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Spectroscopic characterization and microbial degradation of engineered bio-elastomers from linseed oil

Abstract: The microbial degradation of elastomers synthesized through the cationic polymerization reaction of linseed oil, styrene, and divinylbenzene was investigated by using the *Alkaliphilus oremlandii OhILAs* strain. In Fourier transform infrared (FTIR) analysis, the bound oil content in the elastomers was found to vary from 29.63 to 45.5 wt%, whereas the percentage of unreacted oil in the elastomers were in the range of 12.9–38 wt%. In ^{1}H nuclear magnetic resonance spectrum analysis, the unreacted oil and unreacted aromatic components in the elastomers were obtained in the ranges of 13.2–39 wt% and 6.8–16 wt%, respectively. The amount of unreacted oil in the elastomers enhanced the percentage of biodegradation, which varied from 26 to 51 wt%. The biodegradation of elastomers was also confirmed by FTIR and scanning electron micrograph analyses.

Keywords: cationic polymerization; degradation; elastomers; linseed oil; spectroscopic quantification.

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1 Introduction

In recent years, there has been growing interest in biopolymers because of increasing environmental awareness and the need for carbon fixation. This trend encourages researchers to explore new biopolymer resources [1]. Natural oils are versatile renewable resources for producing functional polymeric materials [2–5] because of their low production cost, universal availability, and biodegradability. Larock et al. [6, 7] have produced a variety of polymers by direct polymerization of native natural oil with styrene (ST) and divinylbenzene (DVB) initiated by a cationic initiator, boron trifluoride diethyl etherate (BFE). Wool et al. [3, 8] have synthesized rubbers, rigid composites, and adhesives through the free radical polymerization of a chemically modified oil. Petrovic et al. [9, 10] have developed polyurethanes from soy polyols derived from epoxidized vegetable oils. In recent years, polymers have been synthesized from vegetable oils by using relatively new polymerization techniques such as acrylic metathesis polymerization [11] and ring-opening metathesis polymerization [12, 13].

As biopolymers are biodegradable in nature, the study of biodegradation can provide fundamental information facilitating the design and lifetime analysis of materials in industrial applications, and also predicts their possible degradation after the end use. Biodegradation is the degradation caused by biological activity, especially through the action of microbial metabolism. Biodegradation causes a significant change in the chemical structure of exposed materials, which results in the production of carbon dioxide or mineral salts (mineralization) or new microbial mineral constituents (biomass) [14, 15]. Microorganisms responsible for polymer degradation use polymers as a potential source of carbon and energy during the degradation process in a requisite environment [16]. Biodegradation studies of polymers are generally carried out in different environmental testing conditions, such as in soil, seawater, compost, sludge, simulated microbial culture, etc. [17, 18], followed by the determination of biodegradability by using different testing methods such as weight loss measurement, evaluation of the amount of evolved CO_2 or consumed O_2, evaluation of reaction rate constants, etc. [19, 20]. The most reproducible biodegradation tests are laboratory tests where definite media are used and inoculated with either a mixed microbial population (e.g., from wastewater) or individual microbial strains that may have been especially screened for a particular polymer. Among the different testing methods, weight loss analysis is widely used for the estimation of the percentage of degradation. In this analysis, it may be concluded that weight loss of an exposed polymer will not

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occur because of the removal of its volatile and soluble impurities.

Spectroscopic techniques are very powerful tools for characterizing different polymers [21, 22]. These techniques are very useful for the structural analysis of polymers both qualitatively [23] and quantitatively [24]. Quantitative analysis of polymers, especially copolymers and polymer blends, is very important in studying their structures and different properties [25–28]. Many research works have been reported on the quantitative analysis of polymers by using Fourier transform infrared (FTIR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopies [29–33].

Among the different natural oils, linseed oil is the most abundant and a cheap non-edible oil [34]; it is used mainly for the preparation of paints and varnishes owing to its good drying property [35]. It is also used in enamels, linoleum, oilcloth, patent leather, printer's ink, and as waterproofing material for raincoats, slickers, and tarpaulins [36]. In medicine, linseed oil is used to treat several ailments such as anxiety, prostate problems, vaginitis, weight loss, and certain types of cancer [37].

In the present work, a wide variety of bio-elastomers were developed through cationic polymerization of regular linseed oil. During the cationic polymerization process, ST was used as a comonomer. DVB alone or DVB with dicyclopentadiene (DCP) was used as a cross-linker, and the reaction was initiated by using a modified BFE initiator. From a technical point of view, these bio-elastomers will have mechanical stability, enhanced vibration damping performance, lower production cost, and improved biodegradability. The cheapest monomer, linseed oil, was used at the maximum possible amount in the original feed composition. In addition, the DVB content was also reduced by using low-cost DCP in a controlled manner to obtain more commercially viable elastomers. There are no reports about such a variety of elastomers synthesized from linseed oil, although the vibration damping performance in engineering applications and the mechanical stability of these elastomers have been analyzed [38, 39]. A structural analysis with quantification of the elastomers was performed by using FTIR and ^{1}H NMR spectroscopies. Larock et al. [6, 7] have quantitatively studied only the soluble part after Soxhlet extraction of the oil-based polymers through ^{1}H NMR. In this work, the insoluble part after Soxhlet extraction was quantified by using the attenuated total reflection (ATR) method of FTIR to predict the structure of the main polymer chain. The soluble part after the Soxhlet extraction of these elastomers was also quantitatively analyzed with both ^{1}H NMR and ATR-FTIR. The degradation of the elastomers was studied by using the *Alkaliphilus oremlandii OhILAs* strain in an optimum growth condition. In this microbial degradation process, the bacterial cell biomass was produced using elastomers as a potential energy source. Analysis of weight loss due to degradation gives an idea about the degradation behavior of the elastomers. There is no reported work on degradation studies of vegetable oil-based functional polymers. Thus, this research work provides knowledge about the degradability of linseed oil-based elastomers.

2 Materials and methods

2.1 Materials

The linseed oil used in our study was obtained from the V. M. Oils Private Limited, Kolkata, India. ST, DVB (55 mol% DVB and 45 mol% ethylvinylbenzene), and the BFE complex were purchased from Sigma-Aldrich (USA). DCP was purchased from Merck (Germany). Dichloromethane and concentrated sulfuric acid were purchased from Merck (India). Potassium hydroxide was purchased from SRL Chemical Co. (India).

2.2 Synthesis of the elastomers

The elastomers were synthesized by using the cationic polymerization technique. BFE was used as the initiator molecule; it was modified before polymerization to reduce the reactivity of the initiator for homogeneous polymerization. The initiator was modified by mixing the methyl ester of linseed oil at a weight ratio of 3:5 with constant stirring at 0°C. The methyl ester of linseed oil was used instead of regular linseed oil for better miscibility with the initiator, which caused the uniform distribution of the initiator in the reaction mixtures.

Methyl ester was prepared in two consecutive transesterification steps (i.e., the trans-esterification double-step method) [40]. The acid-base catalyst was used to prevent the saponification of the ester produced. Initially, an alkali agent was dissolved in methanol (1 g KOH in 40 ml methanol) at 45°C. Then, 40 ml of this solution was added to 100 ml of linseed oil with vigorous and constant stirring. The resulting mixture was refluxed in a two-neck 250-ml round-bottomed flask at 65°C under an inert atmosphere for 1 h. The molar ratio of alcohol/oil was 10:1 and the catalyst/alcohol ratio was 0.00178:1. The equivalent volume of these molar ratios were calculated by taking the density of linseed oil to be 0.93 g/ml and its molecular weight to be 890 g/mol, and taking the density of methanol to be 0.7912 g/ml. Then, the mixture was cooled to room

temperature and 60 ml of methanol and 1.5 ml of sulfuric acid (18 mol/l) were added, followed by heating at 30°C. The heating of the system stabilized again in the reflux condition for approximately 1 h. After this period, the system was cooled slowly to approximately 25°C. At the end of this step, the formation of two phases occurred. The two phases were separated and processed further. The upper phase was washed with cold water, and the residual alcohol was removed by evaporation under vacuum. The formation of fatty acid methyl ester was confirmed by ^{1}H NMR.

Polymeric materials were prepared by heating of the desired concentration of regular linseed oil, ST, and DVB in glass molds. The desired amounts of ST and DVB were added to the linseed oil, and the mixture was vigorously stirred. Then, the mixture was cooled and the initiator was added slowly with constant stirring at low temperature (≈0°C). The resulting mixture was transferred to a glass mold (100×100×3 mm) after ensuring homogeneous mixing, and the mold was then sealed with silicone adhesive. Then, the mold was kept at room temperature for 12 h and heated sequentially at 60°C for 12 h, 110°C for 24 h, and finally post-cured at 120°C for 3 h. In our experiment, the linseed oil percentage in the original composition of the samples was varied from 45% to 65%, and the ST and DVB internal ratio was taken as 3:2 in SET I, 1:1 in SET II, and 2:1 in SET III. The initiator was maintained at 8% level in all samples. In SET IV, the samples were identical to the samples of SET I, SET II, and SET III containing 50% and 55% oil, except that the DVB content was partially replaced by DCP. The resulting polymers were washed with ethanol to remove the catalyst. The detailed feed compositions of the different samples are given in Table 1.

Table 1 Detailed feed compositions of the different elastomers.

SET	Sample ID	Linseed oil (wt%)	ST (wt%)	DVB (wt%)	DCP (wt%)	Initiator (wt%)
I	S1Lin45	45	28	19		8
	S2Lin50	50	25	17		8
	S3Lin55	55	22	15		8
	S4Lin60	60	19	13		8
II	S5Lin50	50	21	21		8
	S6Lin55	55	18.5	18.5		8
	S7Lin60	60	16	16		8
	S8Lin65	65	13.5	13.5		8
III	S9Lin45	45	31	16		8
	S10Lin50	50	28	14		8
	S11Lin55	55	25	12		8
	S12Lin60	60	21	10.5		8
IV	D1Lin50	50	25	8.5	8.5	8
	D2Lin50	50	21	10.5	10.5	8
	D3Lin50	50	28	7	7	8
	D4Lin55	55	22	7.5	7.5	8
	D5Lin55	55	18.5	9.25	9.25	8
	D6Lin55	55	25	6.25	6.25	8

2.3 Soxhlet extraction

The elastomeric materials were subjected to Soxhlet extraction to determine the unreacted part in the polymer. During the Soxhlet extraction, 2 g of the bulk polymer was extracted for 24 h with dichloromethane by using a Soxhlet extractor. After extraction, the resulting solution was concentrated by simple distillation at atmospheric pressure and subsequently dried under vacuum to obtain the unreacted part. The insoluble part was dried under vacuum for several hours before weighing.

2.4 FTIR spectrometry

The dried insoluble part and liquid soluble part after Soxhlet extraction were analyzed by the ATR method of FTIR spectrometry. For the microbial degradation study, the elastomers before and after degradation were also analyzed by the ATR method of FTIR. A total of 42 scans at 4 cm^{-1} resolution were collected to obtain an average absorbance spectrum by using a Bruker FTIR spectrophotometer (model: Alpha-E).

2.5 ^{1}H NMR

The extracted soluble part of the polymeric material as well as the linseed oil, methyl ester of linseed oil, ST, and DVB were dissolved in $CDCl_3$. Tetramethylsilane was used as an internal standard. The solution was scanned with a multinuclear FT-NMR spectrometer from Bruker (model: DRX 500) at 500 MHz. The unreacted linseed oil and the aromatic content of the soluble extractable part were calculated from peak integral values of the characteristic peak of the different components in the ^{1}H NMR spectrum [32].

2.6 Microbial degradation study of the linseed oil-based elastomers

For the microbial degradation test of the elastomers, the *A. oremlandii OhILAs* strain was isolated, using the serial dilution method, from rhizosphere soil collected from the University of Calcutta campus (Kolkata, India). The isolated colonies of *A. oremlandii OhILAs* were used to test the

degradability of the elastomers. The degradation of different linseed oil-based elastomeric films (weight 4 g, dimension 10×10×0.8 mm) by the screened microorganism was carried out in a 100-ml growth medium at an optimum pH of 8. The nutrient medium (g/l) was composed of NH_4NO_3 (3), K_2HPO_4 (0.64), KH_2PO_4 (0.2), $MgSO_4.7H_2O$ (0.4), $CaCl_2.2H_2O$ (0.1), $FeSO_4.7H_2O$ (0.01), $Na_2MoO_4.2H_2O$ (0.006), and Na-citrate (0.5). The elastomer was used as a source of carbon and nutrients for microbial cell growth. The elastomer was immersed in the above medium solution. For measurement of the residual weight and extent of degradation, the medium solution and the immersed elastomeric films were sterilized in an autoclave at a pressure of 50 psi and at 121°C temperature for 65 min. Then, these were incubated at 30°C with shaking in a shaker incubator (105 rpm), and the films were withdrawn after a definite reaction time, washed with phosphate-buffered saline, and dried at 50°C to a constant weight in an oven for analysis. The degradation of polymers in the liquid medium was observed by weight loss analysis of the elastomeric film in the definite time interval. The cell density of the growth medium was simultaneously measured through optical density measurement with a UV-VIS spectrophotometer from Optizen (Korea; model: Optizen POP Nano Bio) at a wavelength of 600 nm. In the first stage, the maximum degradation and stationary state of bacterial cells were observed after a 7-day incubation period. In the second stage, the degraded films from the first stage were again added to a fresh culture medium; however, no significant degradation was observed owing to the unavailability of a carbon source for bacterial cell growth. The percentage of degradation (D) was calculated using the equation

$$D=\frac{m_0-m_t}{m_0}\times 100\,\%, \quad (1)$$

where m_0 is the initial weight of elastomeric film and m_t is the weight of the film after t incubation time. The polymer formed in bacterial cells was extracted using the chloroform-hypochlorite method [41]. After complete degradation of linseed oil-based polymer in the culture medium, 10 ml of culture was centrifuged for 10 min at 6000 rpm. The pellet was collected and washed with saline solution followed by centrifugation in the same way. Then, the pellet was disrupted with a sodium hypochlorite solution, followed by centrifugation of the solution and purification in the presence of chloroform and methanol, which resulted in the production of pure poly(3-hydroxybutyrate-co-3-hydroxyvalerate [P(3HB-co-3HV)] with 90% yield of dry cell weight. This polymer was characterized using FTIR and ^{1}H NMR.

2.7 Scanning electron microscopy analysis

The physical changes on the surface of the films were observed with a scanning electron microscope (SEM) (Carl Zeiss, Germany; model: EVO 18), using an acceleration voltage of 15 kV. The samples were observed before and after the biodegradation experiments.

3 Results and discussion

3.1 Soxhlet extraction

The polymeric samples were extracted to determine their insoluble and soluble contents, and the results are shown in Table 2. In all samples of SET I, SET II, and SET III, it was observed that the soluble part after extraction increased with increasing linseed oil content in the original compositions of the samples. The variation of the soluble part after extraction with the increase in linseed oil content in the original sample compositions is shown in Figure 1A. In Figure 1A, in samples with 50% linseed oil content, SET II contained the lowest amount of soluble portion, whereas SET III contained the highest amount of soluble portion, and there was an obvious increase in the soluble portion with an increase in oil content percentage. The samples of SET II had the highest DVB content, whereas the samples of SET III had the lowest DVB content. DVB is very reactive and acts as a cross-linker in the cationic polymerization of linseed oil. Thus, an increase in DVB content increases the stability of the polymer chain; as a result, the solubility of the polymer decreases. In SET IV, the solubility of the sample increased when the DVB content was partially replaced by the less reactive DCP, keeping the linseed oil and ST contents unchanged. For reference, the comparison between sample 2 and sample D1 revealed that the soluble part after extraction increased from 24% to 32% when the DVB content was reduced and replaced by 8.5% of DCP, keeping the linseed oil and ST contents constant. The same results were obtained from the comparison of other samples of SET IV with the samples of SET I, SET II, and SET III having identical compositions. In SET IV, the effect of the cross-linker (DVB/DCP) was also observed. The variation of the soluble part after extraction with the DVB/DCP content is shown in Figure 1B. The soluble part after extraction decreased with an increase in DVP/DCP content. The effects of DVB and DCP contents on the resulting elastomers were the same because both compounds act as a cross-linker in the polymerization reaction.

Table 2 Soxhlet extraction results and quantitative analysis results from FTIR and ^{1}H NMR.

SET	Sample ID	Soxhlet results		FTIR results		^{1}H NMR results	
		Soluble	Insoluble	Bound oil content	Unreacted oil content	Unreacted oil content	Unreacted aromatic part content
I	S1Lin45	20	80	38.4	12.9	13.2	6.8
	S2Lin50	24	76	46.7	13.2	16.4	7.6
	S3Lin55	32	68	43.6	20.5	22.5	9.5
	S4Lin60	40	60	40.1	27.6	29	11
II	S5Lin50	21.6	78.4	45.5	12.5	13.4	8.2
	SLin556	30.4	69.6	44	18.9	20.4	10
	S7Lin60	41	59	39.5	28.5	28.7	11
	S8Lin65	52.4	47.6	34.9	38	39.4	13
III	S9Lin45	25.3	74.7	38.5	13.3	13.4	11.9
	S10Lin50	28.7	71.3	42.3	16.6	16.3	12.4
	S11Lin55	40	60	37.7	25.5	25.5	14.5
	S12Lin60	51.7	48.3	32.8	34.9	38.2	13.5
IV	D1Lin50	32	68	37.8	20.2	20.5	11.5
	D2Lin50	27	73	40.4	17.6	17.6	9.5
	D3Lin50	34	66	36.6	21.4	22	12
	D4Lin55	51	49	28.8	34.2	33.8	17.3
	D5Lin55	37.4	62.6	37	26	26.6	7.5
	D6Lin55	53	47	27.2	36.4	36.4	16.8

3.2 FTIR spectroscopy analysis of the elastomers

The quantitative analysis of the soluble and insoluble parts of the linseed oil-based polymer was carried out by using FTIR spectrometry. The representative FTIR spectra of linseed oil and the soluble and insoluble parts of S5Lin50 are shown in Figure 2. The results of the analytical evaluation of the FTIR spectra are given in Table 3 [42, 43]. The absorbance peak at 1741 cm^{-1} was taken as the characteristic peak of linseed oil and the peak at 690 cm^{-1} was taken as the characteristic peak of the aromatic content. In the FTIR spectrum of the soluble part, the peak intensity at 690 cm^{-1} was lower than the peak intensity at 1741 cm^{-1}. However, in the spectrum of the insoluble part, the reverse was observed. The overlapping absorbance peaks of the -CH_2 rocking vibration and the out-of-plane vibration of *cis*-disubstituted olefins at 720 cm^{-1} in linseed oil disappeared in the spectra of the soluble and insoluble parts. The absorbance values at 1741 cm^{-1} (A_{1741}) and 690 cm^{-1} (A_{690}) were recorded to calculate the unreacted oil content (wt%) in the soluble part as well as the bound oil content (wt%) in the resulting elastomers. The ratio of absorbance at 1741 cm^{-1} to that at 690 cm^{-1} (i.e., A_{1741}/A_{690}) had a higher value in the soluble part than in the insoluble part. The calibration curves for determining the oil content in both the soluble and insoluble parts are shown in Figure 3. The details of the quantitative results are shown in Table 2. The unreacted oil content in the resulting elastomers was in the range of 12.9–38 wt%. The unreacted oil content increased with increasing linseed oil content in the original sample compositions. The unreacted oil and the soluble portion present in the sample helped in the plasticization of the cross-linked insoluble polymers. The insoluble part was the cross-linked elastic part, and it had a major effect on the properties of the elastomers. The bound oil contents in the insoluble part were in the range of 29.6–45.5 wt%. The bound oil content decreased with an increase in linseed oil content and a decrease in aromatic content. This indicates that the main polymer chain was composed of cross-linked polymers of ST and DVB in which the linseed oil molecule was grafted. In the samples of SET IV, DVB was partly replaced by DCP to reduce the cost of the production of the elastomers. These materials were compared with the elastomers of SET I, SET II, and SET III. When DVB was totally replaced by DCP, the resulting products became a highly viscous gel. The bound oil content of D1Lin50 was less than that of S2Lin50 because in D1Lin50, the DVB content was partially replaced by the less reactive DCP. In the polymerization reaction, DCP acted both as a cross-linker and as a comonomer. The main polymer chain consisted of ST-DVB-DCP in which the triglyceride unit of linseed oil was grafted.

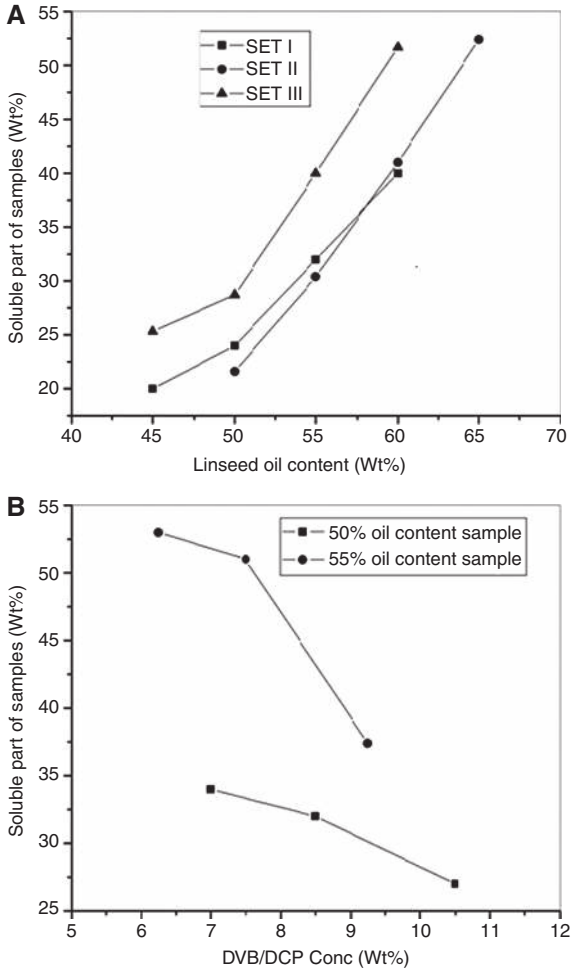

Figure 1 Soxhlet extraction results of different elastomers of (A) SET I, SET II, and SET III, and (B) SET IV.

3.3 ^{1}H NMR characterization of the elastomers

The ^{1}H NMR spectra of linseed oil, methyl ester of linseed oil, ST, DVB, and the soluble extract from the cationic polymer (S2Lin50) are shown in Figures 4 and 5. Here, the ^{1}H NMR spectra of the soluble extracts of S2Lin50 were representative of the soluble extracts obtained from all other samples. The analysis of the ^{1}H NMR spectra is shown in Table 4. In linseed oil, the methylene protons (CH_2) of the glycerol unit detected at 4.1–4.3 ppm was taken as the characteristic peak. In the NMR spectrum of methyl ester (Figure 4A), the appearance of a new peak at 3.7 ppm and the disappearance of the peak at 4.1–4.3 ppm confirmed the formation of methyl ester [39]. The vinylic protons of linseed oil were detected at 5.33–5.39 ppm, and those of ST and DVB were detected at 5.2–6.7 ppm.

The ^{1}H NMR spectrum of the soluble extract of S2Lin50 is the representative spectrum of the soluble extract of all samples. The peak at 4.1–4.3 ppm in the soluble extract of S2Lin50 is due to the ethylene proton of the glycerol unit of the linseed oil monomer and the low molecular weight oligomer of linseed oil. This is basically the characteristic peak in the unreacted linseed oil in elastomers. It was used to calculate the wt% of the unreacted linseed oil present in the soluble extractable part of the elastomers as a monomer and as a low molecular weight oligomer form. The aromatic portion of ST and DVB, the oligomeric portion of these materials, and the ST-DVB copolymer were observed between 7.2 and 7.5 ppm. These peaks were assigned to the aromatic proton of the benzene ring and were distinctive for the calculation of the aromatic content in the soluble extracts. The peaks of the vinylic protons of linseed oil and the aromatic components overlapped partially in the spectrum. The quantitative analysis results are shown in Table 2. The results obtained by ^{1}H NMR corroborated with the results obtained through FTIR. From Table 2, it is clear that in the soluble extract, the unreacted oil content was always higher than the unreacted aromatic content. As linseed oil is the least reactive among the different monomers, a large amount of linseed oil is present in the soluble extract of all samples. The linseed oil content in the soluble extract increased with increasing linseed oil content and decreasing aromatic content in the original sample compositions. The unreacted oil contents of the 45% and 50% oil content samples of SET I and SET III were almost same, and the samples of SET II had the minimum amount of unreacted oil. However, in samples with 55% and 60% oil content, the unreacted oil content was minimum in SET II and maximum in SET III. As linseed oil was grafted in the ST-DVB polymer chain, a large amount of linseed oil remained unreacted in the sample containing a high percentage of oil owing to the lack of reactive groups of ST and DVB. The ST and DVB ratio in SET II was 1:1, in SET III was 1.97:1, and in SET I was 3:2. Thus, the numbers of the available reactive groups of DVB were maximum in SET II and minimum in SET III. As a result, the amounts of unreacted oil were maximum in SET III and minimum in SET II in samples containing a high percentage of oil. The unreacted aromatic contents of the samples of SET I were minimum and of SET III were maximum, although all samples had the same amount of aromatic parts in the original composition. The ratio of ST and DVB is very crucial for a controlled polymerization reaction. DVB is very reactive, and at an early stage of prolonged polymerization a certain amount of DVB homopolymer was formed and then the linseed oil grafted ST-DVB copolymer was formed. When the DVB content is

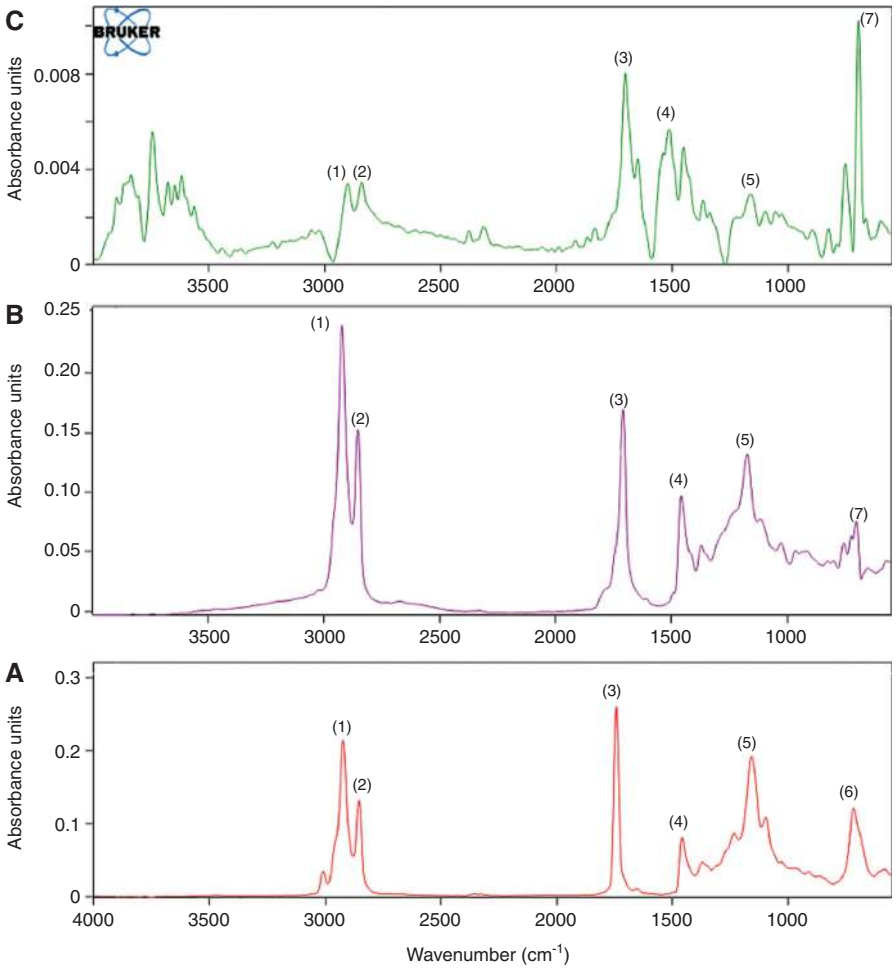

Figure 2 FTIR spectra of (A) linseed oil, (B) soluble extract of S5Lin50, and (C) insoluble extract of S5Lin50.

similar to the ST content, the possibility of homopolymer formation increases; owing to this homopolymer, a phase separation occurred at the early stage of polymerization. This homopolymer was extracted in solvent during Soxhlet extraction. As the ratio of ST to DVB was 1.47:1 in SET I and 1:1 in SET II, a copolymer of ST-DVB was formed in a more controlled manner in SET I than in SET II. Thus, the unreacted aromatic content was higher in SET II than in SET I, although SET II contained a higher amount of reactive DVB than did SET I. In SET IV, the unreacted

Table 3 Evaluation of the FTIR spectra.

Wave number (cm^{-1})	Band origin	Assignments
≈2900 (1)	$-CH_2$	Asymmetric stretching vibration modes of the methylene group of linseed oil
≈2850 (2)	$-CH_2$	Symmetric stretching vibration modes of the methylene group of linseed oil
≈1740 (3)	C=O	Stretching vibration modes of the ester linkage of linseed oil
≈1450 (4)	$-CH_2$	Asymmetric bending vibration modes of the methylene group of linseed oil
≈1150 (5)	C-O-C	Stretching vibration of the ether linkage of linseed oil
≈720 (6)	$-CH_2$	Overlapping of the $-CH_2$ rocking vibration and out-of-plane vibration of *cis*-disubstituted olefins of linseed oil
≈700 (7)		Out-of-plane bending vibration of the aromatic ring of styrene and divinylbenzene

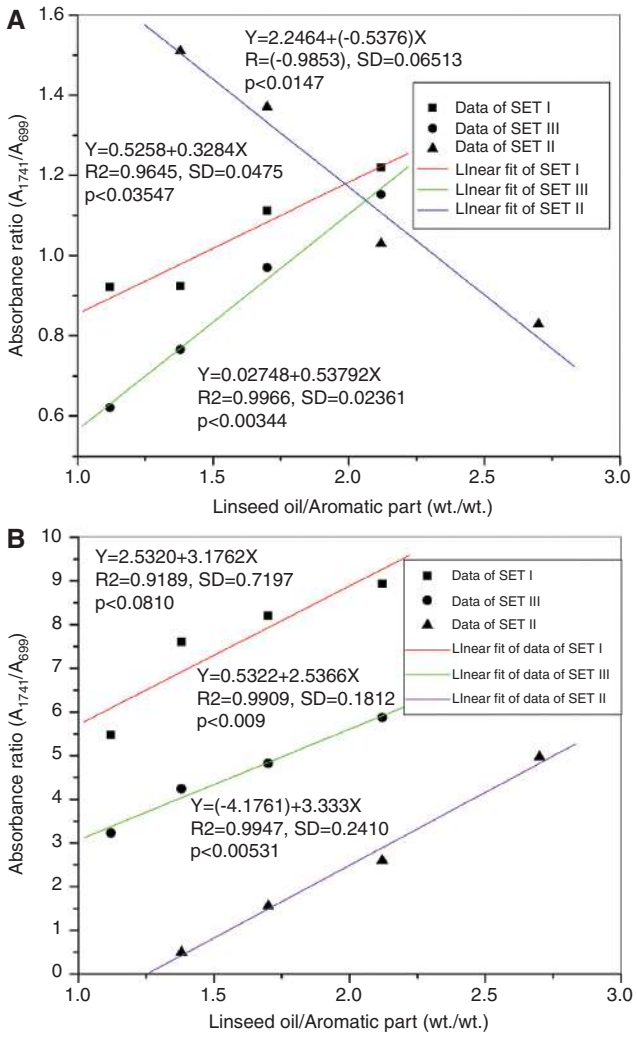

Figure 3 Regression calibration curves of (A) soluble extracts and (B) insoluble extracts.

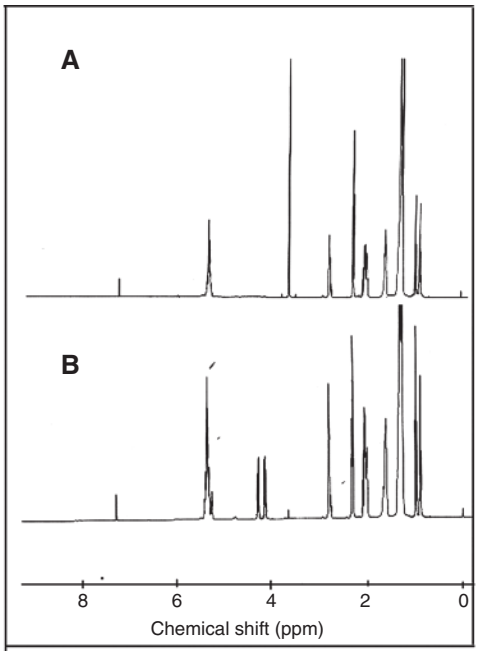

Figure 4 ^{1}H NMR spectra of (A) the methyl ester of linseed oil and (B) linseed oil.

linseed oil and aromatic part contents were directly calculated, and DCP content was indirectly calculated from the ^{1}H NMR spectrum. Sample D1Lin50 of SET IV was identical in composition with the S2Lin50 sample of SET I. The unreacted oil content of D1Lin50 was higher than that of S2Lin50 because in D1Lin50, DVB was partially replaced by the less reactive DCP. The unreacted aromatic contents were also lower in S2Lin50 than in D1Lin50. DVB played the most active role in cross-linking to form the ST-DVB-DCP cross-linked polymer chain in which the linseed oil molecule was grafted. Because of the lesser amount of DVB present in sample D1Lin50, the amount of the unreacted aromatic part became higher. The same comparison can be made for other samples of SET IV with their identical samples in SET I, SET II, and SET III. As discussed earlier, DCP acts both as a cross-linker and a copolymer. Thus, in samples with 50% and 55% oil content, the unreacted DCP content decreased with increasing DCP content in the original compositions of the samples. The effect of the unreacted and bound oil contents on the mechanical and dynamic mechanical behavior of elastomers will be discussed in the next section.

3.4 Analysis of the mechanical and dynamic mechanical behaviors of the elastomers

Elastomers are extensively used as vibration damping materials in machineries, buildings, large civil structures, etc., owing to their unique combination of low modulus and inherent damping properties. Because of the large damping and low stiffness of elastomers, they reduce the mechanical vibrations and transmit forces from vibrating structures and thus prevent fatal breakdown of systems. However, elastomers must have a large enough degree of static stiffness because they should support the vibrating structures. Thus, the mechanical behaviors of elastomers under static and dynamic loading conditions are very important for their practical applications. Mechanical and dynamic mechanical analyses have been investigated in other studies [38, 39] and are reviewed in Table 5. From the table, it is clear that the damping property of elastomers (loss factor) increases from S1Lin45 to S4Lin60 in

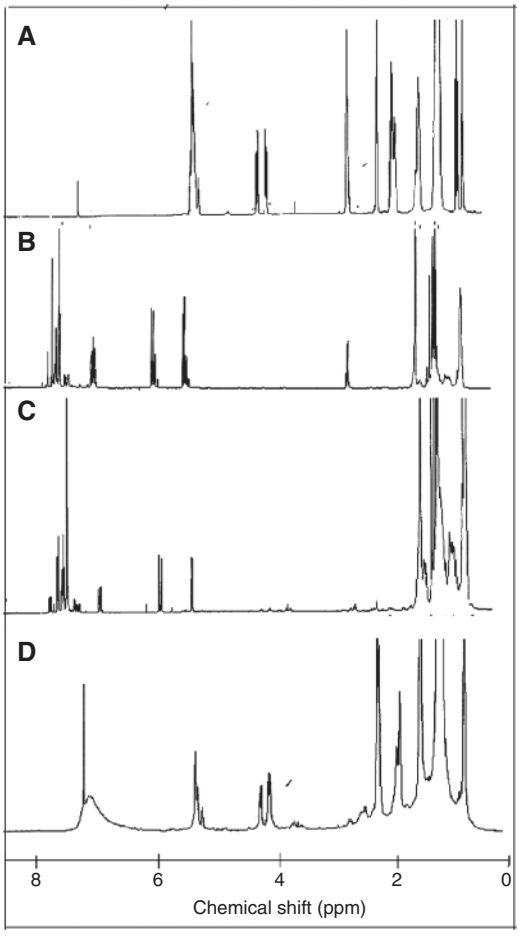

Figure 5 ^{1}H NMR spectra of (A) linseed oil, (B) styrene, (C) divinylbenzene, and (D) the soluble extract of S2Lin50 (Oil50+ST25+17DVB+In8).

SET I and from S5Lin50 to S8 Lin65 in SET II, i.e., with increasing linseed oil content in the original feed composition. The elastomers are viscoelastic in nature; thus, variations in the dynamic mechanical properties and damping properties are controlled by the viscous and elastic contents in the elastomers. In these synthesized elastomers, the plasticized homopolymer majorly contributes to the viscous nature and the cross-linked part to the elastic nature, although the viscous and elastic properties of the polymer network are related to the whole network. The dangling chain ends also have an effect on the viscous property. The viscous part dissipates energy, whereas the elastic part stores energy during excitation according to the theory of viscoelasticity [44]. The amount of the viscous part increases with increasing linseed oil content in the original feed compositions, according to spectroscopic characterization. Thus, the loss factor improved with increasing linseed oil content in the original feed compositions. The cross-linking density, the most significant parameter obtained from dynamic mechanical analysis, decreased with increasing linseed oil content and decreasing DVB content in the original feed compositions. DVB acts as a cross-linker in the polymerization reaction, and the spectroscopic structural analysis revealed that the elastomer was the linseed oil grafted ST-DVB copolymer. The decrease in DVB content reduced the reactivity of the ST-DVB copolymer, which resulted in the reduction of the cross-linking density of the elastomers. When a correlation between cross-linking density and spectroscopy quantification results was made, it was observed that

Table 4 Evaluation of ^{1}H NMR spectra.

Analysis of	Chemical shift (ppm)	Assignments
Linseed oil	0.83–0.93	Terminal methyl protons of saturated, monounsaturated, and n-6 polyunsaturated fatty acid
	0.93–1.03	Terminal methyl protons of n-3 polyunsaturated fatty acid
	1.22–1.32	Methylene protons associated with the saturated carbon atoms of the fatty acid chain
	1.52–1.70	β-Methylene protons from carbonyl carbons
	1.90–2.10	Allyl methylene protons
	2.29–2.32	α-Methylene protons adjacent to carbonyl carbons
	2.75-2.81	Divinyl methylene protons of the fatty acid chain
	4.14–4.29	Methylene protons of the glycerol unit
	5.33–5.39	Vinylic protons (C=C-H) of the fatty acid of linseed oil
Methyl ester of linseed oil	3.7	Protons of the methyl ester moiety
Divinylbenzene	5.2–6.7	Vinylic protons
	7.2–7.5	Aromatic protons
Styrene	5.2–6.7	Vinylic protons
	7.2–7.5	Aromatic protons
Soluble extract of the elastomers	4.1–4.3	Methylene proton of the glycerol unit of linseed oil
	7.2–7.5	Aromatic protons of ST and DVB

Table 5 Review of the mechanical and dynamic mechanical behaviors of the elastomers.

SET	Dynamic mechanical analysis results					Mechanical properties			
	Sample ID	Cross-linking density (mol/m³)	Storage modulus, E' (MPa)	Loss modulus, E'' (MPa)	Loss factor, tan δ	Young's modulus in tension, E_T (MPa)	Elongation at break, ε_T (%)	Young's modulus in compression, E_C	Compression at break, ε_c (%)
I	S1Lin45	434	367	93.8	1.07	10.27	117	30.1	74.6
	S2Lin50	350	320	90.3	1.15	7.23	144	27.6	70.2
	S3Lin55	253	208	83	1.23	5.54	154	24.9	40.2
	S4Lin60	115	7.6	4.8	1.32	1.10	98	21.4	22.5
II	S5Lin50	430	365	98.9	0.79	9.80	91	17.4	57.5
	S6Lin55	281	242	74.5	0.78	5.46	115	6.7	50.9
	S7Lin60	201	10	6	0.87	1.32	83	1.5	52.7
	S8Lin65	104	6.1	3.3	0.95	0.27	144	–	–

(from Tables 2 and 5) the bound oil content decreased but the unreacted oil content increased with the decrease in cross-linking density. Both the storage modulus and the loss modulus increased with an increase in the cross-linking density of the elastomers. As the Young's modulus increases with cross-linking [45], the Young's modulus in tension (E_T) and the Young's modulus in compression increase with an increase in the cross-linking densities of the elastomers. The elongation at break (ε_T) and compression at break (ε_c) increased with increasing linseed oil content in the original feed compositions. The increase in linseed oil content led to an increase in the contents of unreacted oil and the aromatic part in the elastomers, which had the direct effect of softening the cross-linked part of the elastomers. Thus, elongation both in tension and compression improved with an increase in linseed oil content.

The elastomers had loss factors in the range of 0.72–1.32, which are very much higher than the minimum required loss factor for effective damping in different applications [46]. The loss factor values are even higher than those of different conventional polymer damping

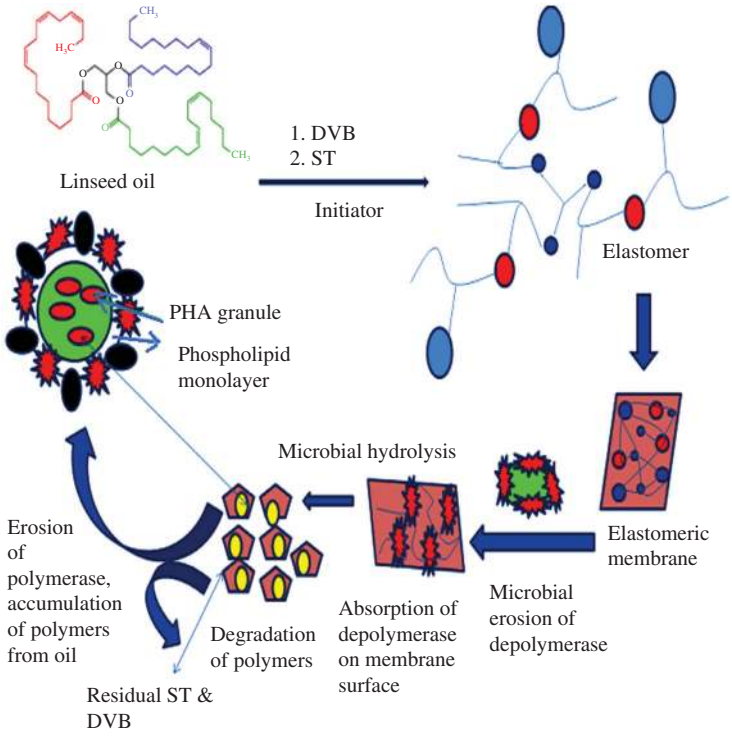

Figure 6 Schematic of the microbial degradation of the elastomers and the formation of a new polymer.

materials such as poly (methyl methacrylate) (PMMA), polytetrafluoroethylene (PTFE), epoxy, neoprene rubber, etc. [47]. The production cost of a 100×100×3 mm elastomeric sheet is approximately 100–150 INR, which is lower than the market price of other commercially available polymeric damping materials.

3.5 Microbial degradation of the elastomers

The linseed oil-based elastomers were degraded through depolymerization by specific depolymerases secreted from the microorganism. The first step of depolymerization was the microbial erosion of depolymerases on the surface of the polymeric membrane; the second step was the hydrolysis of elastomers through their ester linkage, which produced water-soluble intermediates. These intermediates were assimilated and then metabolized by microbial cells. The microorganism used the elastomers as its sole carbon source depending on the molecular structures of the elastomers and accumulated the P(3HB-co-3HV) copolymer in the bacterial cells. The microbial degradation of the elastomers and the accumulation of the copolymer in bacterial cells are schematically presented in Figure 6. The weight loss of elastomers occurred because of microbial degradation, and bacterial cell growth were monitored at regular time intervals to optimize the degradation reaction. The percentage of degradation calculated by using Eq. (1) and the bacterial cell density growth as a function of incubation time are plotted in Figure 7. It is the plot for S8Lin65, which is the representative plot for all samples. From this figure, it is clear that at the initial stage, the degradation was very slow. A small amount of random hydrolytic cleavage of ester linkages occurred at this stage. Such cleavage is non-enzymatic and takes place within the polymer bulk [48]. Meanwhile, the microbe adhered to the surface of the elastomeric film; however, most of the fragments formed at this stage were large and remained relatively immune to microbial attack. After 96 h, the degradation rate increased quickly and considerable weight loss occurred. At this stage, the enzymes randomly split the bonds of the polymeric chains, which resulted in the production of small fragments of carbon molecules. These carbon molecules were assimilated by the microorganism as a potential nutrient for its growth. The degradation percentages of the different elastomers are listed in Table 6. The percentage of degradation increased with increasing linseed oil concentration in the original composition of the samples both for SET I and SET II. As obtained from spectroscopic characterization, the unreacted oil content increased but the bound oil content decreased with increasing linseed oil content in the original sample compositions. The elastomers having a higher linseed oil content were less cross-linked than those elastomers having a lower linseed oil content (Table 5). The microorganism hydrolyzed the ester linkage of unreacted oil more easily than the bound oil as the bound oil was grafted in the ST-DVB copolymer, forming a polymeric network. Also, the enzymatic degradation of structures with fewer cross-links was faster than that of highly cross-linked structures. Thus, the percentage of degradation increased with increasing linseed oil content. The cell density reached a saturation point after 200 h of incubation, and because of the unavailability of carbon source, it was not increased further. As the unreacted oil content in S8Lin65 was maximum and also greater than the bound oil content, it exhibited the maximum percentage of degradation, i.e., 51% among the samples of SET I and SET II. The FTIR spectra of S8Lin65 before and after degradation are shown in Figure 8. It

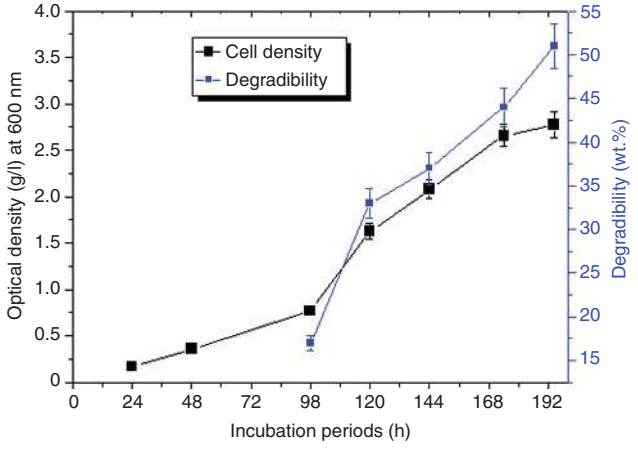

Figure 7 Variation of degradability and cell density as a function of incubation periods.

Table 6 Microbial degradation analysis of the elastomers.

SET	Sample ID	Percentage of degradation at 200-h incubation	Cell density (g/l)
I	S1Lin45	26	1.32
	S2Lin50	31	1.76
	S3Lin55	36	2.17
	S4Lin60	40.4	2.3
II	S5Lin50	30.4	1.67
	S6Lin55	34	2.07
	S7Lin60	42	2.4
	S8Lin65	51	2.8

shows the representative plot for all samples. The absorbance of the characteristic peak of linseed oil (1741 cm^{-1}) decreased after degradation; however, the absorbance of the characteristic peak of aromatic content (690 cm^{-1}) in the elastomers remained unchanged. In the microbial degradation process, the microorganism degraded the unreacted oil and the oil segments of the main polymer chain. Thus, only the absorbance due to the oil content of the elastomer decreased after degradation. The FTIR and ^{1}H NMR spectra of the P(3HB-co-3HV) copolymer produced by the biodegradation process of elastomers are shown in Figure 8. In the FTIR spectrum of P(3HB-co-3HV), the strong absorption peak at approximately 1283 cm^{-1} is associated with the saturated ester linkage of C-O groups. The

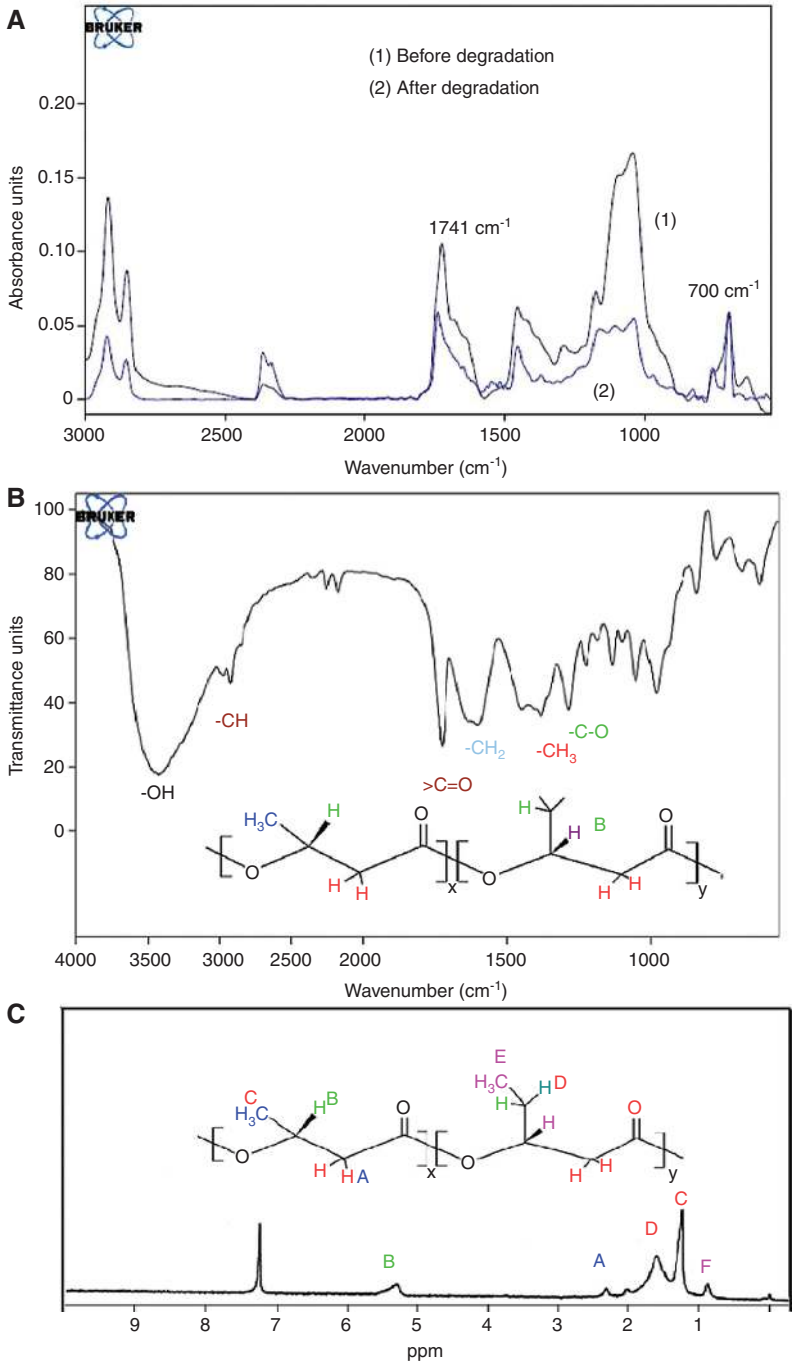

Figure 8 (A) FTIR spectra of S8Lin65 before and after degradation. (B) FTIR spectrum of the produced polymer as cell mass. (C) ^{1}H NMR spectrum of the produced polymer as cell mass.

absorption peak at 1383 cm^{-1} corresponds to the stretching of methyl ($-CH_3$) group and that at 1449 cm^{-1} to the bending mode of vibration of the methylene ($-CH_2$) group. The peaks at 2930 cm^{-1}, 1727 cm^{-1}, and 3429 cm^{-1} are the respective characteristic peaks of the methine (-CH), -carbonyl (C=O), and hydroxyl (-OH) groups. The ^1H NMR spectrum of the polyester produced by the *Alkaliphilus* strain indicates that the methyl protons ($-CH_3$) of the HB side group had a doublet resonance at 1.253 ppm and the methyl protons ($-CH_3$) of the HV side group at 0.878 ppm had a triplet resonance due to the coupling with the adjacent methylene group in the polymeric chain. A group of strong absorption peaks at 1.606 and 2.307 ppm were observed because of the multiple resonance of methylene protons ($-CH_2$) of the HV side group and of the HV-HB bulk structure in the presence of an external magnetic field. Again, the methine proton, -CH (HV and HB bulk structure), linked with more electronegative oxygen atom had a multiplet resonance at 5.340 ppm due to the deshielding effect on the methine proton. From the ^1H NMR analysis, it is concluded that the polyester formed by the *Alkaliphilus* strain is a copolyester of P(3HB-co-3HV). Therefore, the FTIR results of the copolymer formed from the linseed oil-based elastomer are in complete agreement with the earlier reports [49, 50].

The SEM micrographs of the elastomers before and after biodegradation are presented in Figure 9. The degraded samples of S5Lin50, S10Lin60, and S11Lin65 are shown. Degradation occurs randomly on the polymer surface, creating cavities on it that are obvious in SEM images of the degraded samples. On the basis of the roughness in SEM micrographs of the degraded samples, it is clear that S5Lin50 exhibited a low extent of biodegradation, whereas samples containing higher amounts of linseed oil in the original compositions, i.e., S7Lin60 and S8Lin65, showed increasingly higher degradation extents.

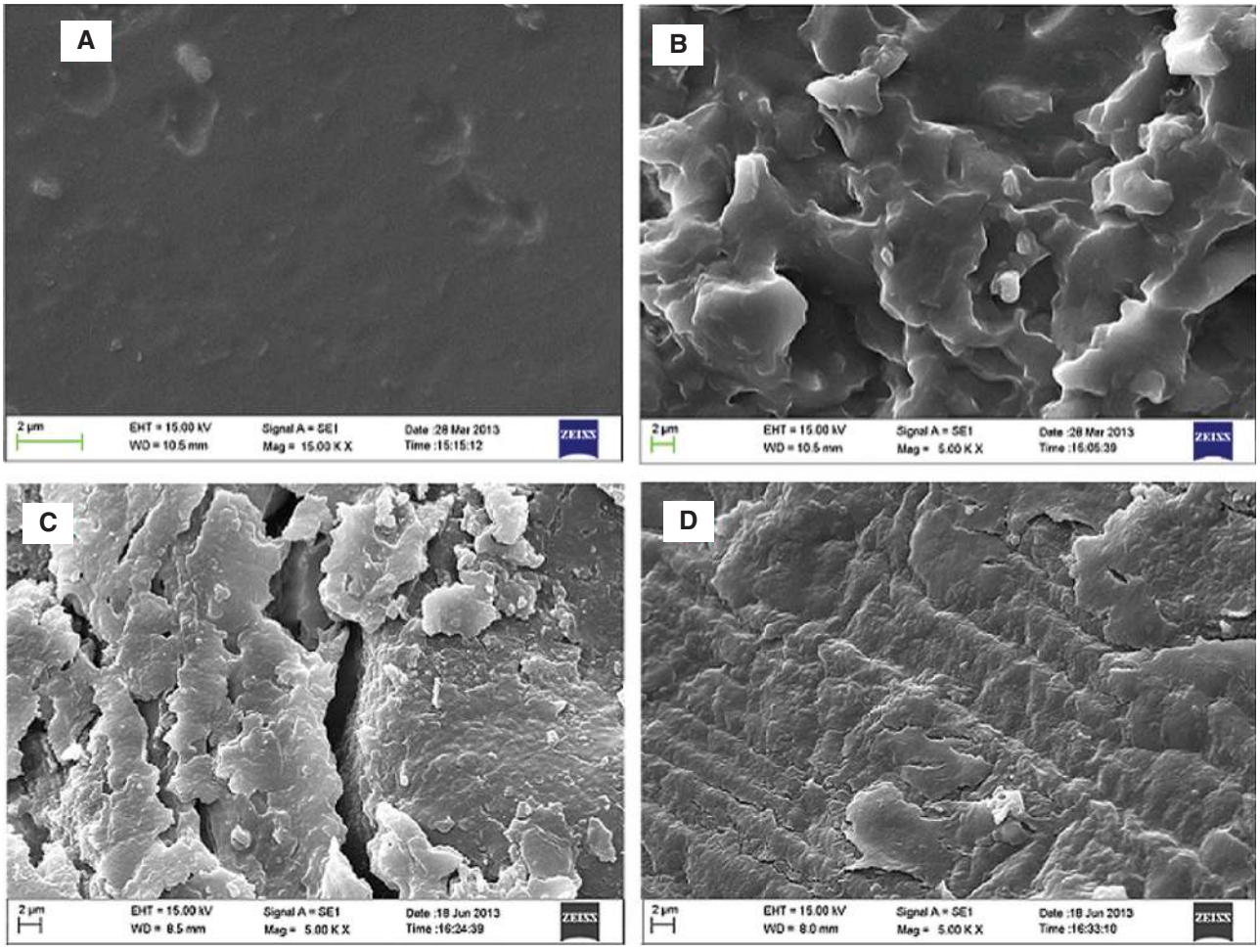

Figure 9 Scanning electron micrographs of (A) S8Lin65 before degradation, (B) S8Lin65 after degradation, (C) S7Lin60 after degradation, and (D) S5Lin50 after degradation.

4 Conclusions

Elastomers synthesized from linseed oil through the cationic polymerization technique have been characterized and analyzed quantitatively by using FTIR and ^{1}H NMR spectroscopies. The solubility of the samples through Soxhlet extraction ranged from 20 to 51 wt%. The bound oil content, i.e., the linseed oil grafted in the polymer backbone, obtained through FTIR ranged from 29.63 to 45.5 wt% and decreased with increasing linseed oil content in the original compositions of the elastomers. The unreacted oil content in the elastomers, calculated by using FTIR, varied from 12.9 to 38 wt%. In ^{1}H NMR analysis, the unreacted oil content ranged from 13.2 to 39.4 wt% and the unreacted aromatic part ranged from 6.8 to 16 wt%. The mechanical and dynamic mechanical behaviors of the elastomers correlated with the spectroscopic quantification results. The elastomers showed mechanical stability and very good vibration damping properties. The microbial degradation of these elastomers, evaluated by using an efficient single microbial strain, ranged from 26% to 51%. The degradation behavior was very much related to the bound and unreacted oil contents obtained from the spectroscopic quantifications. The biodegradability increased with an increase in unreacted oil content in elastomers because of the easily available hydrolyzable ester groups. These bio-elastomers are effective alternatives to non-biodegradable synthetic elastomers in different engineering applications, especially as vibration dampers in machinery, structural engineering, and dynamic systems.

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