodegradation, microorganisms moved and adhered onto the surface of the PVCENC75, followed by further propagation. In the second phase of degradation, the weight of PVCENC75 decreased significantly which was primarily attributed to the biodegradation of ENO. For PVC/ENO inoculated with *P. aeruginosa*, this stage continued for 60 days, indicating higher amount of biodegradation. In the third stage, weight loss rate became slow, which suggested biodegradation of the PVC component of the system. This was catalyzed by the presence of both the ENO component and organoclay. Further the biodegradation was supported by the solution viscosities data of PVC, PVCENC75 and biodegraded samples after 180 days. Francbetti et al. [26] treated PVC film (20 ppm thick) with *Pseudomonas aeruginosa* in nutrient agar medium for a week at 37 °C, they observed apparent changes in flexibility and color. This was correlated to the superficial changes in the polymeric matrix by bacterial action. Infrared measurements on PVC films further confirmed the modifications of the polymeric matrix after bacterial attack. The influence of biodegradation by the *Aspergillus niger* on the diffusion desorption of a dialkylphthalate plasticizer from PVC was studied by Gumargalieva et al. [27]. They observed that the loss of dialkylphthalate accelerates with bio-overgrowth on the PVC. ENO, with its sufficient functionality such as ester, hydroxy, epoxy etc. initiate the biodegradation process. On the other hand clay helps in the adsorption of the bacteria onto its surface bringing it more close to the targeted groups (ester/hydroxy) and consequently causing hydrolysis/breakage, etc. Thus both clay and ENO helps the bacteria to come in close contact with the PVC chains thereby utilizing it as a source of carbon and energy. The biodegradation phenomenon of PVC/linseed oil blend by enzymatic hydrolysis was studied by Riaz et al. [11]. They observed that the degradability of the system increases with the increase in the epoxidized oil in the PVC matrix. They inferred that hydrolysis was responsible for the initial degradation process followed by microbial degradation in the blend films. Further, Romero and Castrejon [28] suggested that a suitable growth condition is necessary for the growth of microorganism on plasticized PVC.The dilute solution viscosities in THF (0.1%, w/v) of PVC, PVCENC75 and *Pseudomonas* sp. and *Achromobacter* sp. inoculated PVCENC75 after 180 days were determined. The viscosities of PVC was 1.8 dL/g, for PVCENC75 1.10 dL/g and for samples after biodegradation of 180 days by *Pseudomonas* sp. was 0.65 dL/g and for *Achromobacter* sp. it was found to be 0.86 dL/g respectively. These decreased indicated and chain scission of the PVC main chains by the studied bacteria. Which was further confirmed by the determination of the chlorine content, for PVC chlorine content was found out to be 52%, for PVCENC75 it was 12.5%, however after 180 days of inoculations there was a decrease in the chlorine content (*P. aeruginosa* 9.4% and *Achromobacter* sp. 10.2%). The

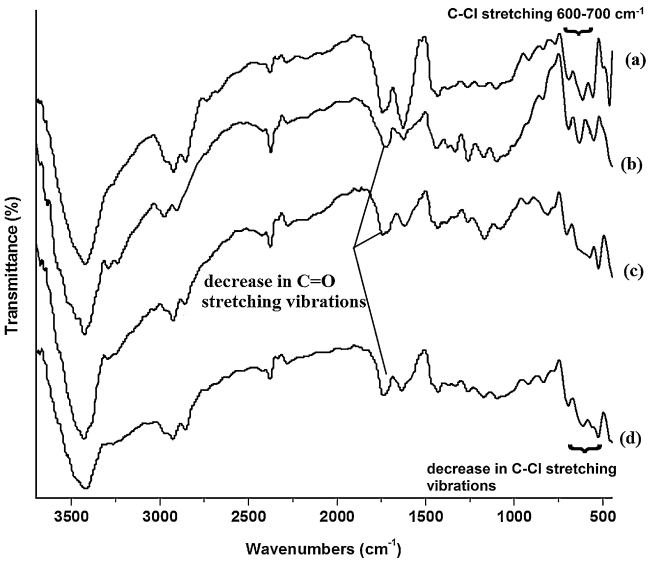

Fig. 4. FTIR spectra of PVCENC75 (a) control, (b) 180 days post treatment, (c) 60 days post treatment and (d) 30 days of post inoculation by *Pseudomonas aeruginosa*.

decrease in the chlorine content indicated the degradation of the PVC matrix.

The biodegradability of any system relies on its constituents. Since the presence of ester in the matrix may make it more susceptible to microbial attack [29–32]; therefore the presence of epoxidized oil in the PVC matrix renders biodegradability. PVC was degraded by the hydrolytic cleavage of the ester groups of ENO and was affected by the chemical structure and molecular arrangement of the polymer chain as well as the state of the polymer surface, since contact with microorganisms represents the first step in biodegradation process. The action of enzymes secreted by bacteria acted that on ENO resulting in the fragmentation of PVC and consequently a greater area of contact with the enzyme made it easier to degrade the system [33]. However, the presence of clay and its role in biodegradation cannot be neglected. As reported clay plays a catalytic role in the biodegradation of biodegradable material. Herein clay may help in absorption of more moisture and also provides an affective support or template for more proximal contact with the functional groups like the ester groups, hydroxyl etc. and consequently helps in hydrolysis of the ester groups of ENO in the PVC matrix [34]. It may be presumed that after absorbing the water, the terminal hydroxyl groups of clay in the presence of microbes caused heterogeneous hydrolysis of the ester groups.

4.4. FTIR analysis

Fig. 4 shows the FTIR spectra of pure PVCENC75 and treated samples with *P. aeruginosa* for 30, 60 and 180 days respectively. Transmittance data, on a common scale, showed that all bands in FTIR spectra of PVCENC75 (Fig. 4) decreased in size after biodegradation for 60 and 180 days. Reduction of band intensity related to carbonyl (1743 cm^{-1}) and ether (1102 cm^{-1}) suggested chain scission [35,36]. The intensity of the C—Cl stretching frequency (600–700 cm^{-1}) post biodegradation decreases. However the decrease in intensity after 180 days is more visible, the decrease can be related to the decrease in the VC–VC (vinyl chloride) units in the PVC main chain. This observation is further supported by the decrease in the chlorine percentage in the post biodegradation with *P. aeruginosa*. In all the above cases the relative absorbance of the carbonyl peaks decreased over the time course investigated. These changes are due to the hydrolysis of the ester bonds and hence the bacterial species grown in the media without any source of nutrients except for the PVCENC75 which was utilized as a source of energy. The FTIR study of *Archomobacter* sp. treated PVCENC75 also showed the similar result.

4.5. Morphology

The SEM images of the film prior to and post biodegradation showed significant changes in the topographical morphology as can be visualized from Figs. 5–7. For polymer prior to biodegradation and in buffer (control) the surface were rather smooth. In contrast, the surface erosion due to bacterial degradation becomes more and more prominent with increase in the test period. As discussed above ENO helps in the degradation process of the matrix as a whole the hydrolytic breakdown of the matrix by the bacterial enzyme instigated the degradation process. For samples taken out after 30 days of inoculation network of crater types structure can be seen to form on the surface of the films with *P. aeruginosa* inoculation (Fig. 5). However, for *Achromobacter* sp., the surface of treated material becomes rough with no surface penetration. The samples after 60 days showed higher surface erosion with significant morphological changes (Fig. 6). After 180 days of inoculation (Fig. 7) the bacterial penetrations to the inner surface can be observed [37]. The surface erosion property was more prominent for samples inoculated in *P. aeruginosa* strain.

Ester groups (O—CO) are known to be more easily hydrolysable than carbon—carbon bonds because of the oxygen adjacent to the carbon which provide a good nucleophilic center of attack for molecules like water in the presence of clay.

4.6. Performance characteristics

The tensile measurements were carried out for all samples and the data are presented in Table 2. For the sake of comparison, the tensile strength value of pristine PVCENC75 is also shown in this table. After 30 days of inoculation the variation of tensile strength is quite visible. *P. aeruginosa* degraded samples showed higher percent (%) of decrease in the tensile strength properties for the stipulated period of observation. After 30 days, a decrease of about 14.63% in tensile strength was observed, whereas samples treated for 60 and 180 days showed 32.70% and 53.83% decrease in tensile strength, respectively. It is noteworthy to mention that PVCENC75 inoculated with *Achromobacter* sp. displayed lower decrease in the tensile strength compared to *P. aeruginosa* (Fig. 8). The two faces were strongly eroded to leave sponge like structures (Figs. 5–7) for samples inoculated with *P. aeruginosa* for 30 and 60 days. The boundaries between these zones were very well defined because the boundaries were mainly composed of crystallite defects or amorphous material which could be preferentially degraded. The presence of both networks like structures and craters might reflect different degrees of degradation; the later reflects a more advanced degradation. The decrease of the tensile strength is practically inferred to the change of this surface morphology which leaves the PVC/ENO matrix with a great deal of voids.

Tensile strength measured after 180 days of inoculation exhibited more than 50% decrease for *P. aeruginosa* inoculated strain. The bacterial degradation seriously affected the structural integrity of the matrix and the chains were broken down into smaller fragments.

The elongation at break also decreased for all the samples post biodegradations as has been confer to the substantial loss of the ENO moiety. The degradation results in structural inhomogeneity, consequently affecting the plasticizer distribution in the matrix. Consequently the loss of plasticizer from the matrix leads to voids and as a result chain breakage occurs even at low stress. As shown in

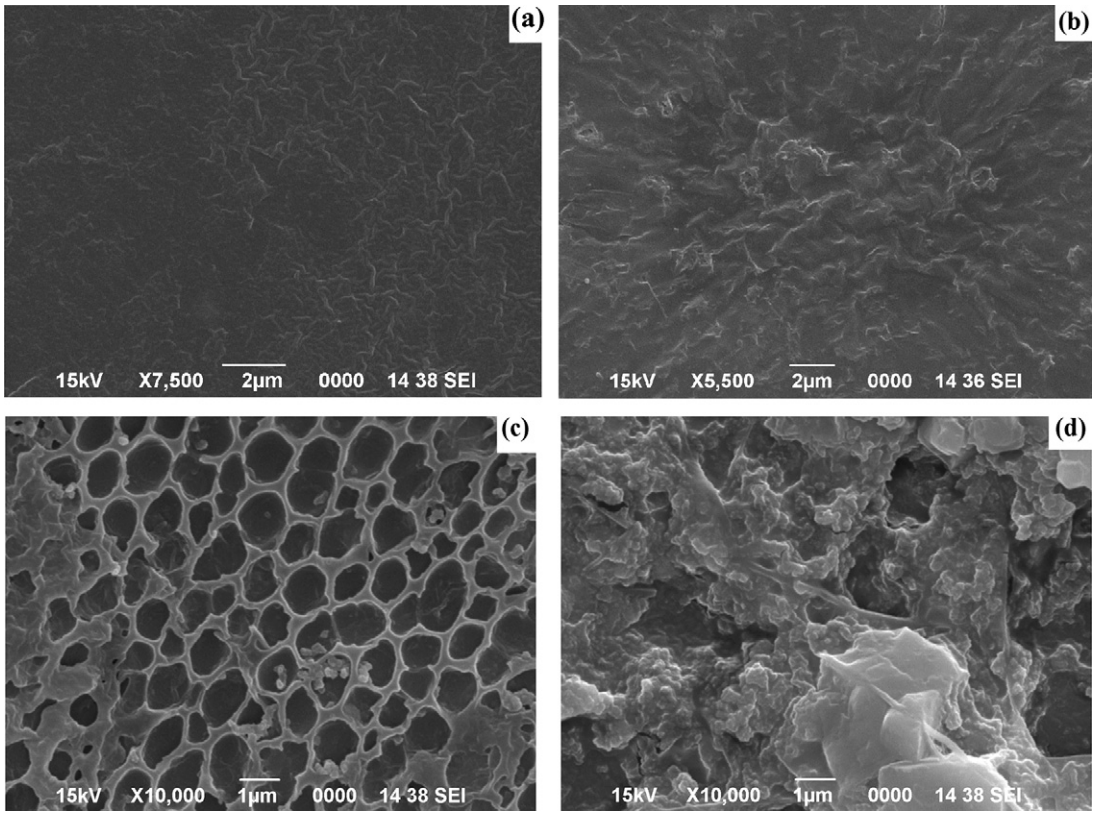

Fig. 5. SEM micrographs for PVCENC75 after 30 days of inoculations with (a) untreated PVCENC75, (b) positive control, (c) *Pseudomonas* sp. and (d) *Achromobacter* sp.

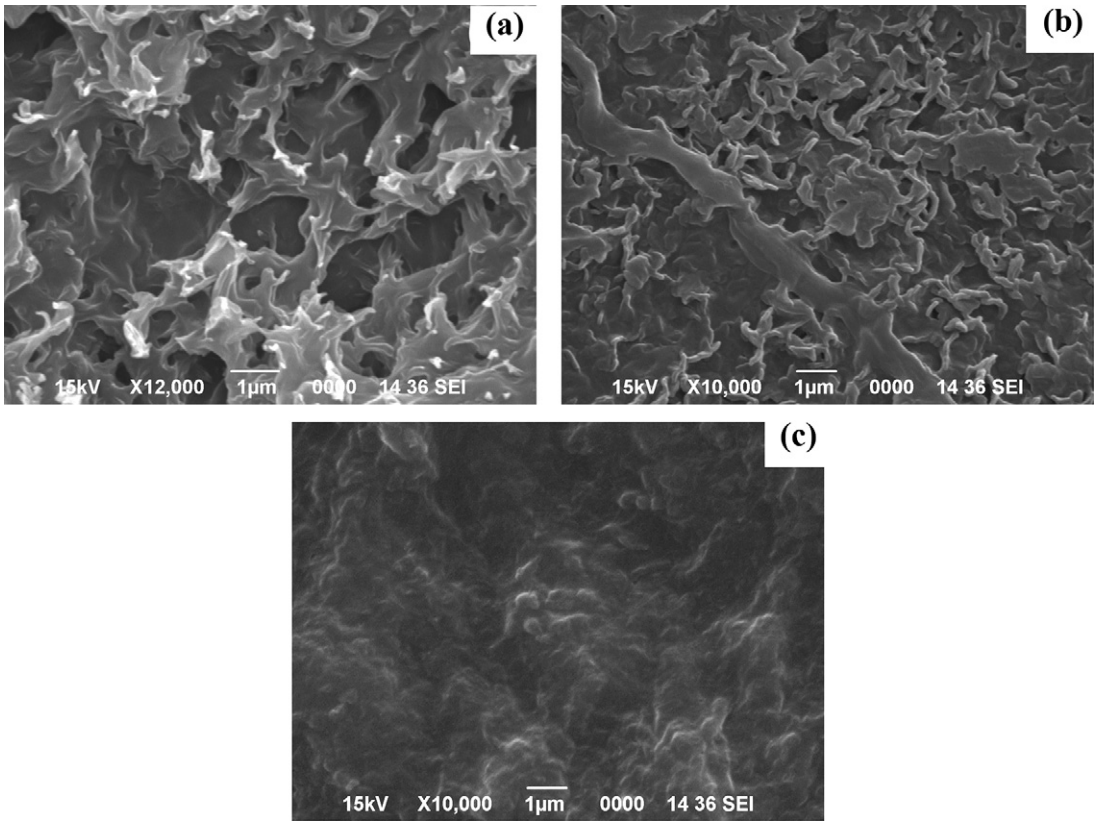

Fig. 6. SEM micrographs for PVCENC75 after 60 days of inoculations with (a) *Pseudomonas* sp., (b) *Achromobacter* sp. and (c) positive control.

Table 2
Performance characteristics for PVCENC75 after biodegradations, values are mean ± S.D. of triplicate determinations.

Samples	Durations (days)	Tensile strength (MPa)	Elongation at break (%)	Impact resistance (cm)	Hardness (shore-A)
PVCENC75	0	11.07 ± 0.51	94.09 ± 0.23	75 ± 0.20	89 ± 0.25
PVCENC75 inoculated with *P. aeruginosa*	30	9.45 ± 0.11	90.12 ± 0.21	70 ± 0.32	82 ± 0.23
	60	7.54 ± 0.34	86.13 ± 0.24	66 ± 0.12	78 ± 0.21
	180	5.11 ± 0.21	56.90 ± 0.32	52 ± 0.36	73 ± 0.29
PVCENC75 inoculated with *Achromobacter* sp.	30	10.34 ± 0.52	89.50 ± 0.38	73 ± 0.15	85 ± 0.48
	60	8.75 ± 0.31	87.21 ± 0.55	69 ± 0.71	80 ± 0.57
	180	6.21 ± 0.22	68.23 ± 0.17	63 ± 0.45	75 ± 0. 67

S.D., standard deviation.

Table 3
Various degradation patterns for PVCENC75 post biodegradation for 180 days.

Samples	Initial degradation temperature, T_0 (°C)			Peak temperature, T_{max} (°C)			End temperature, T_f (°C)		
	T_{01}	T_{02}	T_{03}	T_{max1}	T_{max2}	T_{max3}	T_{f1}	T_{f2}	T_{f3}
Control	–	276	410	–	303	471	–	343	591
PVCENC75 inoculated with *P. aeruginosa*	128	218	427	139	274	467	151	313	502
PVCENC75 inoculated with *Achromobacter* sp.	–	222	405	–	292	446	–	351	476

Table 2 the impact strength and hardness also suffered a significant loss post biodegradation.

4.7. Thermal properties

The change in the thermal stability was carried out for all the samples post biodegradation (Fig. 9). The thermal degradation pattern varied for the test period as well as for the bacterial species tested (Table 3). The decrease in the thermal stability of PVCENC75 was more in case of *P. aeruginosa* treatment compared to *Achromobacter* sp. treatment. After 180 days of biodegradation there was a substantial decrease in the thermal stability of PVCENC75. These results advocate the above observation of polymer biodegradation by the inoculated strains. The structural integrity of the plasticized PVC was greatly affected by the biodegradation, resulting in generation of low molecular weight species more prone to thermal degradation at low temperature. The thermal spectra reveal more weight loss in case of *P. aeruginosa* treated samples as compared to control sample post 180 days of inoculation (Fig. 9). However, in case of *Achromobacter* sp. treated PVCENC75 decrease in the thermal stability was less significant compared to *P. aeruginosa* treated PVCENC75.

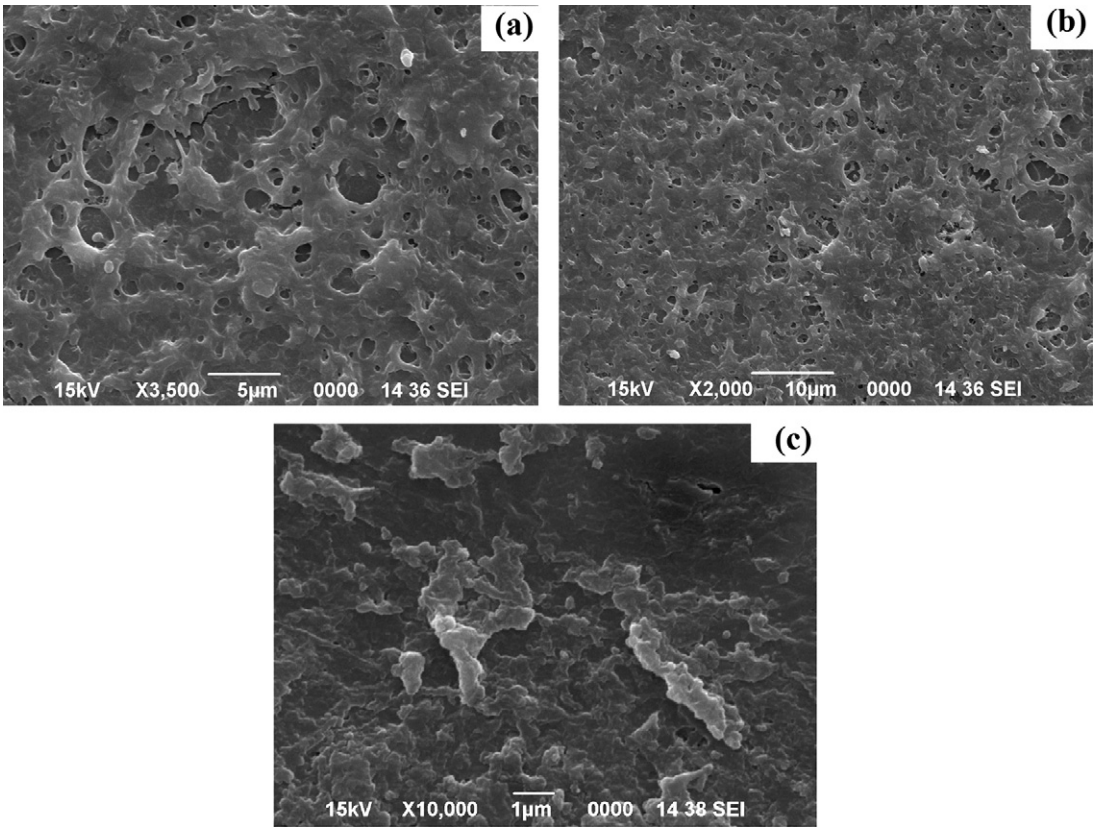

Fig. 7. SEM micrographs for PVCENC75 after 180 days of inoculations with (a) *Pseudomonas* sp., (b) *Achromobacter* sp. and (c) positive control.

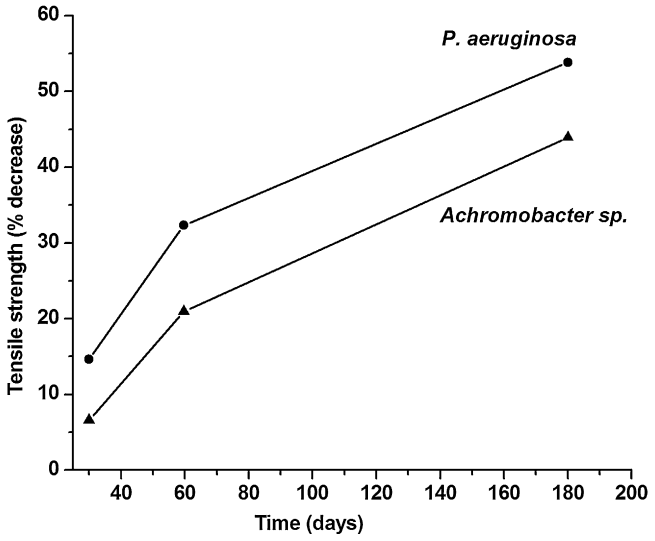

Fig. 8. Tensile curve of PVCENC75 post biodegradation with *Pseudomonas* sp. and *Achromobacter* sp. for 180 days, with standard deviation of ±0.3.

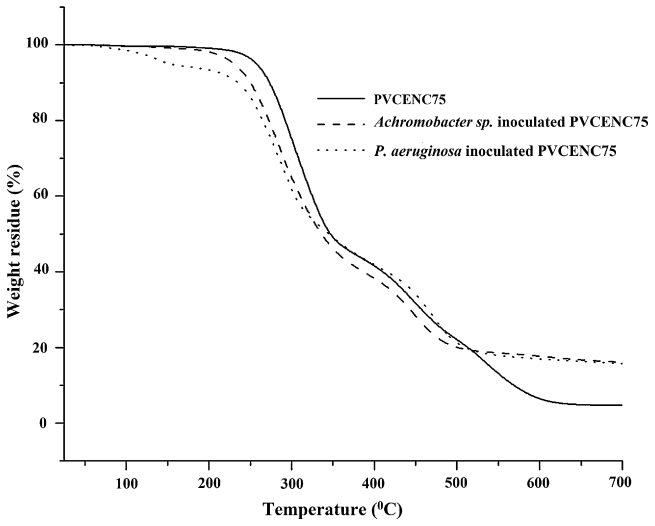

Fig. 9. TGA thermogram of PVCENC75 (a) control, (b) after 180 days inoculated with *Pseudomonas* sp. and (c) after 180 days inoculated with *Achromobacter* sp.

5. Conclusion

The study demonstrated that epoxidized *Mesua ferrea* L. seed oil significantly assisted the biodegradation of PVC and also can successfully be used as a plasticizer in the PVC matrix. PVC with varying content of ENO was subjected to microbial degradation under laboratory conditions. The degradation mechanism can be described as a three-stage process. The first stage involved colonization of microorganisms. The second stage was the degradation of ENO, whereas the final stage was achieved by degrading the PVC component. The biodegradability tests revealed that the composite with ENO content 75 wt% has the best biodegradability; the tensile loss at post 180 days of biodegradation exceeded 50%. The study also demonstrated that both *P. aeruginosa* and *Achromobacter* sp. strains were effective in degrading the system.

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