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Reasearch

Modification of Polystyrene Via Pectin Graft-Copolymerization to Induce Biodegradability in Polymer Composites : Biodegradation and Metal Ion Sorption Studies

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Abstract

Modification of waste plastics with natural polymers offers an attractive route to induce biodegradability therein. The present paper discusses the optimum conditions for the grafting of pectin onto polystyrene. These graft copolymers were characterized with microscopic photographs, FTIR and biodegradability test carried out through soil burial and pure culture method using *Pseudomonas fluorescens* (MTCC-667). After three-month studies, maximum 38.59% and 42.17% degradation has been observed respectively through soil burial and pure culture method. In order to use these polystyrene-pectin based composite samples in water pollution mitigation technologies, the swelling studies and metal ion sorption (As^{+5} and Cr^{6+} uptake) studies were carried out. The percent arsenic uptake by the copolymers was observed 50% after 2h by polystyrene-g-pectin copolymers while maximum sorption of Cr^{6+} ions occurred 50% after 150 minutes. In both the cases, that is sorption of As^{+5} and Cr^{6+}, it is clear that the copolymers have good capacity for the sorption of metal ion and hence can be used for removal, separation, and enrichment of hazardous metal ions in aqueous solutions and can play an important role for environmental remediation of municipal and industrial wastewater.

Keywords: Biodegradation; Pectin; Metal Ion Sorption; Polystyrene

Introduction

Low cost, abundant supply, good overall performance and mechanical properties of the plastics make these materials suitable for variety of applications. However, their resistance to the degradation is great concern leading to the long-term environmental, economical and waste management problems [1-3]. Development of biodegradable plastics or making inert plastic materials degradable through the addition of biodegradable additives, is the emerging options to solve these issues [3]. Biodegradation of polymers could be achieved either by design of a polymer from monomers which are vulnerable to micro-organisms or by incorporation of biodegradable additives or groups in the polymer. This can be achieved by copolymerization of biodegradable monomers/polymers with the non-degradable polymers [4-6]. Grafting of highly hydrophilic monomers or starch, which acted as nutrients for microorganisms, makes the polyethylene films more susceptible to microbial attack. In soil, porosity of these grafted materials increased due to the microbial consumption of starch therein which undergoes degradation into smaller particles [7,8].

The biodegradation has been studied by optical density measurements in the presence of an aerobic bacterium *Pseudomonas species* [6] and by using soil burial method [9,10]. Kaczmarek et al. have investigated the effect of UV irradiation on the degradation of polyethylene and polypropylene/cellulose composites by composting. It has been observed that biodegradation of these composites is hampered by intermolecular cross linking of both components [11,12]. Some fungus species also help in degradation of polyethylene modified with synthetic polyester [13]. In one study, biodegradability of plastic material has been assessed under aerobic and anaerobic conditions. For aerobic conditions, organic fractions of municipal solid wastes have been composted and for the anaerobic process, anaerobic inoculums from a wastewater treatment plant has been used. The biodegradation of the samples have been monitored in terms of mass loss [14].

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Incorporation of polar functionalities onto synthetic polymers induces hydrophilicity which is a step towards induction of biodegradation and use of these polymers in enrichment separation technology [15]. Removal, separation, and enrichment of hazardous metal ions in aqueous solutions play an important role for environmental remediation of municipal and industrial wastewater [16]. Chromium is one of the most toxic of metals, exists in two stable oxidation states viz., Cr(III) and Cr(VI). [17]. Inorganic arsenic occurs with two main oxidation states in natural waters, as As(V) and As(III). The toxicity of As(III) is much higher and more difficult to remove by the conventionally applied physiochemical methods than As(V) [18]. Arsenic and chromium toxicity causes skin, lungs and kidney cancer. These metal ions also responsible for hyperkeratosis, muscular and neurological disorders [19-22]. It is very essential to removal these metal ions from the water bodies and their removal have been reported through various methods [23-27].

Advanced technology in petrochemical polymers has brought many benefits to mankind. Synthetic, petroleum-based polymers are extremely stable and are commonly used in various applications. However, their attractive stability is counterbalanced by two problems; polymers contribute to the demand for expensive imported oil and their great resistance to biodegradation. Petroleum reserves will be exhausted in less than a century. It is therefore, necessary to find another raw material for the fuel area, but also for plastic industry. The shortage and high cost of fossil resources require that alternative resources and processes must take over in the near future. Biodegradable polymers are now being considered as an alternative to the existing petrochemical-based polymers [28]. In view of the environmental pollution caused by the waste polystyrene, we have modified/functionalized the polystyrene with pectin through graft copolymerization to induce biodegradability in it and make it technological important material for water pollution alleviation technology. Pectin is biodegradable hydrophilic polysaccharide derived from plant cell walls, found in fruit and vegetables and mainly prepared from 'waste' citrus peel and apple pomace. The present paper discusses the optimum conditions for the synthesis of graft copolymers, characterization of these polymers with microscope photographs and FTIR, and biodegradation studies of grafted product by soil burial method and microbial method. The present paper also discusses the swelling of the grafted polymers and metal ion sorption (As^{+5} and Cr^{6+} uptake) by these polymers.

Experimental

Materials and Method

Polystyrene (packaging material waste) (PSty), ammonium persulphate (APS) and $K_2Cr_2O_7$ were obtained from S.D. Fine Mumbai-India, Pectin was obtained from SRL Pvt. Ltd. India, Arsenic and chromate testing (sensitive) kit were obtained from Merck-Schuchardt, Germany. Toluene, yeast extract, agar-agar (Bacteriological grade) were obtained from Qualigens Fine Chemicals Mumbai-India. Peptone was obtained from Himedia Laboratories Pvt Ltd. Mumbai-India. *Pseudomonas fluorescens* (MTCC-667) was purchased from Institute of Microbial Technology (IMTech), Sector 39-A, Chandigarh- India.

Graft Copolymerization

One gram of polystyrene (dissolved in 5mL of toluene) was taken in 100 ml round bottom flask placed in a water bath maintained at a definite temperature. A known amount of ammonium persulfate, definite amount of pectin was taken in 10mL water were added and reaction was carried out for a definite time. After three hours, copolymer was extracted by adding non-solvent methanol and was filtered and washed several times with cold water and then with hot water for the removal of the unreacted pectin. The pectin free grafted product was dried in oven at 50°C. Percentage of add-on ($P_{add\text{-}on}$) of pectin onto polystyrene was determined as follows:

$$P_{add\text{-}on} = W_1\text{-}W_0/W_0 \times 100$$

Where W_0 and W_1 are respectively the weights of the PSty and graft copolymers. $P_{add\text{-}on}$ of pectin onto polystyrene has been studied as a function of various reaction parameters such as concentration of APS, time of reaction, temperature, amount of pectin, amount of PSty and water. At optimum reaction conditions, further polymers were synthesized and were used to study the swelling behavior, metal ion uptake studies and biodegradation studies through soil burial and microbial culture method.

Swelling Studies

Swelling studies of the pure and grafted polymers were carried out in aqueous medium by gravimetrically [29]. The equilibrium percent swelling (P_s) of the polymeric networks was calculated as:

$$P_s = (W_s - W_d / W_d) \times 100$$

Where W_s is the weight of swollen polymer and W_d is the weight of dried polymers.

Metal Ion Sorption Studies

Arsenic Uptake

Arsenic uptake studies were carried out according to the procedure reported in the arsenic testing (sensitive) kit of MERCK (cat. No.1.17926.0001). When zinc [Reagent (As-1)] and sulfuric acid [Reagent (As-2)] were added to compounds of arsenic (III) and arsenic (V), it has liberated arsenic hydride, which in turn reacted with mercury(II) bromide contained in the reaction zone of the analytical strip to form yellow- brown mixed mercury halogenides. The concentration of arsenic (III) and arsenic (V) were measured

semi-quantitatively by visual comparison of the reaction zone of the analytical test strip with the field of a color scale. Measuring Range/ Color Scale graduation lies up to : 0.01-0.025-0.05-0.1-0.5 mg/L $As^{3+/5+}$. The chemistry involved in this method is shown in the Fig (1.1).

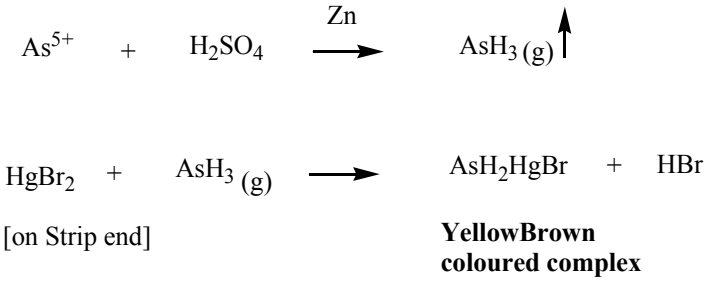


Fig 1.1: Formation of arsenic mercury-halogenide

Procedure

20 mL of 1mg/L As^{5+} solution was taken in a beaker and 100mg of grafted polymer sample was added to it for specific time with constant stirring and then the solution was filtered. The test strip about half way through the slot was inserted in the stopper of the reaction vessel. 10ml pretreated filtrate was injected in to reaction vessel and after that 2 leveled measuring spoonful of Reagent (As-1) were added to the solution and it was swirled, subsequent to this , rapidly 10 drops of Reagent (As-2) were added and reaction vessel was closed immediately with gentle swirling. After 30 minutes (gently swirling two or three times) the test strip was taken out and dipped into water, and color was coincided with the As^{5+} and percent uptake of metal ion was calculated as :

%age uptake of arsenic by the polymer = $(C_i\text{-}C_f / C_i) \times 100$

where C_i is the initial concentration , Cf is the final concentration of arsenic in reaction vessel. Percent uptake of arsenic by the polymer was calculated as function of time.

Chromium Uptake

Chromium uptake studies were carried out according to the procedure reported in the chromate testing (sensitive) kit of MERCK (cat. No.1.10012.0001). In a weakly phosphoric solution chromate ions were reacted with diphenyl carbazide to form chromium(III) and diphenylcarbazone, which have formed a red-violet complex. The concentration of chromate was measured semi-quantitatively by visual comparison of the reaction zone of the test strip with fields of a color scale. Measuring range/ color scale graduation lies up to : 3-10-30-100mg/LCrO_4^{2-} or 1.3-4.5-13.4-44.8mg/L Cr.

A stock solution of known concentration i.e. 30mg/L Cr^{6+} was prepared by dissolving $K_2Cr_2O_7$ in distilled water. The chemistry involved in this method is shown in the Fig. 1.2.

Procedure

10 mL of 30mg/L Cr^{6+} solution was taken in a beaker and 100mg of grafted polymer sample was added to it for specific time at 35°C with constant stirring. Then the solution was filtered. The test vessel was rinsed several times with the pretreated sample. 5ml of pretreated sample was taken to fill the test vessel to the 5ml. Reagent CrO_4^{-2} was added drop wise until the pH of the solution is below 1. The pH of the solution was checked with digital pH meter. The reaction zone of the test strip was immersed in the measurement sample for approximately 1 sec. Excess liquid from the strip was shaked off. Then within 15 sec. colors of the reaction zone was coincided exactly with the color on the label mentioned in the test kit to measure the corresponding value in mg/L CrO_4^{2-}. Polymers were kept in Cr^{6+} solution for different time interval to study the effect of time on the metal ion sorption.

Biodegradation Studies

Biodegradation Studies (Soil Burial Method)

Pure polystyrene and pectin graft polystyrene (PSty-*g*-pectin) films (4×1.5) were buried 5cm in soil at room temperature in 2L capacity jars for three months. Soil was adjusted to 40%-50% moisture contents. After a specified time, samples were taken out from the soil and thoroughly rinsed with distilled water and then dried in oven at 45°C to obtain constant weight. Degradation of the samples was calculated by gravimetric method.

Biodegradation Studies (Microbial Degradation Method)

Procurement of the Microorganism

The freeze-dried culture of *Pseudomonas fluorescens* (MTCC Number 667) was procured from the IMTECH Chandigarh-India.

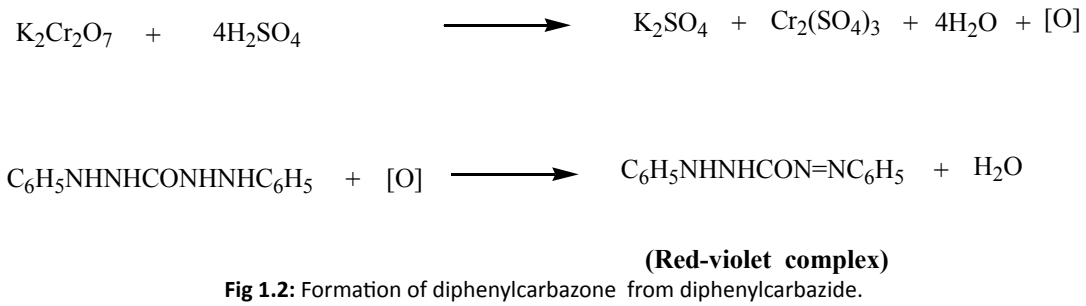


Fig 1.2: Formation of diphenylcarbazone from diphenylcarbazide.

Preparation Nutrient Medium and Revival of Culture

Pseudomonas fluorescens (MTCC Number 667) was revived onto nutrient broth at 30°C at the optimum conditions mentioned in the MTCC literature of IMTECH Chandigarh. The freeze-dried culture of *Pseudomonas fluorescens* (MTCC Number 667) was transferred to sterilized [70% (v/v) ethanol] autoclaved Erlenmeyer flask containing 50ml nutrient broth. (Nutrient broth was prepared with 0.5% peptone (w/v) and 0.2%(w/v) yeast extract in distilled water). Flask was incubated in shaker (160 rpm) at 30°C and growth of microorganism was monitored regularly by taking optical density of the broth at 600nm.

Culture of microorganism was maintained in nutrient broth (pH 7±0.2). The medium for these plates and slants was prepared by mixing 2g agar-agar in 100ml above mentioned nutrient medium. The contents were sterilized by autoclaving at 15 lbs pressure and at 121°C temperature for half an hour. The medium when still hot was poured in sterile petriplates (20ml) and sterile test tubes (10ml), and was allowed to cool so that the agar gets solidified. Plates and slants when completely solidified were incubated at 30°C over night to check any contamination and after that inoculated with the microorganism grown in the nutrient medium under sterile conditions. Plates and slants were incubated at 30°C for growth of *P fluorescens* (MTCC Number 667). When growth appeared, plates and slants were stored in refrigerator at 4°C. Sub-culturing was done at every month to revive the strain of *P fluorescens* (MTCC Number 667).

Microbial Degradation Study

Weighed disinfected [70% (v/v) ethanol] films of pure polystyrene (4×1.5) and PSty-*g*-pectin (4×1.5) were kept overnight at 60°C and were aseptically added to sterilized 50ml culture medium in Erlenmeyer flask (250ml). Culture medium was inoculated with microorganism *Pseudomonas fluorescens* and was incubated at 30°C under shaking (160rpm) for 10 days. Control was maintained with polystyrene films in the microbe-free medium. Three replicates were prepared for each pretreated film. After 10 days the samples were taken out and washed with distilled water and then with 70% ethanol to remove cell mass from the residual films and then dried at 45°C for 24h and weighted. Biodegradation occurred in the samples was calculated in terms of weight loss. The biodegradation study was carried out for three months.

Characterization

Polystyrene and modified polystyrene [PSty-*g*-pectin] were characterized microscopic photographs and Fourier transform infrared spectroscopy (FTIR). Microscopic photographs were taken with LEICA DM LS 2 Microscope (camera number DFC 320) with magnification 200 and FTIR spectra were recorded in KBr pellets on Nicolet 5700FTIR (THERMO).

Results and Discussion

Characterization

The microscopic photographs of PSty film and PSty-*g*-pectin showed the change in morphology occurred after modification of PSty. It was observed that PSty has smooth and homogenous structure while grafted polymers have structure heterogeneity. FTIR spectra of PSty film, PSty-g-pectin has been shown in figs 2.1 and 2.2 respectively to investigate the incorporation of pectin in the polystyrene. absorption band has been observed between 3600-3200 cm^{-1} due to -OH stretching along with some complex bands in the region 1200-1030 cm^{-1} due to C-O and C-O-C stretching vibrations which are the characteristic of the natural polysaccharides. In addition, the absorption bands in the region 930-820 cm^{-1} and 785-730 cm^{-1} are due to vibration modes of pyranose rings of polysaccharides have also been observed.

Effect Of Reaction Parameters on $P_{add\text{-}On}$ of Pectin Onto Polystyrene

Effect of Initiator Concentration

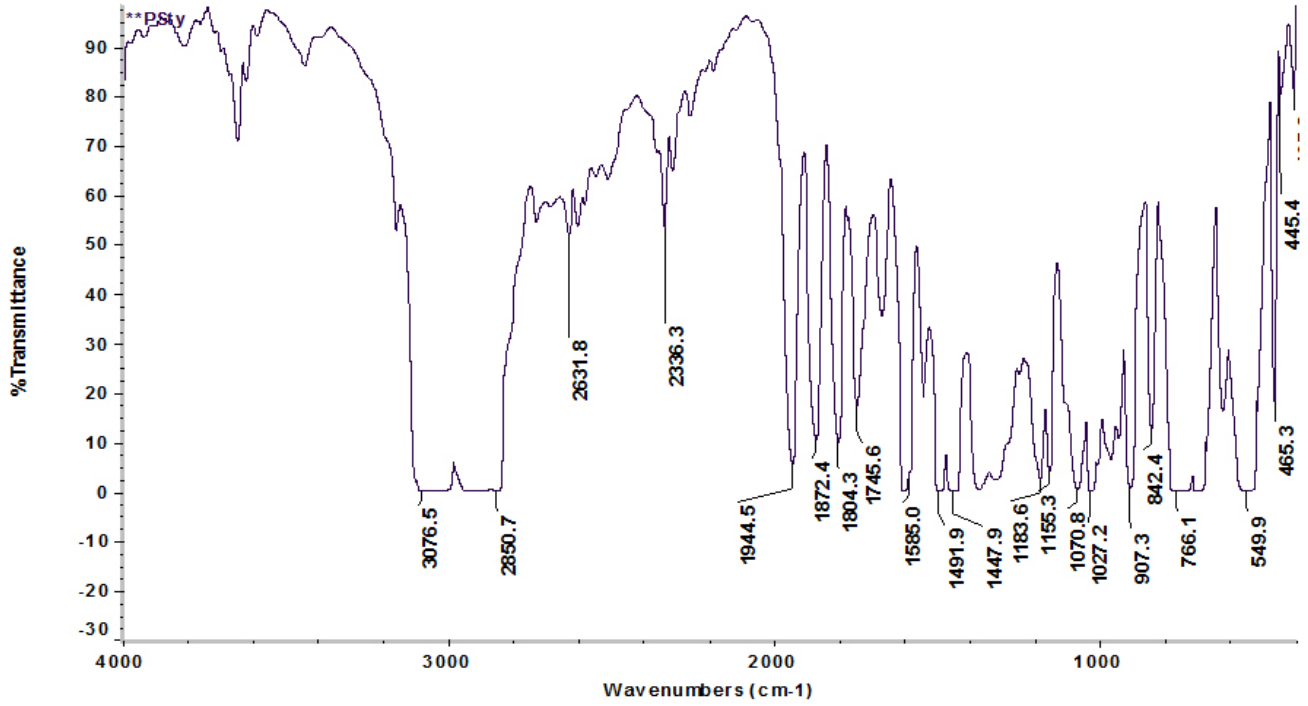
$P_{add\text{-}on}$ of pectin onto polystyrene (Psty) was studied as a function of initiator concentration. The [APS] was varied from 0.22mM to 1.11mM (figure 3.1). It is observed from the figure that $P_{add\text{-}on}$ of pectin onto polystyrene first increases up to 0.44mMole of [APS] and then decreases. Maximum $P_{add\text{-}on}$ 33% was obtained at 0.44mMole of initiator. As the concentration of initiator increases, more and more reactive sites formed on the pectin and polystyrene but beyond 0.44mMole self termination of primary radical started and decreased the $P_{add\text{-}on}$. However at higher concentration some irregular trends have been obtained for unknown reason (Fig. 3.1).

Effect of Time

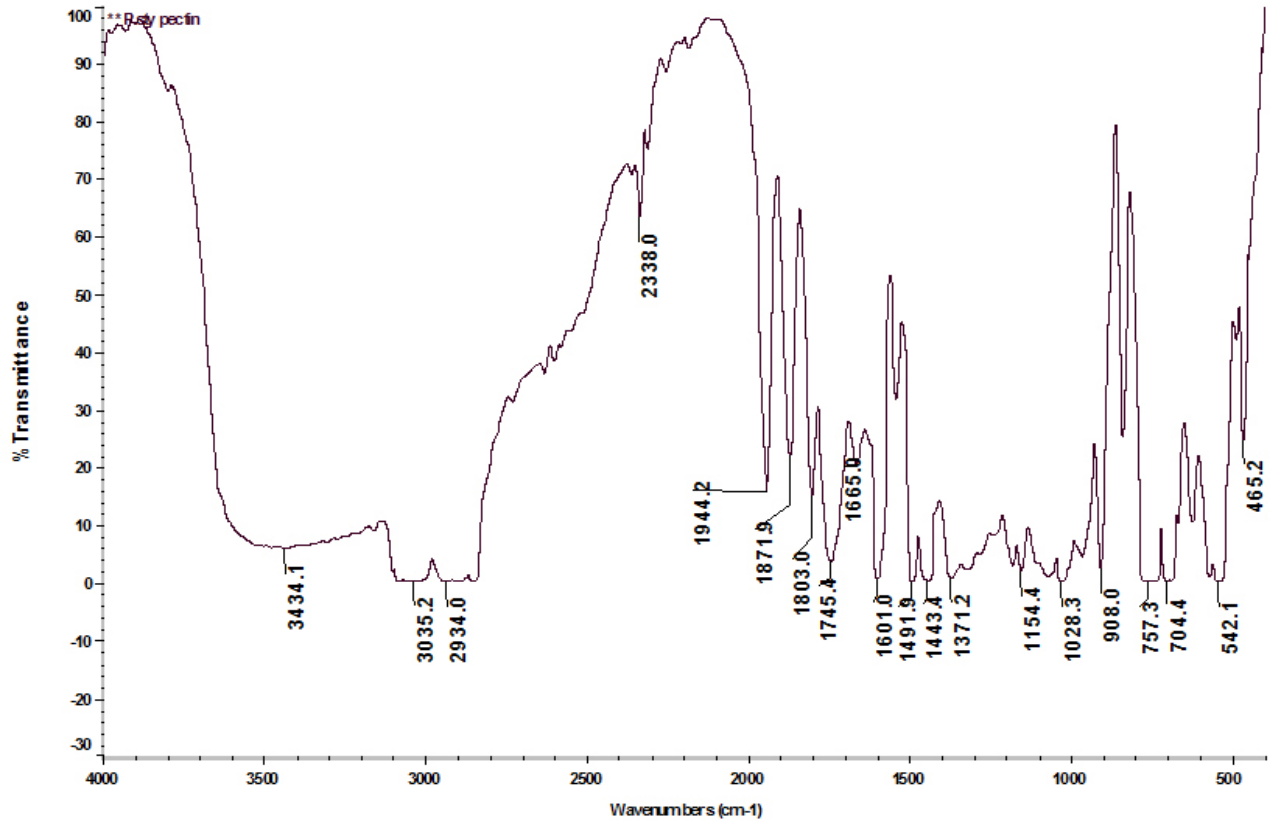
At optimum [APS] (0.44mMole) further polymers were synthesized by varying the reaction time from 1h to 4h. The effect of reaction time on the grafting percentage is presented in the figure 3.2. It is observed from the figure that $P_{add\text{-}on}$ of pectin onto polystyrene increases with increase in reaction time upto 2.5h and after that it decreases. Maximum $P_{add\text{-}on}$ was obtained 43% in 2.5h. This is due to the fact that as in the start of the reaction, rate of initiation and propagation reaction is more as compared to rate of termination reaction.

Effect of Temperature

In order to study the effect of temperature on the graft copolymerization of pectin onto polystyrene, temperature was varied from 55°C to 80°C keeping all other reaction parameters such as concentration of initiator, reaction time and amount of pectin fixed. The results of $P_{add\text{-}on}$ of pectin onto polystyrene are shown in figure 3.3. Initially increase in $P_{add\text{-}on}$ was observed with increase in temperature upto 65°C, after this a sudden fall in $P_{add\text{-}on}$



(2.1)



(2.2)

Fig. 2 FTIR spectra of (2.1) pure polystyrene (2.2) Psty-g-pectin

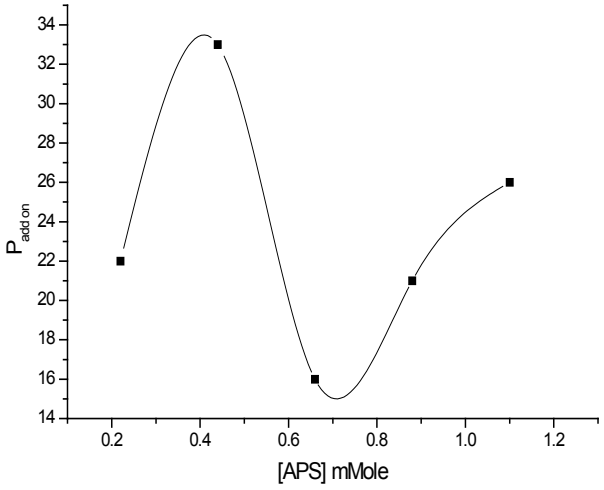


Fig. 3.1 Effect of [APS] on $P_{add\ on}$ of pectin onto PSty. [PSty = 1g, pectin = 1g, reaction time = 2.5h, temperature = 65°C, toluene = 5ml, water = 10ml. ($P_{add\ on}$ = 33%)]

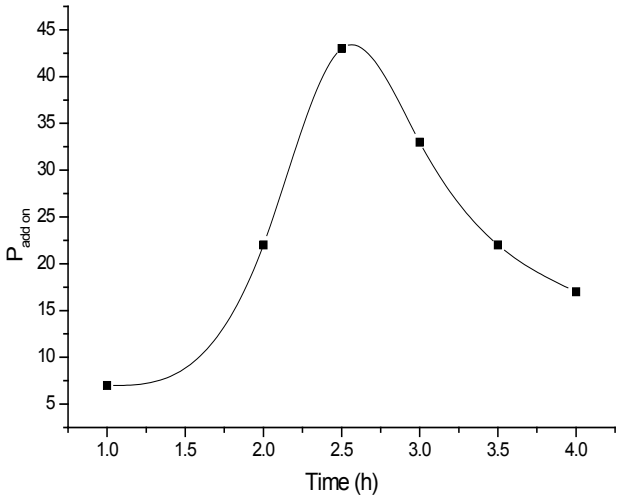


Fig. 3.2 Effect of time on $P_{add\ on}$ of pectin onto PSty. [PSty = 1g, pectin = 1g, APS = 0.44mM, reaction temperature = 65°C, toluene = 5ml, water = 10ml.($P_{add\ on}$ = 43%)]

is observed. It is due to the reason that increases in temperature increases rate of decomposition of initiator which leads to the formation of more free active sites on the pectin and polystyrene. It also increases the rate of collision of reaction species in the reaction system. Decrease in $P_{add\text{-}on}$ at higher temperatures is also attributed to premature termination of growing polymeric chains as well as occurrence of various chain transfer reactions.

Effect of Amount of Pectin

The effect of different amount of pectin on $P_{add\text{-}on}$ of pectin onto polystyrene was studied by varying the amount of pectin from 0.2g to 1.2g. in the reaction system. The results of $P_{add\text{-}on}$ of pectin onto polystyrene are shown in figure 3.4. As the amount of pectin increases the $P_{add\text{-}on}$ first increases and then decreases. This increase in $P_{add\text{-}on}$ due to increase in amount of pectin is due to availability of more active sites on the pectin for the formation of the graft polymers and when all the sites get occupied decrease in grafting has been observed. Maximum $P_{add\text{-}on}$ 43% is observed when 1.0g of pectin was used in the reaction mixture.

Swelling Studies

The swelling studies of PSty-g-pectin were carried out in distilled water at room temperature for 24 hrs and results are presented in the figure 4. Maximum 60% swelling is observed after 24h water treatment. Swelling results indicates that these copolymers of pectin and polystyrene can be used in water pollution alleviation technologies. The swelling in the grafted samples occurred due to hydrophilicity induced by the hydrophilic pectin into polystyrene.

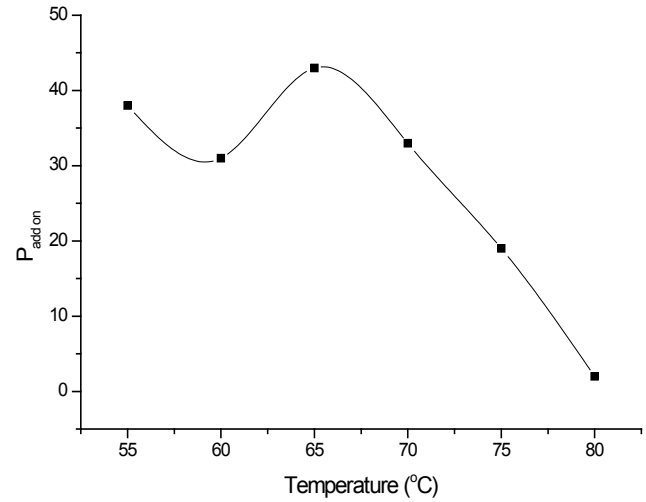


Fig. 3.3 Effect of Temperature on $P_{add\ on}$ of pectin onto PSty. [PSty = 1g, pectin = 1g, APS = 0.44mM, reaction time = 2.5h, toluene = 5ml, water = 10ml.($P_{add\ on}$ = 43%)]

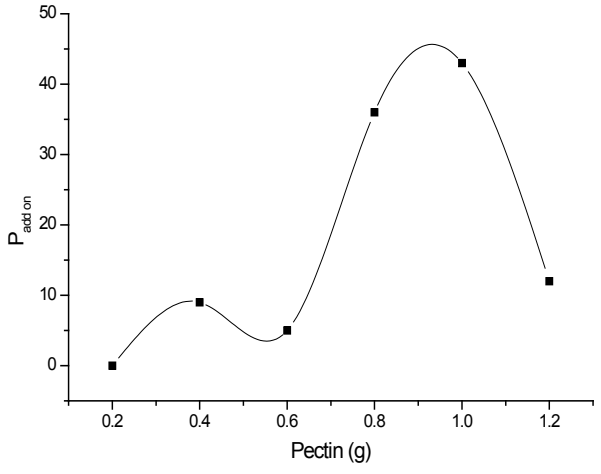


Fig. 3.4 Effect of amount of pectin on $P_{add\ on}$ of pectin onto PSty. [PSty = 1g, APS = 0.44mM, reaction time = 2.5h, temperature = 65°C, toluene = 5ml, water = 10ml.($P_{add\ on}$ = 43%)]

The incorporation of hydrophilic moieties in the first step towards the induction of biodegradability in the plastic.

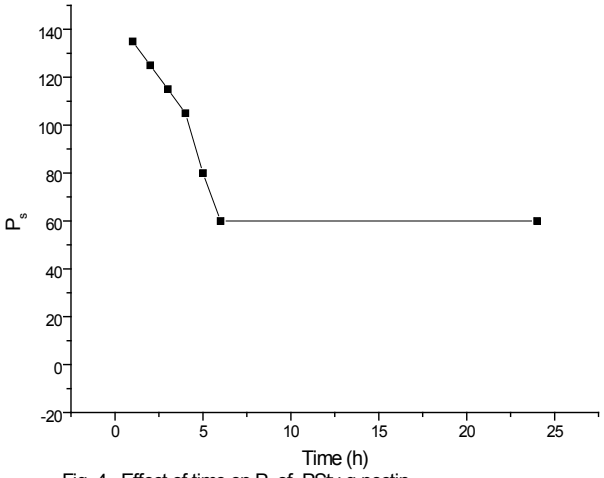


Fig. 4. Effect of time on P_s of PSty-g-pectin.
[PSty = 1g, pectin = 1g, APS = 0.44mM, reaction time = 2.5h, temperature = 65°C, toluene = 5ml, water = 10ml. ($P_{add\ on}$ = 43%)]

Metal Ion Sorption

The percent arsenic uptake by the copolymers was studied as a function of time of polymer sample kept in the solution of As^{+5} ions and results are shown in the figure 5.1. The metal ion sorption capacity of a polymer increases with the increase in time of sorption up to certain limit and maximum sorption 50% occurred after 2h by [PSty-g-pectin].

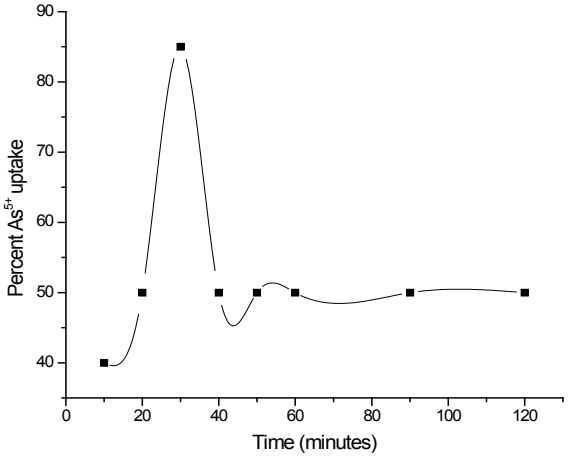


Fig. 5.1. Effect of time on percent As^{5+} uptake by PSty-g-pectin.
[PSty = 1g, pectin = 1g, APS = 0.44mM, reaction time = 2.5h, temperature = 65°C, toluene = 5ml, water = 10ml. ($P_{add\ on}$ = 43%)]

The percent Cr^{6+} by the copolymers was studied as a function of time of polymer sample kept in the solution of Cr^{6+} ions and results are shown in the figure 5.2. The metal ion sorption capacity of a polymer increases with the increase in time of sorption up to certain limit and maximum sorption 50% occurred after 150 minutes. In both the cases, that is sorption of As^{+5} and Cr^{6+}, it is clear that the copolymers have good capacity for the sorption of metal ion and hence can be used for removal, separation, and enrichment of hazardous metal ions in aqueous solutions and can play an important role for environmental remediation of municipal and industrial wastewater

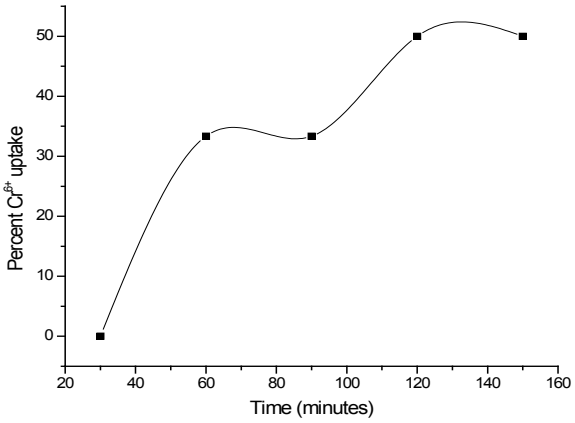


Fig. 5.2. Effect of time on percent Cr^{6+} uptake by PSty-g-pectin.
[PSty = 1g, pectin = 1g, APS = 0.44mM, reaction time = 2.5h, temperature = 65°C, toluene = 5ml, water = 10ml. ($P_{add\ on}$ = 43%)]

Biodegradation Studies

Biodegradation Studies (Soil Burial Method)

In three months of soil burial study, 38.59±0.69 % degradation has been observed for the PSty-g-pectin having 43% of grafting. The results of soil burial studies are presented in figure 6.

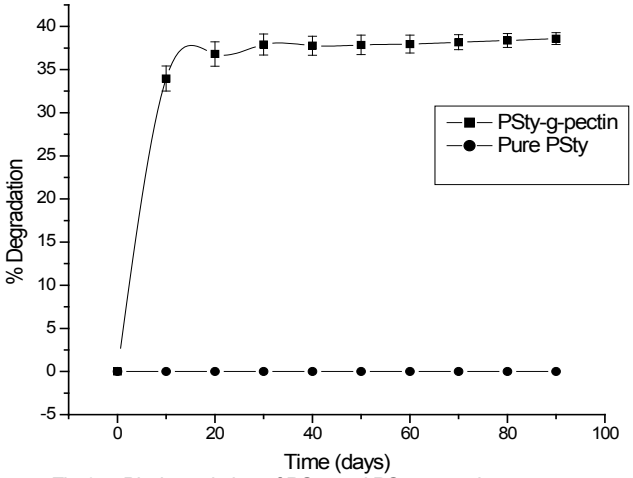


Fig.6. Biodegradation of PSty and PSty-g-pectin (Soil Burial method).
[PSty = 1g, pectin = 1g, APS = 0.44mM, reaction time = 2.5h, temperature = 65°C, toluene = 5ml, water = 10ml. ($P_{add\ on}$=43%)]

This is due to the reason that when the samples were buried in the soil, the growth of microorganism occurred on the samples. Pectin is grafted on the polystyrene and at the point of attachment, scissoring of polystyrene chains may occur and some chains at the site of grafting may cut off and decrease the number of carbon

atom in the chains present in the polystyrene. This observation is attributed to the reason that pectin being a natural polymer is more susceptible for bacterial and fungal growth. Apart from weight loss, change in color of the pectin modified PSty films has also been observed. The change in color of grafted polystyrene film after soil burial is clearly visible in the microscopic photographs taken with LEICA DM LS 2 Microscope (camera number DFC 320) with magnification 200**.** The microscopic photographs of grafted polystyrene after soil burial studies are presented in the figures 7. The surface changes after soil burial studies are clearly visible in the microscopic photographs. FTIR spectra have showed that with passage of time pectin portion of the grafted product has been consumed by the soil microorganisms (figure 8).

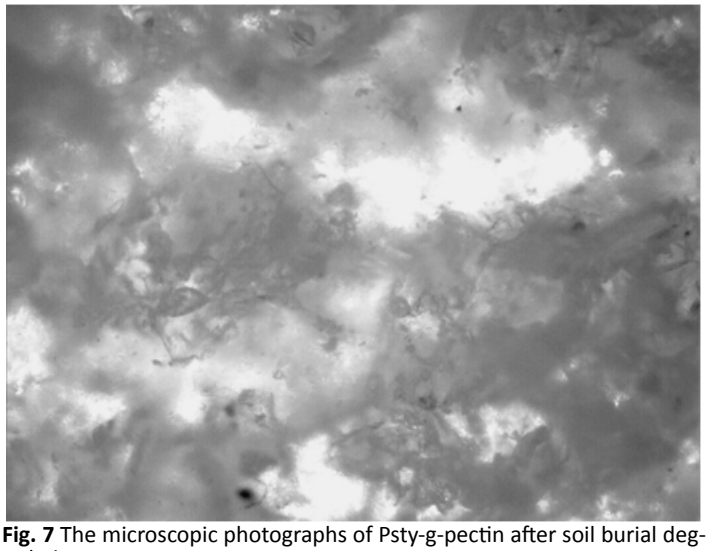


Fig. 7 The microscopic photographs of Psty-g-pectin after soil burial degradation.

Biodegradation Studies (Pure culture Method)

In three months of microbial degradation study, PSty-g-pectin samples have showed 42.17±4.63% degradation while pure polystyrene has showed 3.58±0.6%. The degradation through soil burial method is also due to the scissoring effect. The results of microbial degradations are presented in figure 9. Apart from weight loss, change in color of the copolymer film has been observed after microbial degradation.The microscopic photographs of microbial degradation are presented in the figures 10. FTIR spectra showed that with passage of time pectin portion of the grafted product has been consumed by the soil microorganisms (Figure 11). FTIR spectra of the soil burial degraded and microbial degraded PSty-g-pectin films indicated substantial reduction of the hydroxyl peak occurring at 3434.1 cm^{-1}. The decrease in the intensity of this peak indicates the degradation of surface pectin.

Conclusion

From the above discussion it has been concluded that waste polystyrene when modified via graft copolymerization with pectin, has induced the hydrophilicity and degradability in the plastic waste. From metal ion sorption study it is evident that these polymeric networks developed from the waste polystyrene can be used for the removal, separation, and enrichment of hazardous metal ions in aqueous solutions and can play an important role for environmental remediation of municipal and industrial wastewater. It is the reusability of the waste polystyrene for the use in water technology. This intention could be further explored for the use of waste polystyrene for the sorption of other metal ions from the water bodies and simultaneously induce the degradation in the inert material.

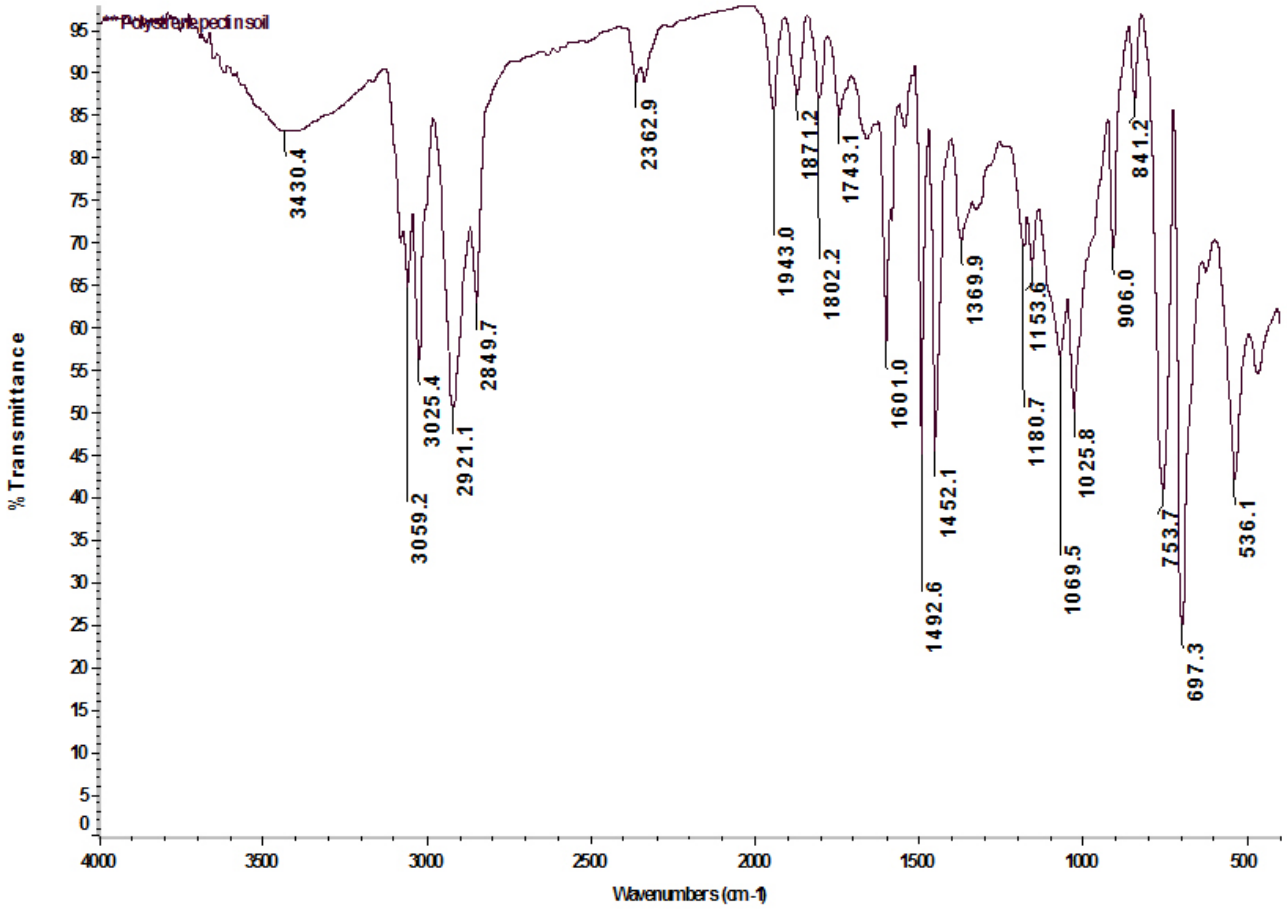


Fig.8 FTIR spectra of Psty-g-pectin after soil burial degradation

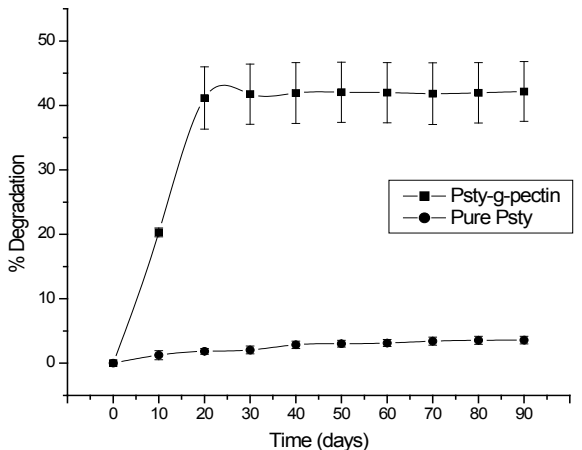


Fig. 9. Biodegradation of PSty and PSty-g-pectin (microbial degradation method).
[PSty = 1g, pectin = 1g, APS = 0.44mM, reaction time = 2.5h, temperature = 65°C,toluene = 5ml, water = 10ml. ($P_{add\ on}$=43%)]

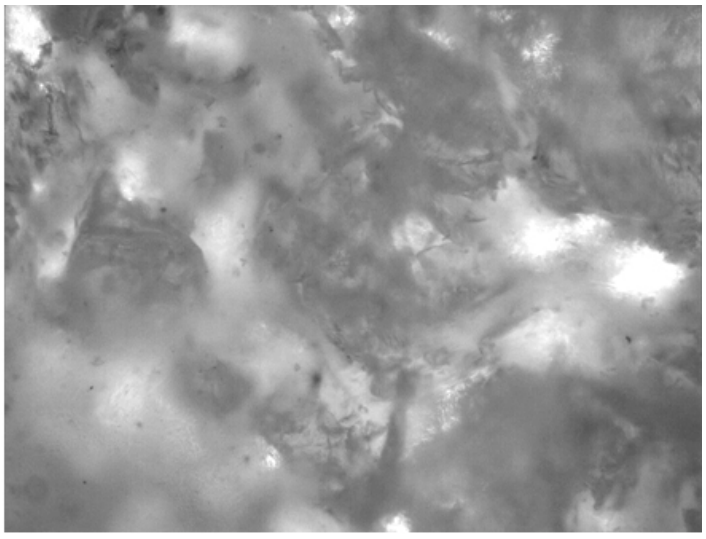


Fig. 10 The microscopic photographs of Psty-g-pectin after microbial degradation.

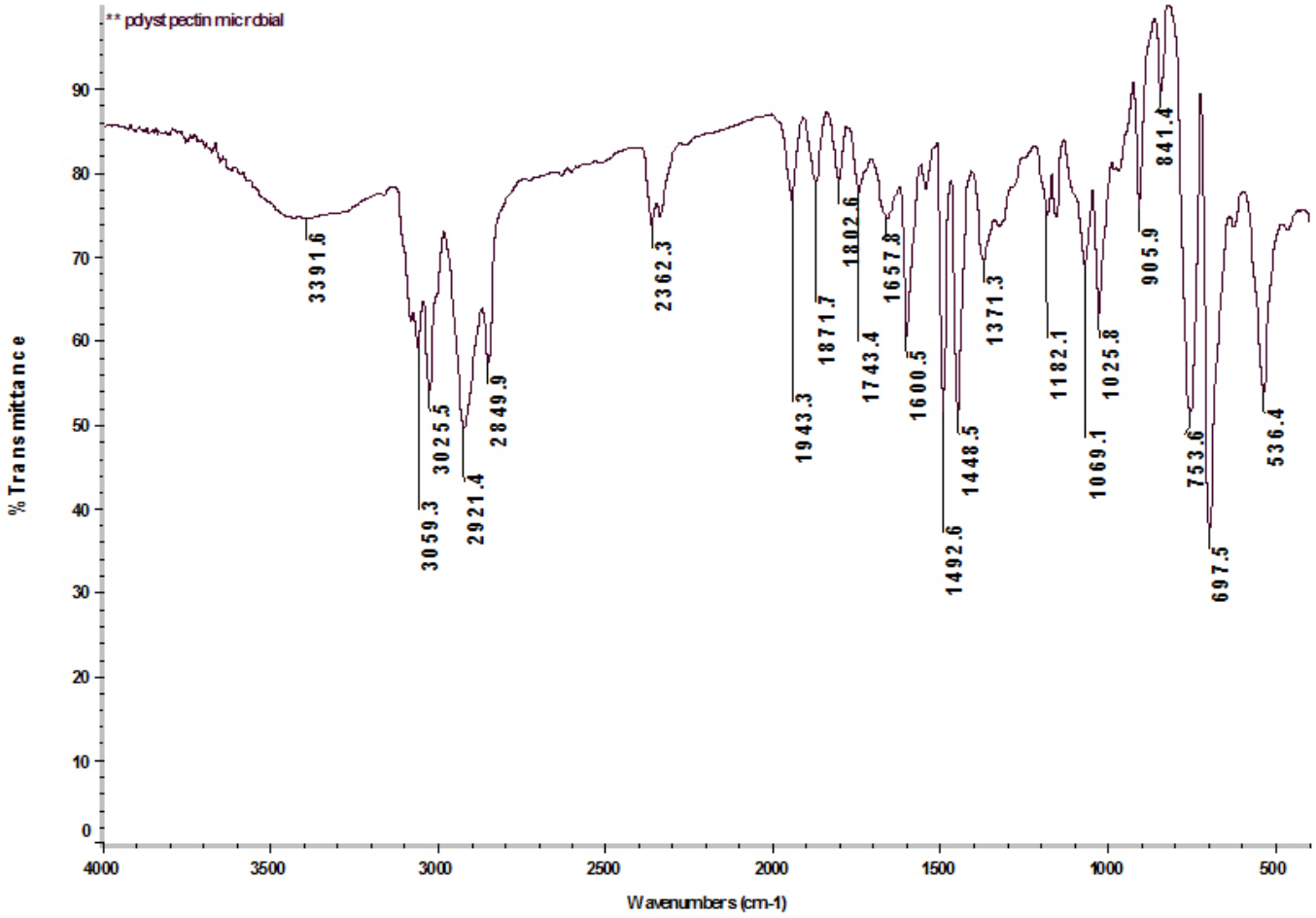


Fig.11 FTIR spectra of Psty-g-pectin after microbial degradation.

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