ISSN : 0974 - 7478 Volume 10 Issue 1

Macromolecules

An Indian Journal

Full Paper

MMAIJ, 10(1), 2014 [30-36]

Biodegradation study of DOC isolated mixed proteins-LDPE blend based plastic sheets

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ABSTRACT

The biodegradability of plastic sheets made of mixed proteins – LDPE blend was investigated. Mixed proteins – LDPE blend based plastic sheets were subjected to microbial degradation using *Pseudomonas aeruginosa*. At regular interval of 4 days, culture growths and soluble protein concentrations have been measured throughout the biodegradation study. Results from the biodegradation showed that, plastic sheets prepared from mixture of soybean, castor and rapeseed proteins – LDPE blend could support the growth of *P. aeruginosa*. Biodegradable plastic sheet with composition (70:10:20 mixed proteins & 10% LDPE) degraded much faster than other eight biodegradable plastics sheets under the conditions examined.

KEYWORDS

DOC proteins;
LDPE;
Bio-based plastics;
Biodegradability;
Microbial degradation.

INTRODUCTION

With the advances in technology and the increase in the global population, plastic materials have found wide applications in every aspect of life and industries. However, most conventional plastics such as polyethylene, polypropylene, polystyrene, poly (vinyl chloride) and poly (ethylene terphthalate), are non biodegradable, and their increasing accumulation in the environment has been a threat to the planet. Majority of the commodity plastics are non-biodegradable and cause a problem of waste accumulation. To overcome all these problems, some steps have been undertaken. The first strategy involved production of plastics with high degree of degradability. So Biodegradable plastics are now emerging as one of the available options to solve environmental problems related to plastics accumulation.

Biodegradable plastics are polymers or polymer blends, which in addition to possessing properties similar to conventional plastics are susceptible to "natural" enzyme hydrolysis or other chemical attack. Research and development of biodegradable plastics from renewable resources including protein[1,2] starch[3,6] and microbial fermentation products[7,9] have been conducted recently. Among this plant proteins are an important source of raw material for both food and non-food uses. These plants protein is relatively low in cost, renewable and readily available. The annual production of protein isolates and

protein concentrates reaches 1 million tonnes world-wide. The possible uses of soy proteins for adhesive[10,11] coating polymers[10,12] plastics[13,17] Composites[18,20] and edible packaging films[21,23] have been investigated recently.

The following methods have been used to assess the biodegradation of plastics:

Changes of mechanical properties in the soil[24]. Biological oxygen demand (BOD) changes or CO_2 evolution in sludges[25,26]. Molecular changes by enzymatic or microbial treatment[27,29]. Mechanical property changes or CO_2 evolution in soil[30,32]. Gas evolution by anaerobic digestion[33]. Mechanical property changes in several compositing soil conditions[34,35].

In this study, in addition to soya protein, rapeseed protein, castor protein and LDPE were also used for the preparation of biodegradable plastics. Also, soy protein, rapeseed protein and castor protein were isolated from deoiled cakes of soybean, castor and rapeseed, which is by-product of oil processing industry. So in this study we have produced important biodegradable plastic sheets from the waste material of oil processing industry. The mechanical properties were determined. Biodegradation behavior of resultant plastic sheets by microbial treatment was also studied for 52 days. Microbial degradation of plastic sheets prepared from mixed proteins – LDPE blend was carried out using *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa is a gram-negative, non-fermentative, aerobic rod, belonging to the bacterial family Pseudomonadaceae[36]. The organism can usually be isolated from soil and water as well as the surfaces of plants and animals. It is found throughout the world, wherever these habitats occur, so it is quite a "cosmopolitan" bacterium. *P. aeruginosa* possesses metabolic versatility. Organic growth factors are not required, and it can use more than 30 organic compounds for growth. The bacterium is often observed growing in "distilled water," which is evidence of its minimal nutritional requirements[36]. Moreover, *P. aeruginosa* can produce a wide range of proteinases and is often implicated in the process of food spoilage[37].

MATERIALS AND METHODS

Deoiled cake (DOC) of soyabean, castor and rape seed were procured from Gujarat Ambuja export Ltd, Kadi, Mehsana, Gujarat, India. LDPE was procured from Reliance Industry, Baroda, Gujarat, India. NaOH, HCL, Polyethylene glycol (PEG_{400}) and used were of laboratory grade.

Analysis of deoiled cake

Deoiled cake (DOC) of soybean, castor and rapeseed were analyzed by standard BIS methods (BIS: 548 (Part-I) 1994). The result of analysis is shown in TABLE 1.

TABLE 1 : Analysis of DOC

Deoiled cakes	Oil Content (%)	Protein Content (%)	Moisture content (%)	Fiber content (%)
Soybean	1.04	40.01	8.58	7.54
Castor	0.66	28.87	7.12	23.15
Rape seed	1.59	41.68	7.70	8.05

Isolation of proteins from deoiled cakes

The proteins from deoiled cakes were extracted with 0.5% aqueous sodium hydroxide solution (1:20 w/v cake: alkali). The soybean, castor and rape seed cakes were extracted at 50°C for 3 h with 0.5% aqueous sodium hydroxide solution. The alkali extract was filtered and the residue was discarded. The filtrate was brought to the isoelectric point by addition of dilute HCL solution. The protein thus precipitated was filtered and dried.

Physical blending of mixture of proteins and LDPE

Physical blending of mixture of proteins and LDPE were done in various proportions i.e. 80:10:10, 70:20:10 and 70:10:20 ratio of the soy protein, castor protein and rapeseed protein respectively and 10%, 20% and 30% of LDPE by wt. of soy protein isolate. Blending ware done on two roll mill at 110°C for 45 min. By using different compositions of proteins and LDPE, plastic sheets were prepared by using a compression molding machine at 90°C and15 kg/cm^2 pressures was applied for 5 min then for 15 min without pressure.

Biodegradation study of different plastic sheets

An experiment consisting of nineteen treatments (TABLE 2) was used to characterize the biodegradation of the mixed proteins - LDPE blends by *P. aeruginosa.*

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The microbial degradation study was continued for 52 days. Triplicates were done for each treatment.

TABLE 2 : Summary of culture conditions

Solution	Composition	Inoculum (ml)	Saline Solution (ml)	Protein sheet
1b	80:10:10 & 10% LDPE	0	200	1 Piece
2b	80:10:10 & 20% LDPE	0	200	1 Piece
3b	80:10:10 & 30% LDPE	0	200	1 Piece
4b	70:20:10 & 10% LDPE	0	200	1 Piece
5b	70:20:10 & 20% LDPE	0	200	1 Piece
6b	70:20:10 & 30% LDPE	0	200	1 Piece
7b	70:10:20 & 10% LDPE	0	200	1 Piece
8b	70:10:20 & 20% LDPE	0	200	1 Piece
9b	70:10:20 & 30% LDPE	0	200	1 Piece
1bb	80:10:10 & 10% LDPE	4	196	1 Piece
2bb	80:10:10 & 20% LDPE	4	196	1 Piece
3bb	80:10:10 & 30% LDPE	4	196	1 Piece
4bb	70:20:10 & 10% LDPE	4	196	1 Piece
5bb	70:20:10 & 20% LDPE	4	196	1 Piece
6bb	70:20:10 & 30% LDPE	4	196	1 Piece
7bb	70:10:20 & 10% LDPE	4	196	1 Piece
8bb	70:10:20 & 20% LDPE	4	196	1 Piece
9bb	70:10:20 & 30% LDPE	4	196	1 Piece
Ps	---	4	196	---

All the sheet samples in the saline solution (0.85% NaCl) were autoclaved at 121^{O}C for 15 min, inoculated with *P. aeruginosa* and incubated at 30^{O}C with shaking at 120 rpm on JEIOTECH. SI 600 R temperature controlled incubator shaker (Koria) for 52 days. A biological control (only *P. aeruginosa* without plastic sheet of mixed proteins - LDPE blends) and negative control (only plastic sheets of mixed proteins - LDPE blends without *P. aeruginosa*) were also taken. Appropriate aliquots of samples were removed and analyzed periodically. The bacterial growth and soluble protein content were measured at the interval of 4 days.

Bacterial growth

One ml samples were taken out at regular time interval and growth of bacteria in terms of optical density at 600 nm was determined.

Soluble protein determination

Biodegradation of organic polymers was defined as the degradation and assimilation of organic polymers by the action of living organisms, primarily fungi and bacteria[40]. Upon degradation, protein molecules were broken down into small peptides and amino acids, which remain soluble in the trichloroacetic acid (TCA) solution. Hence, the percentage of soluble protein can be used as a way to evaluate the rate of biodegradation.

Soluble protein contents of the samples were determined based on the method described by scientists[39]. Two ml of the samples were taken out periodically and diluted in an equal amount of 6% TCA solution. After standing 30 min at room temperature, the precipitate was removed by centrifugation at 8000 rpm (KUBOTA-6200, JAPAN) for 15 min. The supernatant was used for soluble protein determination. An optical density of soluble protein was measured at 750 nm using SHIMADZU UV-1800 spectrophotometer (Japan). The soluble protein concentration of the sample was obtained from the standard curves prepared using BSA as reference.

Percentage of degradation

The initial weight of the each plastic sheet was taken before biodegradation study start. After 52 day of biodegradation, again the weight of each plastic sheet was taken after drying at 100^{O}C for 48 h in oven. The percentage recovery of total solids for each plastic sheet was calculated using following equation[40].

$$\text{Weight loss (\%)} = \frac{W_1 \times 100}{W_0}$$

Where, W_1 = The total solid weight of each plastic sheet recovered after biodegradation study; W_0 = The initial total weight of corresponding plastic sheet before biodegradation study.

RESULTS AND DISCUSSION

The plastic sheets using three different compositions of mixed proteins in the ratio 80:10:10, 70:20:10 and 70:10:20 and different percentages of LDPE were prepared using 50% PEG_{400} (w/w) of SPI quantity as plasticizer.

The effect of culture conditions on the variation of *P. aeruginosa* population over incubation time is illustrated in Figures 1, 2, 3.

In order to accurately interpret the variation in soluble protein due to the microbial degradation, net

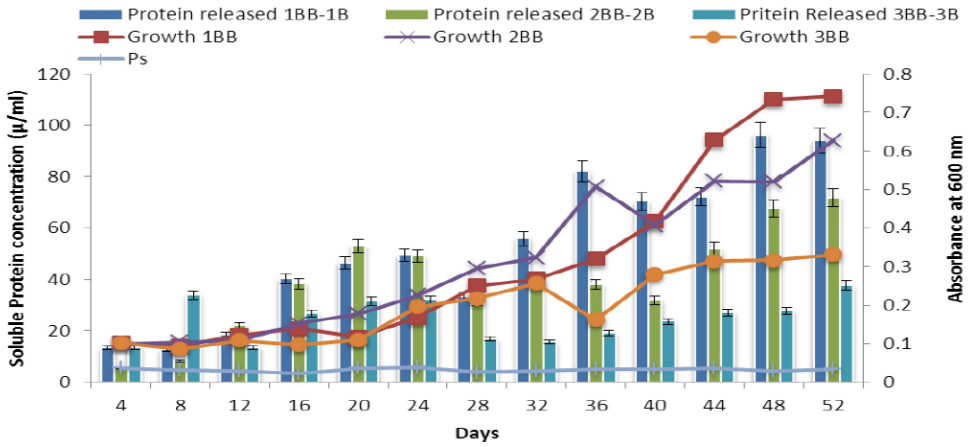

Figure 1 : Biodegradation of mixed protein sheet (80:10:10)-LDPE blends by *P.aeruginosa*

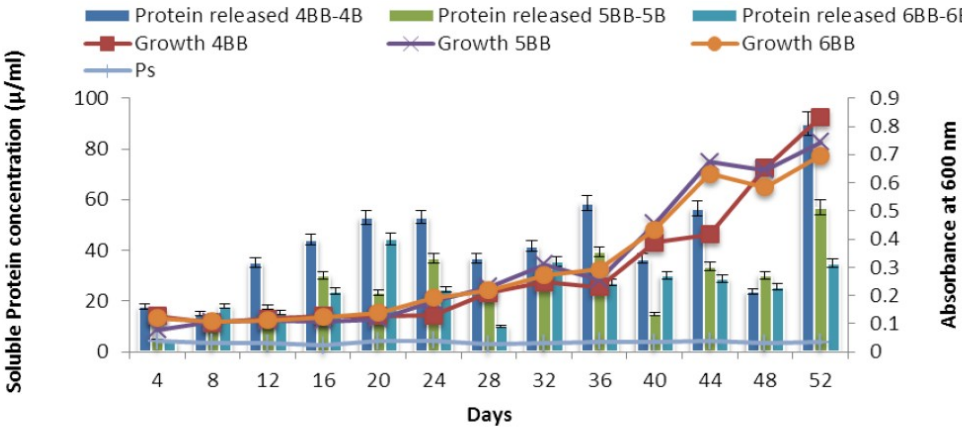

Figure 2 : Biodegradation of mixed protein sheet (70:20:10)-LDPE blends by *P.aeruginosa*

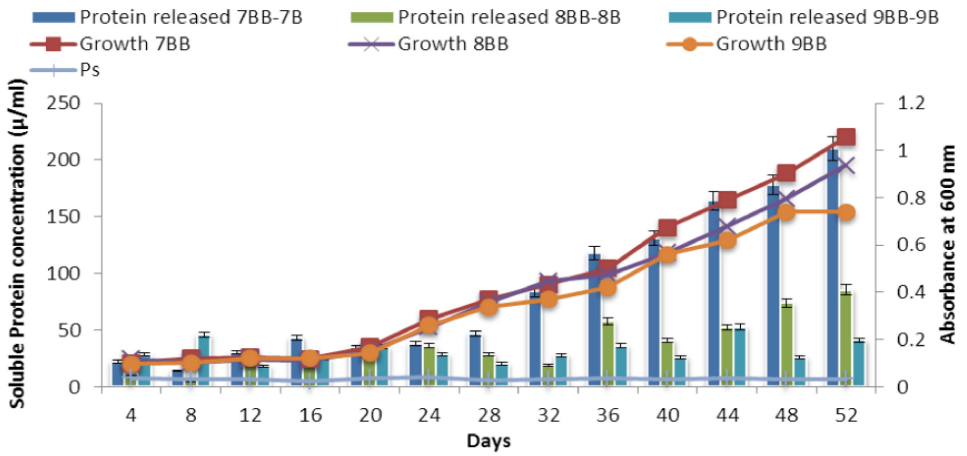

Figure 3 : Biodegradation of mixed protein sheet (70:10:20)-LDPE blends by *P.aeruginosa*

changes of soluble protein concentration were computed by subtracting the values for solutions containing only the films from the corresponding solutions containing the films and the bacteria (1bb-1b, 2bb-2b, 3bb-3b, 4bb-4b, 5bb-5b, 6bb-6b, 7bb-7b, 8bb-8b and 9bb-9b). From Figure 1, optical densities of bacterial population in solution 1bb, 2bb and 3bb were found to be 0.742, 0.627 and 0.331 respectively at 52 days of incubation. Highest soluble protein concentration was observed in 1bb-1b followed by 2bb-2b and 3bb-3b at 52 days of incubation which suggested that maximum biodegradation occurred in 1bb-1b compared to 2bb-2b and 3bb-3b. Figure 2 shows an increase in bacterial population in terms of optical densities of so-

lution 4bb (0.833), 5bb (0.744) and 6bb (0.696) at 52 days of incubation. Highest soluble protein concentration was observed in 4bb-4b, while lowest soluble protein concentration was observed in 6bb-6b. So one can say that highest biodegradation occurred in 4bb-4b compared to 5bb-5b and 6bb-6b. In solution 7bb, the population of bacteria was increased gradually and reached up to 1.056 O.D. at 52 day. In solution 8bb, the population of bacteria was continuously increased from 20days and reached up to 0.937 O.D. at 52 day. In solution 9bb, the population of bacteria was increased gradually and reached up to 0.739 O.D. at 52 day. Highest soluble protein concentration was observed in 7bb-7b, while lowest soluble protein concentration was observed in 9bb-9b. It showed that highest biodegradation occurred in 7bb-4b compared to 8bb-8b and 9bb-9b (Figure 3). In solution Ps, which contained inoculums alone in the sterile saline solution, the population of bacteria was increased very slowly and maximum reached up to 0.039 O.D. Growth of *P. aeruginosa* under the above condition (traces of nutrients from inoculum) is an evidence of its capability of surviving under minimal nutritional requirements (Baron, 1996). No bacterial growth was observed in solutions 1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b and 9b through the experiment, which confirms that the films were sterile prior to inoculation. From the Figure 1, 2 and 3, it was observed that 10% addition of LDPE in mixed proteins-LDPE blends gave good biodegradation compare to 20% and 30% addition of LDPE. It means 1bb-1b, 4bb-4b and 7bb-7b gave good biodegradation than that of others.

In Figure 4, we compared the results of 1bb-1b, 4bb-4b and 7bb-7b. Maximum soluble protein concentration was found in 7bb-7b compare to 1bb-1b, 4bb-4b. So biodegradable plastic sheet with composition (70:10:20 mixed proteins & 10% LDPE) showed good biodegradation. It means in this study, 70:10:20 mixed proteins with 10% LDPE composition was best composition for the preparation of biodegradable plastic sheets.

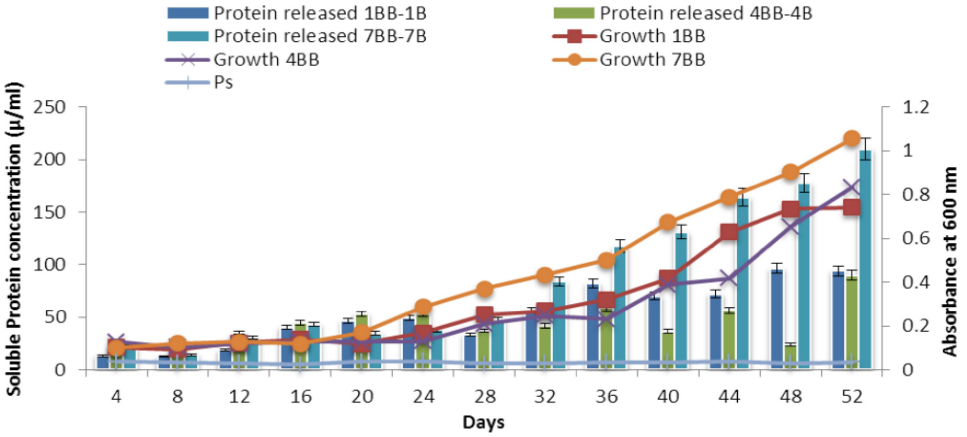

Figure 4 : Comparison of biodegradation of protein sheets 1bb, 4bb and 7bb

Microorganisms require sources of carbon, nitrogen and micronutrients for their growth. Therefore, the composition of the material will determine the speed of the biodegradation process. Furthermore, the extent of covalent cross-linking also affects the rate of biodegradation of protein films[41]. Jane et al., have demonstrated that covalent cross-linking decreases the biodegradability of composite starch-protein films[41].

Percentage of degradation was determined by weight loss method. Percentages of degradation of nine biodegradable plastics were shown in TABLE 3.

TABLE 3 : Percentage of degradation

Composition	Total Degradation (%)	Degradation due to Microorganism (%)
80:10:10 & 10% LDPE	51.53	30.87
80:10:10 & 20% LDPE	50.14	36.81
80:10:10 & 30% LDPE	52.86	36.20
70:20:10 & 10% LDPE	64.20	49.87
70:20:10 & 20% LDPE	51.93	33.23
70:20:10 & 30% LDPE	53.00	30.34
70:10:20 & 10% LDPE	65.73	51.47
70:10:20 & 20% LDPE	58.80	43.33
70:10:20 & 30% LDPE	69.53	58.23

It was shown that actual biodegradation of plastic sheets due to *P.aeruginosa* was maximum in plastic sheet with composition (70:10:20 mixed proteins & 10% LDPE), while minimum biodegradation was observed in plastic sheet with composition (70:20:10 mixed proteins & 30% LDPE).

CONCLUSION

Experimental results demonstrated that mixed proteins – LDPE blends based biodegradable plastics sheets were successfully degraded using microorganism *P. aeruginosa*. Biodegradable plastic sheet with composition (70:10:20 mixed proteins & 10% LDPE) was degraded much faster than other eight biodegradable plastic sheets under the conditions examined, suggesting that the rate of biodegradation is associated with the composition of film-forming materials and the extent of covalent cross-linking in the resulting protein films.

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