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Towards biodegradable polyolefins: strategy of anchoring minute quantities of monosaccharides and disaccharides onto functionalized polystyrene, and their effect on facilitating polymer biodegradation†

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A hypothesis was developed, and successfully tested, to greatly increase the rates of biodegradation of polyolefins, by anchoring minute quantities of glucose, sucrose or lactose, onto functionalized polystyrene (polystyrene-co-maleic anhydride copolymer) and measuring their rates of biodegradation, which were found to be significantly improved.

The continuing growth in consumption of non-degradable polymers has led to severe problems of plastic waste disposal by land filling or incineration. One of the alternatives to overcome this problem is the development of biodegradable polymers. Natural polymers are still some way from being developed as viable alternatives to petroleum derived polymers. This realization has led to voluminous researches on additives for polyolefins that can cause degradation of these polymers.[1] However, these additives are toxic, can leach out, and also affect the other additives needed to process polyolefins. On the other hand, hardly any successful research has gone into designing new plastics or effecting minor chemical modifications of polyolefins or similar polymers with attachment of sugar molecules[2–6] that can render the polymer molecule intrinsically biodegradable or bioassimilable, and convert these large volume polymers into environmentally benign materials.

In this paper we present our results on a new strategy to vastly improve the rates of biodegradation of functionalized polystyrene. Polystyrene, functionalized with maleic anhydride (14% by weight), was used as a model polymer, onto which we chemically anchored minute quantities of various monomeric sugars like glucose, lactose or sucrose.‡ Instead of using a mixture of several microorganisms, we chose three pure soil bacterial cultures§ (*Serratia marcescens*, *Pseudomonas* sp., and *Bacillus* sp.) for studying their individual growth patterns on these new polymers in comparison to their growth in glucose solution or unmodified polymer.

Fig. 1 shows that the rates of increase in optical density in sugar-linked polymers (reflecting material degradation) is much higher than the starting polymer and also higher than the glucose control.¶

The advantages of using pure cultures in biodegradation studies of polymers helps to identify the types of soil bacteria that preferentially attack a particular type of sugar-linked polymer. This is useful in designing biodegradation culture media for biodegrading such systems.

The IR spectrum of poly(styrene-co-maleic anhydride) showed strong bands at ~1772 and 1857 cm^{-1} due to symmetric and antisymmetic stretching vibrations of the anhydride carbonyl group. It also shows the characteristic peak corresponding to cyclic anhydrides at 1220 cm^{-1}. The spectrum of the poly(styrene-co-maleic anhydride) chemically linked with sucrose *via* ester linkages shows a reduction in the intensity of the 1780 cm^{-1} peak along with the appearance of a new broad peak at ~1728 cm^{-1}, which arises due to the merging of

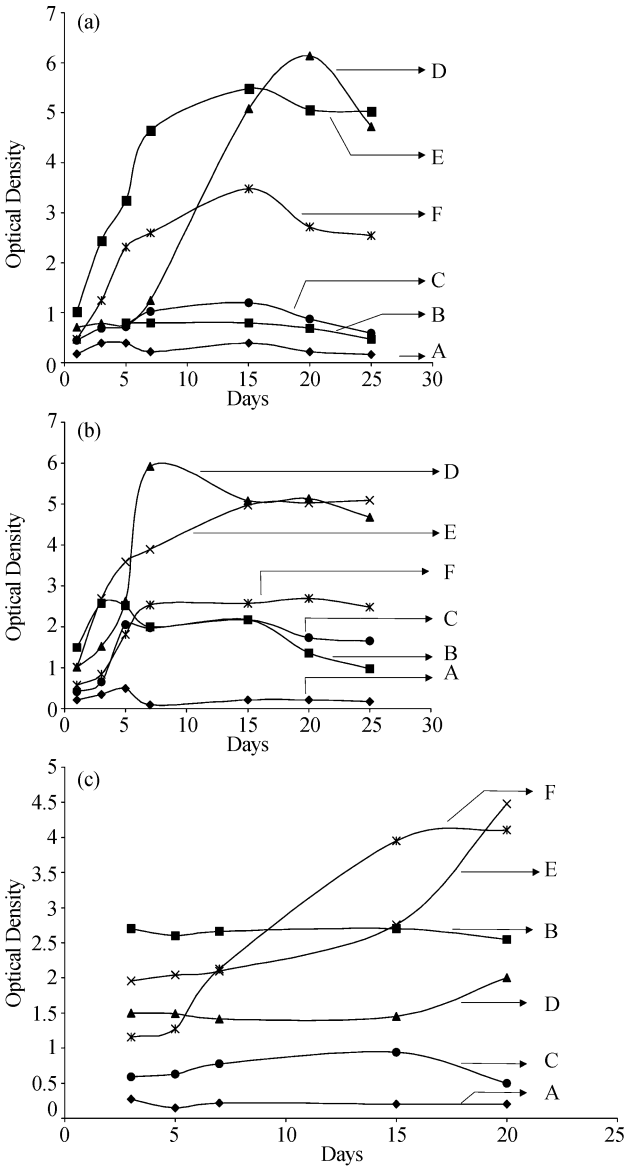

Fig. 1 Growth pattern of (a) *Pseudomonas* sp. (NCIM 2220), (b) *Serratia* sp. (NCIM 5061) and (c) *Bacillus* sp. (NCIM 2812) in the presence of carbohydrate-linked polystyrenes (each point on the graph represents an average of three values, and the reproducibility was ±5%). A = Control 1: control without any source of carbon; B = control 2: control with glucose as the sole carbon source; C = control 3: unmodified poly(styrene-co-maleic anhydride); D = lactose-linked polystyrene; E = glucose-linked polystyrene; F = sucrose-linked polystyrene.

† Electronic supplementary information (ESI) available: experimental details and weight loss data. See http://www.rsc.org/suppdata/cc/b2/b209254a/

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the ester and carboxylic carbonyl groups produced by ring-opening of the anhydride after reaction with the hydroxyl of the carbohydrate. The biodegraded poly(styrene-co-maleic anhydride) linked with sucrose shows a sharp decrease in the intensity of the 1600 and 1583 cm^{-1} bands, and the anhydride carbonyl band at 1780 cm^{-1} disappears. Changes can also be observed in the 1150–1200 cm^{-1} region. There seems to be an apparent increase in the intensity of the ester carbonyl peak at $\sim$1728 cm^{-1}. This could be a result of the contribution of the carboxyl group of the hydrolyzed maleic anhydride groups in the polymer. This fact has been proved by hydrolyzing the poly(styrene-co-maleic anhydride) and comparing the spectra with that of the unhydrolyzed polymer. The changes in the IR spectrum indicate that some chemical changes have indeed occurred in the polymer after biodegradation. Weight loss of the polymers after biodegradation and GPC data showing reduction in the molecular weight of the degraded product support the spectral results on biodegradation.

In conclusion, we have proved our hypothesis that anchoring of minute quantities of saccharide moieties onto polyolefins would greatly improve their rates of biodegradation that can make these polymers acceptable to a society desiring eco-friendly materials. We feel this is a general strategy that can be applied to all polyolefins, and on-going detailed research in our laboratory in this area will be published in due course. The dramatic changes in growth pattern of individual bacteria on the unmodified polymer as well as on the saccharide modified polymers can have wide-ranging ramifications in the design of sugar based biodegradable polymers, the biodegradation media, and the mechanism of biodegradation of sugar-laced polymers by these bacteria.

Notes and references

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‡ *General procedure* for grafting of sugars onto poly(styrene-co-maleic anhydride): a solution of the polymer in dry DMF was added to a solution of the sugar and 4-dimethylaminopyridine in dry DMF slowly over a period of 1 h at 47–50 °C in the presence of dry nitrogen. The mole ratios of maleic anhydride in the polymer to sugar were either 1 : 3 in case of lactose and sucrose and 2 : 1 in case of glucose. The reaction mixtures were stirred at 47–50 °C for 18 h. In case of lactose the reaction mixture was stirred over a period of 4 h at 60–65 °C. The products were precipitated in brine, washed with water several times till they were free of chloride and dried in a vacuum oven. FTIR (Shimadz 8300) in KBr: $\sim$1730 cm^{-1}(–*C*OOR–) (the acid and the ester carbonyl bands merge together), reduction in the intensity of band at 1780 and 1857 cm^{-1} (anhydride >C=O). NMR (^{1}H and ^{13}C) (Bruker) showed only traces of sugar in the polymer molecule. Quantification of the sugar content of the polymer by NMR was therefore very difficult. Therefore, silylation of the carbohydrate hydroxyls was carried out to quantify the extent of sugar incorporated. Another method based on phenolsulfuric acid assay was also used to quantify the carbohydrate content of the polymers. By these methods, the amounts of carbohydrate incorporated in the polymer was found to be in the range of 1.0–3.7 wt%. This data, as well as the FTIR data, proves the chemical linking of the sugars onto the polymer in minute quantities.

§ All the organisms listed in Fig. 1 were obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. Cultures were routinely maintained in Nutrient agar slopes (Beef extract, 1.0%; NaCl, 0.5%; peptone, 1.0% and Agar 2.0%). Test organisms used were: *Bacillus* sp. NCIM 2812, *Pseudomonas* sp. NCIM 2220 and *Serratia marcescens* NCIM 5061.

¶ Determination of biodegradability of polymers using aerobic micro-organisms: testing of the samples: cultures were grown in Minimal medium containing (g l^{-1}): $(NH_4)_2SO_4$, 2.0; K_2HPO_4, 14.0; KH_2PO_4, 6.0; trisodium citrate, 1.0; $MgSO_4{\cdot}7H_2O$, 0.2. The pH of the medium was adjusted to 7.0 prior to sterilization. The medium was sterilized at 121 °C for 20 min. The test samples were surface sterilized with 70% ethanol at a concentration of 0.5% for 2 h and then added separately to the sterilized medium. The cells of the cultures grown in 10 mL nutrient broth for 24 h at 30 °C were suspended in 10 ml of saline and this suspension was used as an inoculum. Approximately 0.1 mL ($\sim 10^8$ cells) were inoculated into 20 ml of the minimal medium in 100 ml conical flasks. The flasks were incubated at 28 °C with shaking at 180 rpm. The growth (optical density) was monitored over a period of four weeks using a Systronics 117 spectrophotometer. The polymer was separated from the cells by filtration using Whatman filter paper. The residue was further washed many times with water followed by washing with 70% ethanol. It was dried at 50 °C and analysed by IR spectroscopy and the percentage weight losses of the polymers were recorded. For recording the weight losses, thick films of the sugar-grafted poly(styrene maleic anhydride) were made and treated with the bacteria in the same manner.

1 *Degradable Polymers: Principles and Applications*, ed. G. Scott and D. Gilead, Chapman & Hall, London, UK, 1995.
2 Y. Tokiwa, H. Fan, Y. Hiraguri, R. Kurane, M. Kitagawa, S. Shibatani and Y. Maekawa, *Macromolecules*, 2000, **33**, 1636.
3 P. Rios and H. Bertorello, *J. Appl. Polym. Sc.*, 1999, **64**, 1195.
4 C. Alvarez, M. Strumia and H. Bertorello, *Polym. Bull.*, 1988, **19**, 521.
5 R. Bahulekar, T. Tokiwa, J. Kano, T. Matsumura, I. Kojima and M. Kodama, *Carbohydr. Polym.*, 1998, **37**, 71.
6 B. Lee, A. L. Pometto III, A. Fratzke and T. B. Bailey Jr., *Appl. Environ. Microbiol.*, 1991, **57**, 678.