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ORIGINAL PAPER

Biodegradation of Poly(ε-caprolactone) (PCL) Film by *Alcaligenes faecalis*

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Abstract The biodegradation behavior of PCL film with high molecular weight (80,000 Da) in presence of bacterium *Alcaligenes faecalis* and the analysis of degraded polymer film have been carried out. Thin Films of PCL were prepared by means of solution casting method and the bacterial degradation behavior was carried in basal medium, in presence of bacteria with time variation after UV treatment. It was observed that after UV treatment the degradation of polymer film was increased and the degradation rate followed a three steps degradation mechanism. The degraded polymer film was analyzed by means of Differential Scanning Calorimeter (DSC), Thermo Gravimetric Analyzer (TGA) and Fourier Transform Infrared Spectroscope (FTIR). DSC results revealed that at the initial stages of the degradation up to 15–20 days, the bacterium preferentially degrades the amorphous parts of the polymer film over the crystalline zone. Thermo gravimetric analysis highlighted the low temperature stability of degraded films with extent of degradation. FTIR results showed the chain scission mechanism of the polymer chains and also supported the preferential degradation of amorphous phase over crystalline phase in the initial stages of the degradation.

Keywords Poly(ε-caprolactone) · Biodegradation · Thermal analysis · FTIR

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Introduction

Use of plastic as packaging materials has increased rapidly over the last 20 years. The annual disposal of plastics has raised the demand for biodegradable plastic materials. The disposal of these materials has become a concern, because of their persistence in the environment [1, 2]. Selective uses of biodegradable polymers in certain applications might help to reduce the environmental impact of plastic materials.

Poly(ε-caprolactone) (PCL) is a semi crystalline linear aliphatic polyester derived from a ring opening polymerization of ε-caprolactone, having relatively low melting point, 60 °C [3]. It is completely biodegradable by microorganisms in marine, sewage sludge, soil and compost ecosystem, which makes it a useful material in drug delivery systems [4–6]. Due to its biodegradability and biocompatibility, PCL has attracted much attention in medical field mainly as controlled release drug carriers. According to literature, the degradation of this aliphatic polyester in a living environment can result from enzymatic attack or from simple hydrolysis of ester bonds, or both [7, 8].

Biodegradability of polycaprolactone was observed in the presence of microorganisms in diverse environments. Several parameters, such as polymer molecular weight, crystalline morphology, film thickness and degradation conditions were found to influence polycaprolactone

biodegradation. This degradation is due to microorganisms that secrete extracellular depolymerases to degrade PCL and use it as a nutrient. Several studies have been carried out to evaluate the degradation and biodegradation of PCL and it has been shown that the PCL degrading bacteria are widely distributed in nature [8, 9], but has not been investigated in great detail. Chen et al. [10] found that lipase accelerates the degradation of PCl micro-particles but by comparison of degradation rate of both film-like and micro-particles-like PCL, they observed that surface area did not had any great influence on the degradation rate of the PCL sample. Rutkowska et al. [6] have reported the biodegradation of PCL in seawater. Murphy et al. [11] confirmed that depolymerase produced by the fungus *Fusarium moniliforme* to be a cutinase. However, the enzymes involved in the degradation of PCL have not been studied in detail. Oda et al. [12] isolated five strains as fungi, which were able to degrade both poly(3-hydroxybutyrate) (PHB) and PCl and one of the strains, D218, were identified as *Paecilomyces lilacinus.* Some properties of PCL depolymerase from *P. lilacinus,* D218, were also studied, laterally similar type of results have been reported by the same author but with a different microorganism, *Alcaligenes faecalis* [13].

Researchers have extensively studied the biodegradation mechanism of PCL [14–17]. It is usually assumed that the various PCL depolymerases attack preferentially the amorphous regions of the semi crystalline PCL, citing as evidence the well-known inverse dependence of degradation rate on PCL crystallinity. It is also reported that the degradation mechanism is a combination of endo- and exo-cleavage accompanied by broadening in molar distribution [18, 19]. However, the fact that degradation rates depend on a large number of different variables e.g., polymer chemical composition, physical state, molecular weight and molecular weight distribution, solvent or melt cast films, crystallinity, type of microorganism, and temperature, makes the evaluation of the degradation characteristics of bacteria a very challenging goal [15–17, 20].

PCL is well known for its hydrolytic and enzymatic biodegradability, but very little information is available about the bacterial degradation of high molecular weight PCL in presence of single bacterium. The aim of the present work is to study the degradation behavior and thermal stability of high molecular weight PCL films in the presence of a well-known bacteria *A. faecalis*. Weight loss was measured gravimetrically as a function of the degradation time. Changes in the polymer films after exposure to degradation conditions were evaluated by Thermo Gravimetric Analyzer (TGA), Differential Scanning Calorimeter (DSC) and FTIR analysis.

Experimental

Materials

PCL, of average molecular weight of 80,000 Da and ε-caprolactone was purchased from Sigma–Aldrich Chemical Co. Inc. Solvents, dichloromethane and chloroform were purchased from S. D. fine chemical (India) HPLC grade and used as it is.

Preparation of Polycaprolactone (PCL) Film

PCL films were prepared in glass petri dishes by dissolving pre-weighed polymer into chloroform and left for air drying for 2–3 days till a constant weight was observed, further it was dried in a vacuum oven at 30 °C to remove the traces of moisture if present.

Bacterial Culture

A. faecalis (MTCC-3104) was obtained from the stock collection of IMTECH Chandigarh, India. It was grown in 100 mL of basal medium in 250 mL Erlenmeyer flask containing 0.01% yeast extract, 0.01% $(NH_4)_2SO_4$, 0.01% KH_2PO_4, 0.01% K_2HPO_4, 0.005% $MgSO_4 \cdot 7H_2O$, 0.001% KCl, 0.0001% $CaCl_2 \cdot 2H_2O$, and 0.0001% $FeSO_4 \cdot 7H_2O$ in doubly distilled water supplemented with necessary amount of ε-caprolactone (99.9% pure) and named as seed culture, adjusted to pH 7 [12, 13]. For a solid medium, 1.5% (w/v) agar was added.

UV Treatment of PCL Films

The prepared PCL films were exposed at distance of 57 cm below, under UV lamp of power 30 watts for different time and stored carefully before feeding it to the pre-grown bacterial culture.

PCL Films Degradation Assay

The UV treated films of PCL were fed to pre-grown bacterial culture for different time intervals to study the degradation behaviour. The films were taken out carefully and washed thoroughly by doubly distilled water, to remove any media components or bacterial cells if present on the surface of the films and were completely vacuum dried at 30 °C till a constant weight was obtained. The weight loss was studied by gravimetric, thermal and spectroscopic analysis were also carried out.

Characterization of Degraded PCl Films

Infrared spectra of samples (pure and degraded films) were obtained using a Shimadzu (Japan) FTIR-8700

spectrophotometer in simple transmission mode. The number scan was fixed to 100 with a resolution of 4 cm^{-1}. All the scans were carried out within the same predefined range.

The thermal behavior of pure and degraded films was investigated with Rheometric scientific (USA) Thermo-Gravimetric Analyzer (TGA) model 1500. All scans were carried out from 10 to 800 °C at the heating rate of 10 °C min^{-1} under liquid nitrogen atmosphere. The thermal properties of the pure and degraded polymer were analyzed by using TA instruments DSC Q10. Samples were tested by heating from 30 to 90 °C, at a rate of 10 °C min^{-1} under nitrogen atmosphere.

Results and Discussion

Effect of UV Treatment

The effect of "UV exposure time" on degradation of PCL films in bacterial media (Fig. 1) played an important role on the degradation of PCL films. The radiated PCL films were fed to the pre-grown bacterial culture for a period of 12 days and it was observed that a minimum 4 h of radiation was required to get optimum polymer degradation. Without UV treatment there is found a negligible weight loss of the film, approximately 1 wt.%. The results could be attributed to the extent of bacterial cell adhesion and cell spreading on the film surfaces as the surface chemistry of the substrate changes on exposure to UV [21, 22]. Some of the major ways through which microorganisms deteriorate synthetic polymers are fouling (which is an unwanted deposition and growth of microorganisms on surfaces), degradation of leaching components and corrosion including hydration and penetration. The polymer surface deterioration is an interfacial process, and can involve microorganisms that can colonize on the polymer surfaces as bio-films. These bio-films consist of cells embedded in a polymer matrix of their own origin, mainly containing polysaccharides and proteins [23, 24].

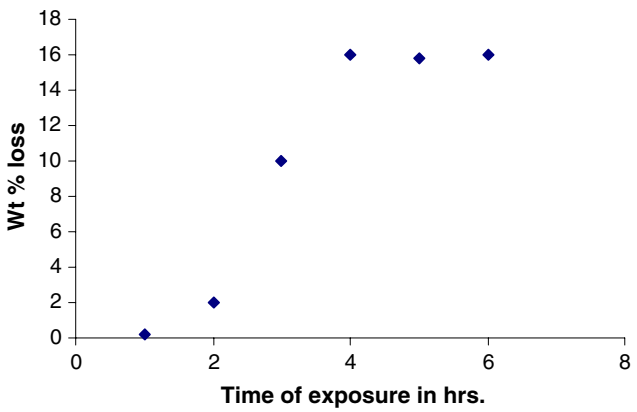

Fig. 1 Effect of UV treatment on bacterial degradation of PCL films

Gravimetric Analysis of Polymer Degradation Assay

UV treated PCL films were fed to 150 mL of basal media inoculated with 24 h grown seed culture in 500 mL Erlenmeyer flask and were analyzed at different time intervals along with weight loss % of PCL (Table 1). The result clearly revealed an increase in the disintegration of PCL film with time (Fig. 2). It was also found that the weight-loss profile exhibits three conspicuous steps (Fig. 3) that appear to arise from separate degradation mechanisms. In first stage, there is a low rate weight loss where predominantly the chain scission of polymer chains occurred due to bacterial action. While, in second stage, the high weight loss was due to chain end scission, which was then converted into different low molecular weight compounds. Finally in the last stage moderate rate of weight loss was observed showing the onset of degradation [25].

Thermal Analysis of Degraded Polymer Films

The thermal properties of the pure and degraded PCL films were analyzed using DSC and TGA. Thermo-grams of DSC and TGA of all the samples were taken in order to characterize the thermal properties of pure and degraded PCL films. DSC analysis (Fig. 4) clearly indicates that the melting point of pure Polycaprolactone was around 60 °C and the amorphous region of the polymer was being degraded prior to the crystalline region [16, 26]. Further, the crystallinity increased from 46.88 to 54.25% in 45 days (Fig. 5). This result also revealed that the rate of increase in crystallinity is parallel to the rate of degradation of the PCL film. The rate of increase in crystallinity was quite fast at first, but with increasing weight loss it became slow. The amorphous part of the PCL thin film was degraded more rapidly than the crystalline part. In the crystalline part of

Table 1 Weight loss and degree of crystallinity of PCL film during the course of bacterial degradation

Time in days	Weight loss in %	Crystallinity from DSC thermograms (%)
0	0.0	46.88
5	0.5	47.21
10	2.0	48.88
15	17.0	53.02
23	53.0	53.51
30	72.0	53.79
45	83.0	54.25
68	100.0	–

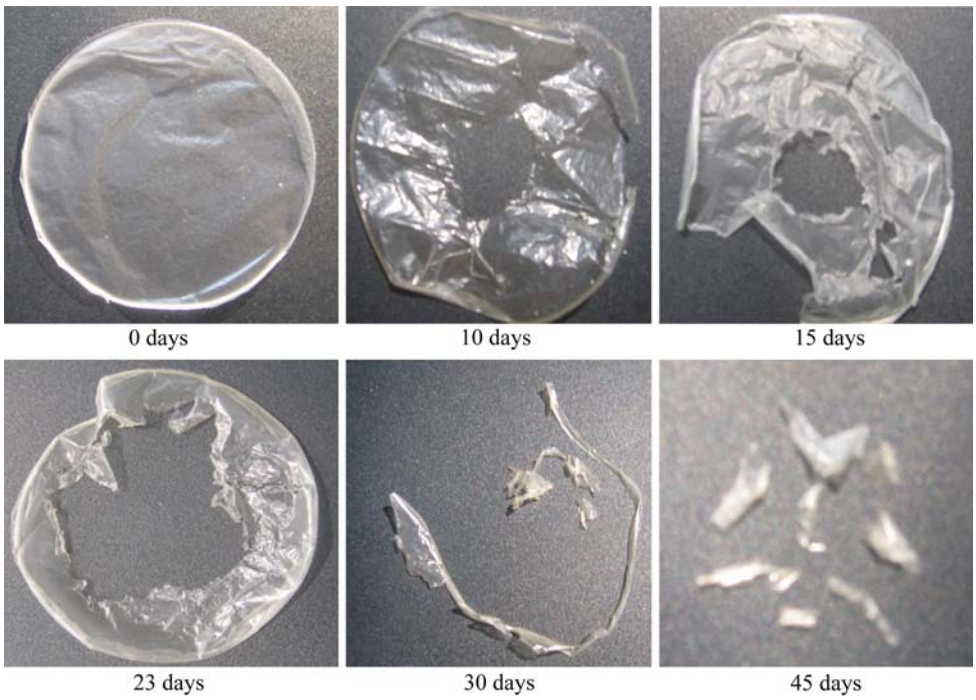

Fig. 2 Photographs of PCL films after 0, 10, 15, 23, 30 and 45 days of degradation

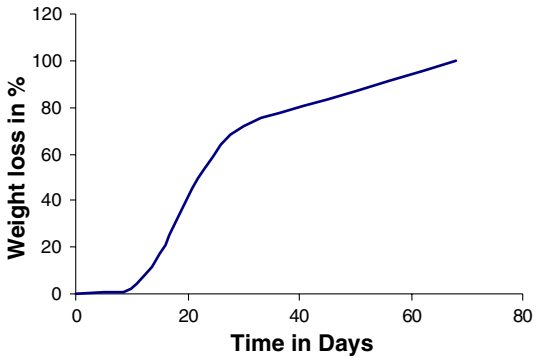

Fig. 3 Weight losses in wt.% of PCL films with exposure time

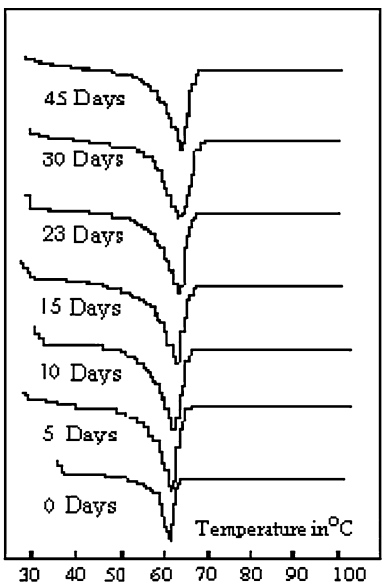

Fig. 4 DSC Thermograms of PCL films after 0, 5, 10, 15, 23, 30 and 45 days of degradation

the material, the molecules are regularly arranged in lamellae, which consist of folded molecules. Since the amorphous part is degraded faster than the crystalline part, it is mainly the crystalline part that remains after degradation. This suggests that the extra-cellular hydrolase enzyme of bacteria *A. faecalis* attack preferentially the amorphous or less-ordered regions at initial stages of degradation than the crystalline or more-ordered regions because the enzymes are able to migrate more readily into the less-ordered regions than the more-ordered regions [13]. In this case however, one must assume that in this thin layer crystalline and amorphous regions are degraded indiscriminately; otherwise the progressive dissolution of the film could not take place. The total crystallinity of PCL was reported to be 139.5 J/g [27].

TGA result (Fig. 6) showed the shifting of 'weight loss transition temperature' from higher to lower side with respect to degradation time. It clearly revealed that higher the exposure time for the PCL films to bacteria, higher the disintegration of the polymer chain, thus shifting the degradation temperature to lower side [28, 29]. Due to chain scission the overall molecular weight of the polymer decreased with the broader molecular weight distribution, which caused the shifting of the lower degradation temperature to further lower side (Table 2). This shift tends to decrease as the exposure time of the PCL films was

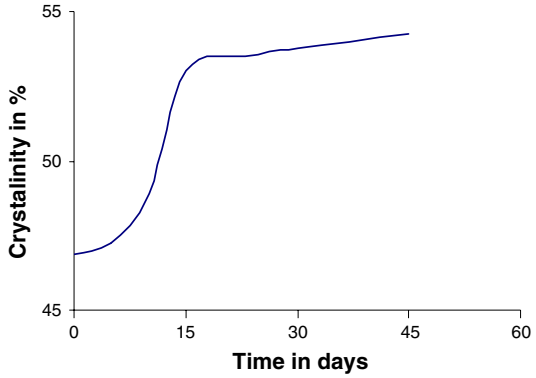

Fig. 5 Crystallinity of degraded PCL films with different exposure time

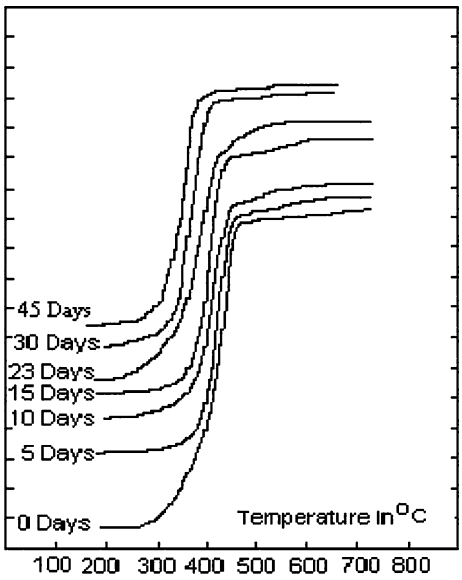

Fig. 6 TGA Thermogram of PCL films after 0, 5, 10, 15, 23, 30 and 45 days of degradation

increased to the bacterial medium. The increased rate of reduction in the molecular weight has also been explained as the preference of the degradation near the chain ends, which in the most cases are situated in the amorphous parts of the material [30].

Infrared Spectroscopic Analysis

The infrared spectrum (Fig. 7a) of the solvent cast PCL film in 500–4,500 cm^{-1} region before bacterial degradation, strong bands such as the carbonyl stretching around 1,727 cm^{-1} and symmetric and asymmetric CH_2 stretching mode around 2,943 and 2,864 cm^{-1} can be easily identified (Table 3) [31, 32]. According to Coleman and Zarian [33], the band at 1,294 and 1,167 cm^{-1} are assigned to the backbone C–C and C–O stretching modes in the crystalline and amorphous PCL, respectively. Taking into account the 77% degree of crystallinity of PCL [33], we can justify the presence of the strong band at 1,294 cm^{-1} in the obtained PCL infrared spectrum. The infrared spectrum of PCL film with duration of exposure (Fig. 7b–d) also gave the same picture as non-degraded film. The infrared spectrum of PCL film after 30 days or more has not been reported, because the degraded film was too opaque to take the infrared spectrum. In this spectrum all the characteristic peaks are found with strong crystalline band at 1,294 cm^{-1}.

Incubation of the PCL film with bacterium *A. faecalis* for 23 days showed a marked reduction in carbonyl index, which is the ratio between the absorbance peak of carbonyl at 1,726 cm^{-1} to that of CH_2 at 1,466 and 1,398 cm^{-1} [34]. It was found that the exposure to bacteria of PCL film in both calculations reduced the carbonyl index by 70% (Table 4). The reduction in carbonyl index indicates that the chain cleavage of the polymer chain occurs through carbonyl linkage. The bacterium *A. faecalis* is known as the extracellular depolymerase which degrades PCL polymer by the enzyme, lipase. The enzyme attacks the carbonyl groups and cleavages the polymer chains to reduce the molecular weight of the polymer [18, 29].

The crystallinity index was calculated as the ratio between the absorbance peak of the bands at 1,294 and 1,167 cm^{-1} [32, 35], found out to be 45.95% of PCL film before degradation and 52.32% (Table 4) after 23 days of exposure to bacterial medium which are very close to the DSC results (Table 1). The result clearly indicates the preferential bacterial degradation of amorphous region over crystalline parts at initial stages of degradation of the PCL films, which further support the DSC results.

Table 2 TGA results of bacterial degraded PCL film

Time in days	Mass loss transition temp (°C)	1st decomposition temp (°C)	2nd decomposition temp (°C)
0	428.66	382.11	446.43
5	411.28	378.61	440.24
10	408.87	364.68	438.28
15	400.56	462.00	436.71
23	395.15	347.76	434.45
30	391.39	340.56	411.79
45	385.39	333.28	403.82

Conclusions

The bacterial degradation of the PCL film is greatly influenced on ultra-violet radiation treatment; as to achieve

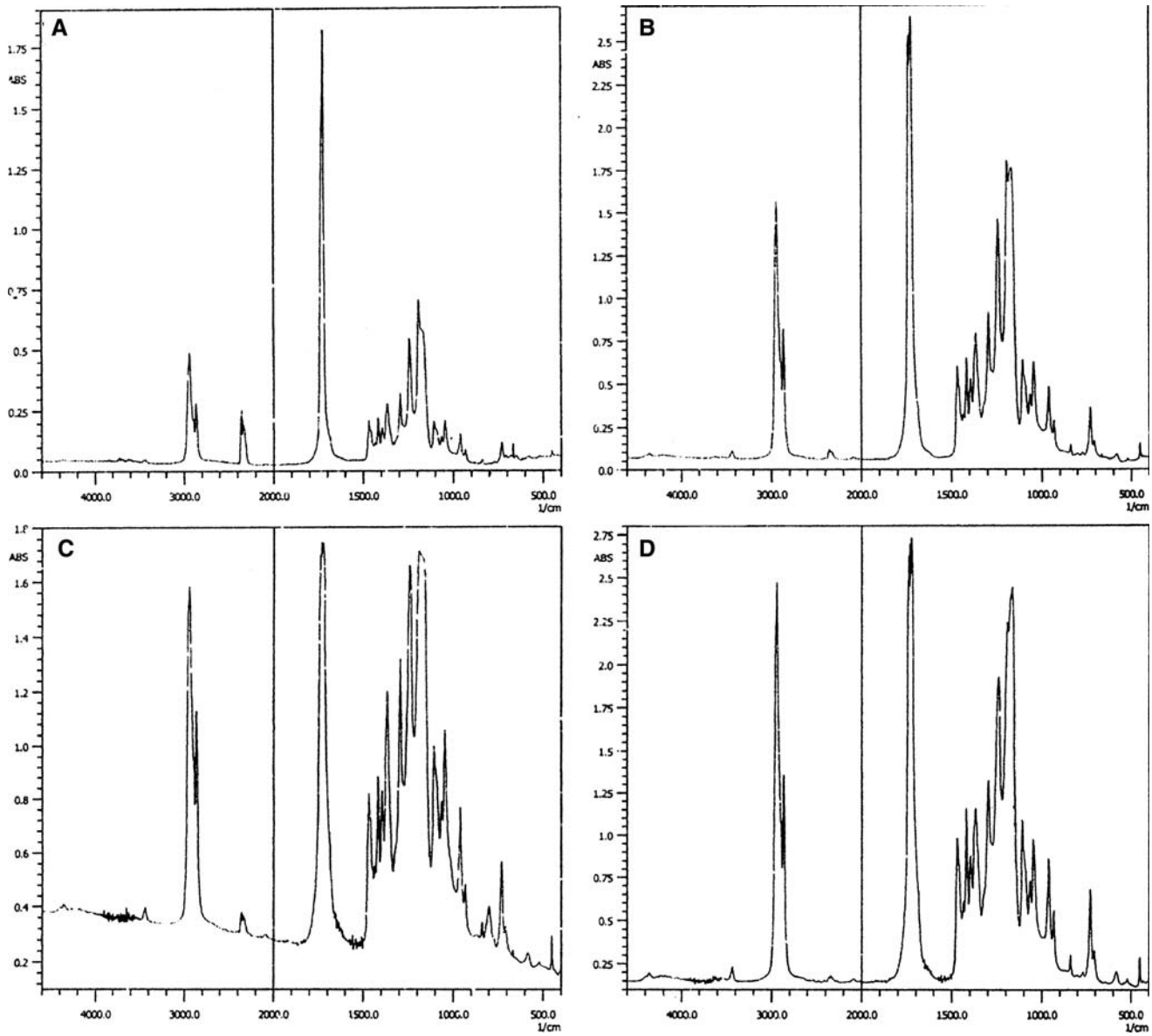

Fig. 7 Infrared spectrum of solvent cast PCL film before and after bacterial degradation in the region of 500–4,500 cm^{-1} for (**a**) 0 days, (**b**) 5 days, (**c**) 10 days and (**d**) 15 days

Table 3 Characteristic infrared spectra bands of PCL film

Band position	Vibrator
2943	Asy-CH_2 stretching
2864	Sym-CH_2 stretching
1727/1735	Carbonyl stretching
1294	C–O and C–C stretching in the crystalline phase
1240	Asymmetric COC stretching
1190	OC–O stretching
1167	C–O and C–C stretching in the amorphous phase

the maximum degradation, the polymer film should be treated for minimum of 4 h. The weight loss of PCL films by bacteria *A. faecalis* followed a three-stage degradation rate with exposure time. The thermal stability of the biodegraded PCL films decreases with increasing exposure time. Thermogravimetric analysis results show low temperature stability of the films. This has been interpreted as the bacteria cleavage the polymer chains and increases the molecular dispersity. During the course of the studies it has also been found that at initial stages of degradation a preferential degradation of the amorphous part of the

Table 4 Infrared spectroscopic results

Duration of exposure time in days	Crystallinity index by IR analysis (%)	Carbonyl index from the ratio of absorbance peaks at 1,726 and 1,466 cm^{-1}	Carbonyl index from the ratio of absorbance peaks at 1,726 and 1,466 cm^{-1}
0	45.95	8.6133	3.6687
5	47.22	4.3649	1.6804
10	48.08	4.2448	1.5740
15	51.80	2.7672	1.1056
23	52.32	2.6028	1.0817

material over crystalline part, as the crystallinity increased in the biodegraded samples measured by DSC thermo grams. The results have also been supported by FTIR analysis.

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