



Fungal degradation of carbohydrate-linked polystyrenes

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Abstract

A series of carbohydrate molecules like glucose, sucrose and lactose were linked to maleic anhydride functionalized polystyrene by polymer analogous reactions to produce biodegradable polymers. Pure culture system was used for evaluating the biodegradability of these sugar linked polystyrene-maleic anhydride copolymers by known fungal test organisms. Weight loss measurements after fungal treatment confirmed the biodegradability of the carbohydrate-linked polymers, and it was observed that the degree of susceptibility to degradation varied with the type of test organism as well as with the type of sugar. FTIR spectra confirmed the degradation of the polymer.

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1. Introduction

Due to the excellent processing properties, polyolefins have occupied a special status as commodity plastics. However, the major drawback of polyolefins is that they are non-biodegradable and hence pose severe problems of their disposal after their useful life. With the aim of developing biodegradable polymers based on polyolefins, blending of starch with polyolefins (particularly with polyethylene) has been much explored and also put in practice in a limited way (Griffin, 1974, 1977a,b,c; Otey, Westhoff, & Doane, 1987). The intention of such blending procedures was that after disposal, degradation of starch in the blend would create voids and weaken the integrity of the polyethylene and result in its degradation. However, the main drawback of this methodology is that attainment of such properties demands larger volumes of starch (in the range of 30% or higher) due to which the physical properties of the polyethylene have to be compromised (Lenz, 1993).

There have been several reports of chemically modifying polystyrenes with non-polymeric sugars. Polystyrenes with pendant sugar residues have been synthesized by polymerization of vinyl benzyl sugars. Polystyrene

derivatives with maltose, lactose and maltotriose substituents on each phenyl ring were synthesized by coupling the corresponding oligosaccharide lactones with *p*-vinylbenzyl amines followed by radical polymerization (Kobayashi, Sumitomo, & Ina, 1983, 1985; Kobayashi, Sumitomo, Kobayashi, & Akaike, 1998; Kobayashi, Tsuchida, Usui, & Akaike, 1997). These polymers were water soluble, and are potential biomedical materials wherein the oligosaccharide moieties are used as recognition signals. However, there is no scope to develop these into bulk plastics as substitutes for polyolefins. Sugars have also been incorporated into silicone rubbers in order to improve their wettability and biocompatibility.

The present paper pertains to synthesizing biodegradable polymers based on functionalized polystyrene, by chemically linking carbohydrate molecules onto the polymer, by polymer analogous reactions, and testing their biodegradation by using pure fungal cultures.

2. Experimental

2.1. Materials

Poly(styrene-co-maleic anhydride) (PS-MAH) (maleic anhydride content 14% by weight) was obtained from Aldrich, USA. All solvents were obtained from SD Fine

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Chemicals, Pune, India, and were distilled and dried before use. The microorganisms used *Aspergillus niger* NCIM 1025 (ATCC 9642), *Trichoderma* sp. NCIM 1297 (ATCC 9645) and *Pullularia pullulans* NCIM 1049 (ATCC 9348) were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India.

2.2. Synthesis of sugar linked PS-MAH (general procedure)

PS-MAH, dissolved in anhydrous *N,N'*-dimethyl formamide, was added to a stirred solution of the sugar, e.g. glucose, sucrose, or lactose, etc. and the catalyst, viz. 4-dimethylaminopyridine taken in a three-necked round bottom flask provided with a magnetic stir bar, thermowell, addition funnel and a dry nitrogen balloon at a temperature 45–65 °C over a period of 1/2–1 h. The mole ratio of the maleic anhydride content of the polystyrene-maleic anhydride copolymer versus the sugar was either 1:3 or 2:1. In case of lactose, only a 1:3 ratio was investigated; the lactose sugar was not completely soluble in DMF under the reaction conditions. Stirring was done for 4–18 h at temperatures of 45–65 °C. The amount of sugar incorporated into the polymer by this method was generally between 0.2 and 3.5 by weight. The reaction mixtures were precipitated in brine, washed several times with water till free of chloride and dried in a vacuum oven. These polymers were found to be degraded by both bacterial as well as fungal cultures.

2.3. Use of colorimetry for quantification of the sugar content in the poly(styrene maleic anhydride) linked with glucose, sucrose, and lactose: the phenol–sulfuric acid reaction method

The sugar content of the polymer was found by phenol sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Glucose, sucrose, lactose, and the sugar linked poly(styrene maleic anhydride) polymers were dried overnight in a vacuum oven at ~60 °C. A standard plot for glucose/sucrose/lactose was plotted using different concentrations (0.01–0.1 mg/ml) of the respective sugar in water. The solutions of different concentrations were prepared as follows (Table 1).

To 2 ml of each sugar solution was added 1 ml of 5% aqueous phenol followed by a rapid addition of 5 ml concentrated sulfuric acid. In a similar way, a blank was prepared using distilled water instead of sugar solution. The solutions were placed in a water-bath at 35–40 °C for 30 min. The absorbency of the sugar solutions was recorded at 490 nm and a standard plot was plotted. Each of the accurately weighed samples (glucose/sucrose/lactose linked poly(styrene maleic anhydride)) in the range of 15–25 mg was taken in a 50 ml beaker to which were added 4 ml water and 2 ml 5% aqueous phenol solution followed by a rapid addition of 10 ml concentrated sulfuric

Table 1

Preparation of sugar solutions of different concentrations using a stock solution of 0.1 mg/ml

Sr. no.	Solution	Amount of solution 1 added (ml)	Amount of distilled water added (ml)	Final concentration (mg/ml)
1	Solution 1 ^a	25	–	0.10
2	Solution 2	20	5	0.08
3	Solution 3	15	10	0.06
4	Solution 4	10	15	0.04
5	Solution 5	5	20	0.02
6	Solution 6	2.5	22.5	0.01
7	Blank solution	–	25	0

^a Solution 1 was prepared by dissolving 10 mg of the sugar (glucose/sucrose/lactose) in 100 ml water.

acid. The solutions were placed in a water-bath at 35–40 °C for 30 min. The solutions were filtered through a sintered disc to remove the undissolved polymeric backbone. The absorbency of these solutions was recorded at 490 nm and the unknown concentrations of the sugars were calculated from the standard plot. Using these concentrations the mole percentages and the weight percentages of the sugars in the polymers were calculated. The range of sugar content in the polymer was found to be between 0.2 and 3.5 wt% (Table 2).

2.4. FTIR spectra

FTIR spectra were recorded on a Shimadzu 8300 spectrometer. For PS-MAH, its fungal treated product and sucrose linked PS-MAH, transparent films were cast from chloroform or tetrahydrofuran solution, and were used to record the spectra. For biodegraded products of sucrose linked PS-MAH, films could not be cast, and their spectra were recorded in KBr.

Table 2

Table showing number of moles of the sugars in the polymers (wt% are in brackets) calculated by colorimetric experiments

Polymer	Moles and weight percentages of sugars			
	1	2	3	Average
PS-MAH linked to glucose	0.198 (0.338)	0.15 (0.26)	0.116 (0.198)	0.155 (0.265)
PS-MAH linked to sucrose	0.952 (3.05)	0.811 (2.6)	0.818 (2.63)	0.860 (2.76)
PS-MAH linked to lactose	0.351 (1.18)	–	–	0.351 (1.18)

PS-MAH, poly(styrene maleic anhydride).

Table 3

Weight loss of different sugar linked polystyrene-maleic anhydride (PS-MAH) copolymers in presence of different fungal strains

Sample	%Weight loss by different strains			
	<i>Aspergillus niger</i> NCIM 1025	<i>Penicillium ochro-cloron</i> NCIM 1219	<i>Pullularia pullulans</i> NCIM 1049	<i>Trichoderma</i> sp. NCIM 1297
Control (without any carbon source)	–	–	–	–
PS-MAH	0.0	0.0	0.0	0.0
Lactose linked PS-MAH	2.8	12.0	9.2	6.4
Glucose linked PS-MAH	20.4	9.6	5.2	2.8
Sucrose linked PS-MAH	0.0	10.0	14.0	19.6

2.5. Test microorganisms

The selected microorganisms *A. niger* NCIM 1025 (ATCC 9642), *Trichoderma* sp. NCIM 1297 (ATCC 9645) and *P. pullulans* NCIM 1049 (ATCC 9348) were standard fungal strains (ASTM Standards, G-21, 1980) and *Penicillium ochro-chloron* NCIM 1219 (IS 9000, Part X, 1979) recommended for testing the fungal resistance. All the microorganisms used for the test were obtained from our culture collection, NCIM. These cultures were routinely maintained on Potato Dextrose Agar (PDA) slopes.

2.6. Testing of the samples

The cultures were grown in minimal medium (ASTM Nutrient Salts Medium) containing (g/l): KH_2PO_4 0.7; K_2HPO_4 0.7; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.7; NH_4NO_3 1.0; NaCl 0.005; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.002.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.002.; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.001. The pH of the medium was adjusted to 6.5 ± 0.2 and sterilized at 121°C for 20 min.

The test samples (250 mg/50 ml) were surface sterilized with 70% ethanol for 2 h and added aseptically to the sterilized medium.

The inoculum was prepared by suspending the spores from fully grown cultures on PDA slopes in 10 ml of sterile saline. This suspension (1.0 ml) was added to 50 ml of minimal medium in 250 ml Erlenmeyer conical flasks. Flasks were incubated at 30°C for 10 weeks under stationary condition.

Test samples were harvested, washed in sterile distilled water several times to remove mycelia and spores followed by three washings with 70% ethanol and drying at 37°C for 48 h.

3. Results and discussion

Test organisms used in the present study were standard cultures used for testing samples for their fungal resistance. Table 3 shows that the percent weight loss (indicating biodegradability) of the sugar linked polystyrene-maleic

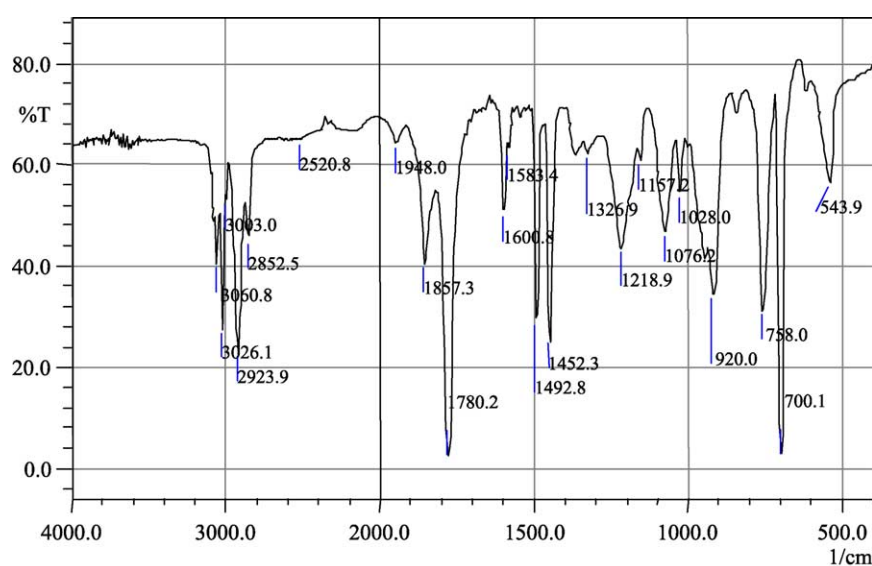


Fig. 1. FTIR spectrum of PS-MAH.

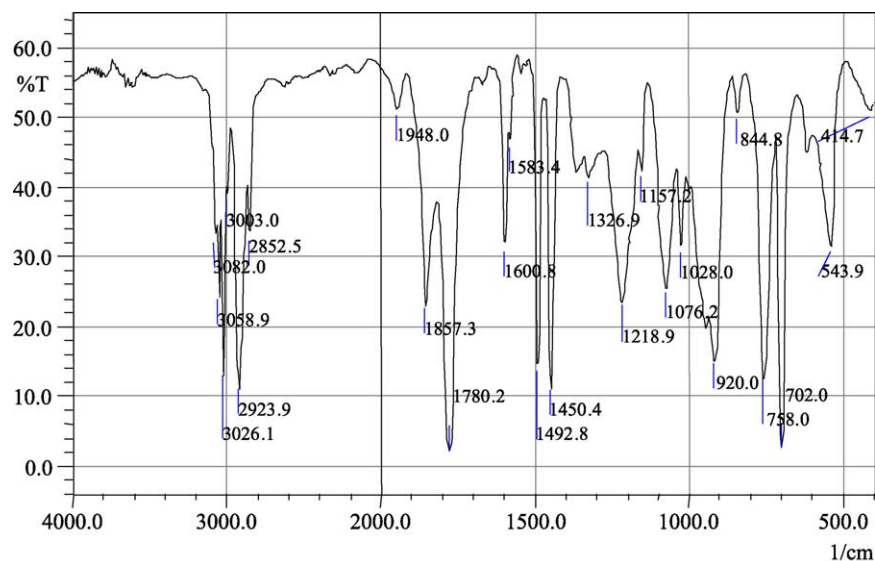


Fig. 2. FTIR spectrum of PS-MAH treated with *Aspergillus niger* NCIM 1025.

anhydride (PS-MAH) copolymer varies with the type of mono- or disaccharide attached. Thus, we observed that the PS-MAH without any sugar linked to it was not degraded by any of the four fungal species used in this study. Lactose linked PS-MAH showed maximum degradation with *Penicillium ochro-cloron* NCIM 1219 (12%), and the least with *A. niger* NCIM 1025 (2.8%). In the case of glucose linked PS-MAH, the maximum weight loss was with *A. niger* NCIM 1025 (20.4%), and the lowest with *Trichoderma* sp. NCIM 1297 (2.8%), while for the sucrose linked PS-MAH, the greatest degradation was caused by *Trichoderma* sp. NCIM 1297 (weight loss 19.6%), while *A. niger* NCIM 1025 caused no degradation at all. Thus, each fungal microorganism has specific

preference for different saccharide types linked to PS-MAH.

The test cultures used in the present study are organisms widely found in the environment. However, use of pure culture system offers the advantage of reproducibility of results, and would aid in designing biodegradation systems for different polymers after their disposal as waste materials.

Figs. 1 and 2 show the FTIR spectra of PS-MAH and its fungal treated (*A. niger* NCIM 1025) product. There is no change in the spectra, indicating no chemical changes. This is supported by the fact that no weight loss was observed. Fig. 3 shows the FTIR spectrum of sucrose linked PS-MAH, while Figs. 4 and 5 are the spectra of the latter

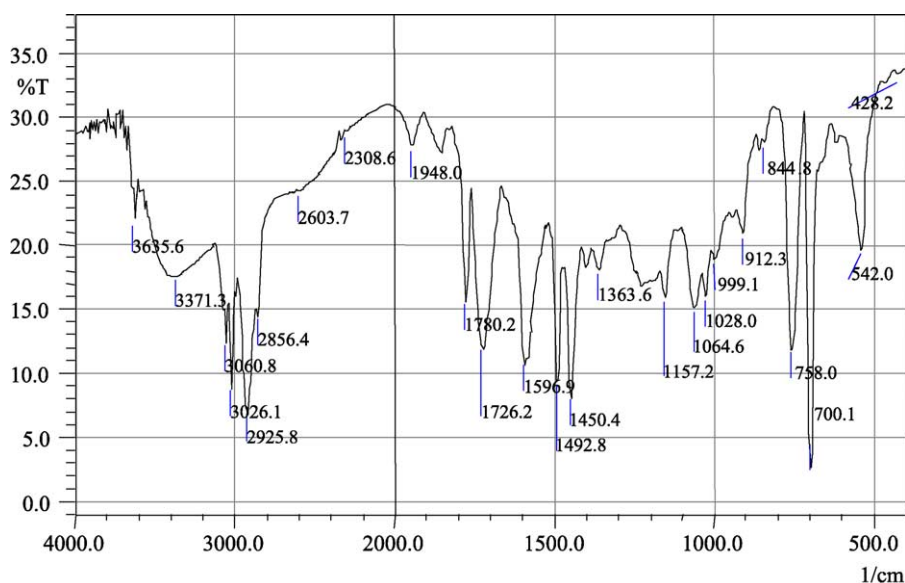


Fig. 3. FTIR spectrum of sucrose linked PS-MAH.

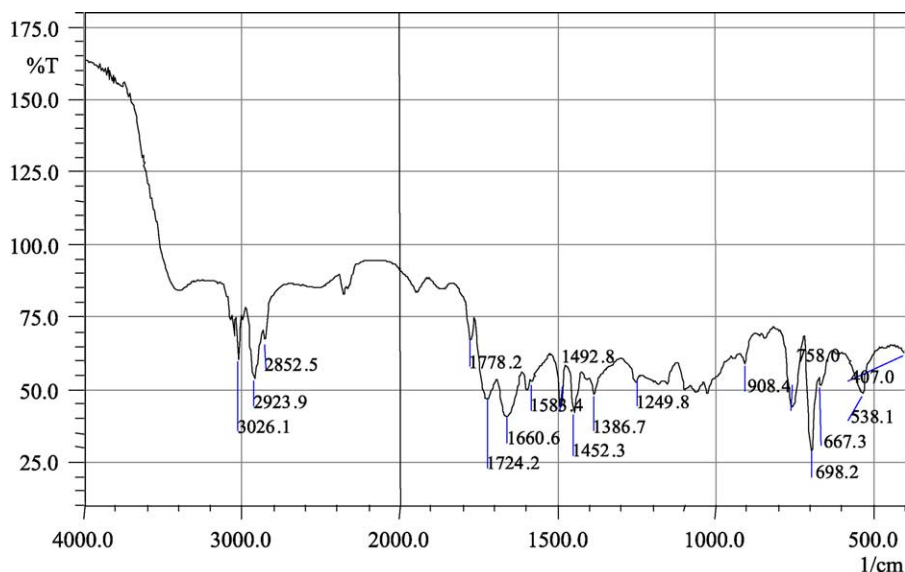


Fig. 4. FTIR spectrum of sucrose linked PS-MAH biodegraded by *Pullularia pullulans* NCIM 1049.

biodegraded by *P. pullulans* NCIM 1049 and *Trichoderma* sp. NCIM 1297, respectively. Comparing Fig. 3 with Figs. 4 and 5, we find that the fungal treated polymer samples have greatly reduced intensity of the 1780 cm^{-1} anhydride peak and an increase in the carbonyl peak at $\sim 1724\text{ cm}^{-1}$ due to ring opening of the maleic anhydride group. The carboxyl region at $2500\text{--}2900\text{ cm}^{-1}$ and the hydroxyl region beyond 3200 cm^{-1} also show broadening and increased intensity. Changes are also seen in the $1000\text{--}1400\text{ cm}^{-1}$ region. The spectrum shows an additional peak at 1660 and at 1387 cm^{-1} . These clearly show that the chemical structures of the sucrose linked PS-MAH are changed after fungal treatment. This is supported by weight loss data. The other carbohydrate-linked polymers also showed very similar

results and their spectra are therefore not presented here. The overlapping spectra in the expanded form ($1000\text{--}2000$) are also provided for comparison, which clearly depict the changes mentioned (Figs. 6–8).

4. Conclusions

It has been shown in this study that otherwise non-biodegradable synthetic polymers can be incorporated with structural features which can induce biodegradability. Thus, chemically linking small amounts of carbohydrate molecules with synthetic polymers like polystyrene-maleic anhydride leads to new polymers that are partially

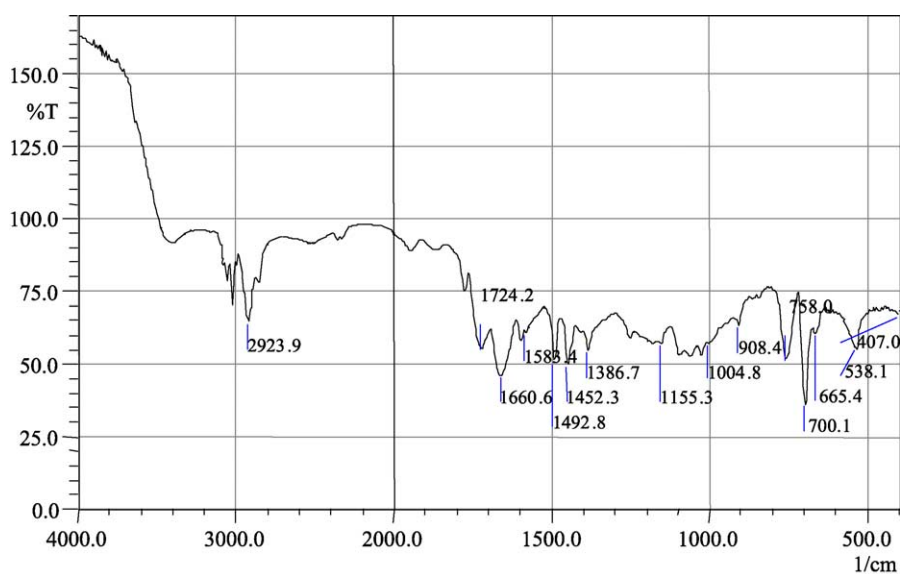


Fig. 5. FTIR spectrum of sucrose linked PS-MAH biodegraded by *Trichoderma* sp. NCIM 1297.

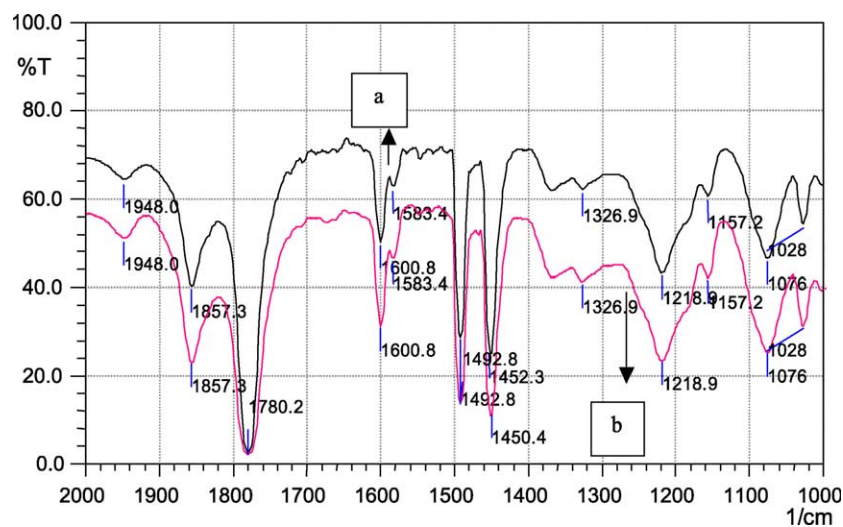


Fig. 6. Overlapping IR spectra of poly(styrene maleic anhydride) and its degraded product by *Aspergillus niger* NCIM 1025.

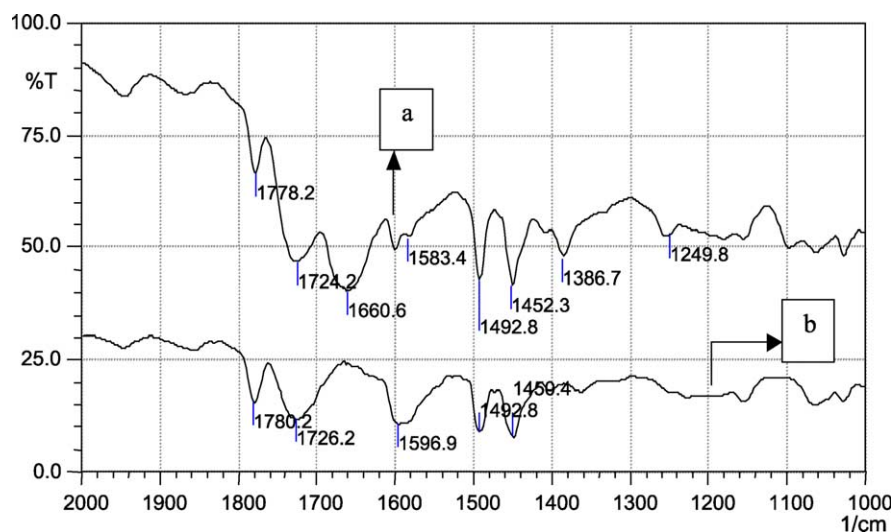


Fig. 7. Overlapping IR spectra of poly(styrene maleic anhydride) and its degraded product by *Pullularia pullulans* NCIM 1049.

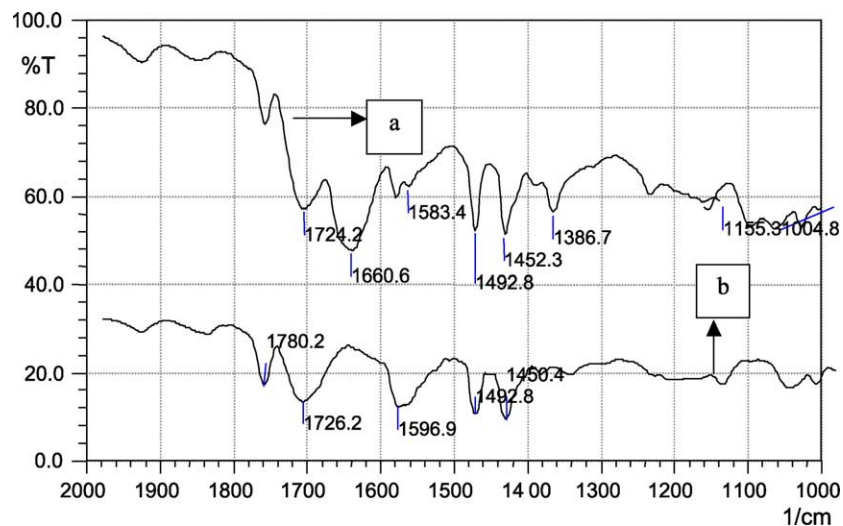


Fig. 8. Overlapping IR spectra of poly(styrene maleic anhydride) linked by sucrose and its degraded product by *Trichoderma* sp. NCIM 1297.

degraded by fungal cultures. The role of the carbohydrate molecule is the key to the further biodegradation of the synthetic polymer. The differences in the weight losses of the different types of carbohydrate-linked polymers, as well as the type of fungal culture used will throw useful new light on the design of new biodegradable polymer systems. More work is needed to fully biodegrade the modified synthetic polymer, and work in this direction is proceeding in our laboratory.

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