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BIODEGRADABLE COMPOSITE FILMS BASED ON WASTE GELATIN

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SUMMARY: Gelatin, a naturally occurring polymer, is currently used in various applications comprising manufacturing of pharmaceutical products, x-ray and photographic films development and food processing. However, gelatin scraps generated in the different manufacturing processes may constitute a concern for the environment. Basically speaking, waste disposal deriving from plastics based on synthetic as well as semisynthetic polymeric materials, is becoming an increasingly difficult problem for their unfavorable volume-to-weight ratio and extremely wide variability of type, shape and composition of post consume plastic items that hinder the way to a general unique option for a simple and economically feasible management.

As a partial solution to the global issue of plastic waste, in recent years much interest has been devoted to the formulation of environmentally degradable plastic items.

Biodegradable mulching films were formulated from blends and composites based on waste gelatin and other natural waste such as sugarcane bagasse or synthetic materials such as PVA. Also, crosslinked films were produced using external or inherent crosslinker. The films were produced either by casting method or spraying on soil surface. The composites were submitted to biodegradation trials. The results showed that the films were biodegradable and the crosslinking could delay and predeterminate their biodegradation rate and extent.

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1. Introduction

Plastics have gained a unique position in materials technology for a number of reasons.

Initially they were used as substitutes of paper, glass and metal because of their better physical properties 1) and because plastics are more economical than metals, wood and glass in terms of manufacturing costs, weight to strength ratio, and cost/performance convenience 2). Plastics have also achieved a dominant position in agriculture and horticulture, which has merited the new description 'plasticulture' to describe the use of plastics in greenhouses, tunnels and irrigation and in protective covers for crops (mulching films) 1).

Plastic waste may represents however a serious concern for the environment because of its recalcitrance to microbial attack 3,4). Recent estimates indicate that more than 200 million tons of municipal solid waste (MSW) is generated annually in the United States 5).This makes polymer waste management an urgent problem, needing environmentally compatible and friendly solutions, both short and long term, as soon as possible 2).

It is generally recognized that there is no single, simple solution to waste management but there are several options which comprise either reduction of the amount of material entering the waste stream and/or deal with its ways of disposal 6). Since plastic waste has caused serious, but often emotionally exaggerated environmental concern, there are nowadays increasing demands to develop environmentally degradable polymeric materials 7-9) The search for biodegradable alternatives to stubbornly bio resistant thermoplastics has led to the exploitation of the complementary properties of natural and synthetic polymers as blends, block copolymer, or graft copolymers 10).

Gelatin and starch are of particular interest since these biopolymers have no adverse impact on human or environmental health 11).

Gelatin, a naturally occurring polymer, is currently in use in various industrial applications. Gelatin scraps generated in the different manufacturing processes may constitute a concern for the environment due to strong swellability in water medium and high carbon and nitrogen content, that lead to high oxygen demand once they reach the sewage drainage system and waste water treatment plants.

The use of mulching films in particular, has led to a very substantial increase in the yields of soft fruits and vegetables in many areas all over the world, because plastics films not only helps to increase the temperature of the soil, but also reduce the usage of irrigation water and fertilizers with consequential beneficial economical and environmental returns 1). The disposal of agricultural mulching films, can represent a key in the economy of the overall productive cycle, primarily caused by the advent of automated harvesting. Cropping equipment's are often rapidly put out of action by plastic-based litter on the surface of the soil and residual undegraded plastics, once buried, may survive for long time and interfere with the root growth of subsequent crops. The intensive and costly labour to remove such debris from the fields by manual collection in large-scale plasticulture, may result in a drastic reduction of the benefits connected with the increased crop yields.

Herein, we report the development of biodegradable blends and composites based on waste gelatin and other natural or synthetic waste materials that can be used in agriculture for the fabrication of environmentally sustainable mulching films, and soil conditioners. The composite based on waste gelatin could constitute the basic matrix for the formulations of good biodegradable mulching and soil conditioning films and at the same time be helpful in solving the problems with the correct management of an yet energy reach plastic waste.

2. Experimental

2.1 Materials and methods

Materials used in this study were either waste material or commercially available materials. Polyvinyl alcohol (PVA) and scraps from the production of blown films for hydrosoluble biodegradable packaging and sizing wraps commercialized under the trade mark of Hydrolene¨ were supplied by Idroplast SpA-Italy. The PVA had an average molecular weight of 67 KD and degree of hydrolysis of 88 % and was used without further purification, also PVA with 98 % hydrolysis was used as received from the same supplier. Waste and virgin gelatin was kindly supplied by Rp Scherer Egypt. The elemental microanalysis of waste gelatin, gave: carbon (43.55 %), nitrogen (11.62 %) and hydrogen (6.59 %). Brazilian sugarcane bagasse was kindly supplied by Prof. Miele, of the Faculty of Agriculture of the University of Pisa. Prior the use it was dried in an oven at 50 ^{o}C for 24 h and then granted with a blade grinder. The granted bagasse was sieved and the fraction passing through a mesh sieve (0.212 μm) was collected. The carbon, nitrogen, phosphorous and organic substance content of the bagasse were determined by Kjeldahl method and were as following: organic substance (83.80%), carbon (48.72 %), nitrogen (0.34 %) and phosphorous (0.02 %).
Perlite, a naturally occurring siliceous stone, was supplied from BPB Italia S.p.A., Italy.
Air compressor: Films were sprayed on soil surface using a Fini compressor (Italy), type E.C. attack OL 195 23050 EU RC working at 3 bar pressure and with 2.5 mm nozle.

Scanning Electron Microscope (SEM)

SEM inspection were carried out on a Jeol T300 apparatus. The film samples were prepared for SEM by critical point drying, sputtered with gold and observed at 10 keV[12).

2.2 Film preparations

Films were prepared by casting of water dipersions of all the ingredients or direct spraying according to a proprietary procedure. Table 1 shows the composition of the prepared films.

Tab. 1. Composition of films based on waste gelatin (WG), PVA (P), bagasse (B) and crosslinking agent (X).

Film Sample	Waste Gelatin	PVA	Bagasse	Crosslink Ratio (GLU)	Visual Characteristics	
	wt-p[a]	wt-p[a]	wt- p[a]	wt- p[a]	Colour	Appearance
VGP0	0	0	0	0	colourless	rigid
WGP0	100	0	0	0	dark-red	very flexible
WGP2	10	90	0	0	light pink	flexible
WGP4	20	80	0	0	pink	flexible
WGP6	30	70	0	0	deep pink	flexible
WGP14	80	20	0	0	deep red	highly flexible
WGPX4	80	20	0	0.25	pale brown	very flexible
WGPB10	80	20	20	0	brown-red	very flexible
WGPB13X	80	20	20	0.25	brown	flexible
WGB2	80	0	20	0	red-brown	flexible
WGBX1	80	0	20	0.25	brown	very flexible
WGX1	100	0	0	0.25	faint-brown	flexible

[a] wt-p = weight parts

2.3 Coating of loamy soil surfaces

In this series of experiments the dispersions in water of the various components were sprayed on pots containing the loamy soil with an overall surface area of 154 cm^2 or by slow water evaporation from liquid films confined onto teflonized surfaces. The preparatory were aimed at testing the conditioning and mulching properties of the films obtained by in situ spraying on the soil samples. In Table 2 are collected the compositions of the films sprayed on the loamy soil samples. The pots were left outdoor under open air conditions. A pot containing the loamy soil without any film was used as a control.

Tab. 2. Composition of sprayed films based on waste gelatin (WG), PVA (P), bagasse (B) and a low molar mass or high molecular weight dialdehyde crosslinking agent (X).

Film Sample	Waste Gelatin	PVA	Bagasse	Crosslink Ratio (GLU)
	wt-p	wt-p	wt-p	wt-p
WGB5S	50	-	50	-

WGB5XS	50	-	50	0.25
WGPBS	40	10	50	-
WGPBXS	40	10	50	0.25

2.4. Crosslinking of waste gelatin containing films

Crosslinked films were prepared according to a proprietary procedure [13] by using high and low molar mass dialdehydes as crosslinking agents. Table 3 shows the composition of the prepared films.

Tab.3. Crosslinked films based on waste gelatin and crosslinking agents (X).

Film	Waste Gelatin	Crosslink Ratio	Visual Characteristics	
Sample	wt- p	wt- p	Colour	Appearance
WGX1	100	0.25	faint brown	very flexible
WGX3	100	1.0	coffee like	very flexible
WGX5	100	2.5	brown-yellow	very flexible
WGX7	100	5.0	coffee like	very flexible

2.5 Biodegradation studies

The biodegradation tests were carried out using a new respirometric procedure set up in the laboratory of the Department of Chemistry and Industrial Chemistry of the University of Pisa[14]. The test was carried out in 1 litre Erlenmeyer flasks containing a culture medium consisting of a bottom layer constituted by 20 g of Perlite and 35 ml water. On top of that layer a mixture of soils (forest and loamy soils) and grinded Perlite (5 g soil mixture and 7.5 Perlite) and 7.5 ml of water was layered. The test samples (6 strips 0.5 cm x4.9 cm) of known weight were placed on the top of the soil-perlite layer. The samples were then covered by another layer of the mixture of soil-perlite moistened with 7.5 ml water. Finally a layer consisting of 20 g of wet perlite was added. The Erlenmeyer flasks containing the samples were connected to a system for set for the determination of carbon dioxide developed within the time.

The flasks were discontinuously aerated with carbon dioxide-free air. The carbon dioxide-free air was passed through two flasks, the first flask contained a super saturated solution of barium hydroxide and the second one contained filtered saturated solution of barium hydroxide. The two barium hydroxide flasks were used for trapping any traces of carbon dioxide in the air before passing to the flask containing the sample and the inoculum. Then the carbon dioxide-free air was humidified by passing through a flask containing water. The air flow was allowed to the different test flasks and regulated with plastic valves at a rate of approximately 30 ml/min.

At regular intervals barium hydroxide solutions in the CO_2 absorbers, were titrated with standard solution of HCl. The amount of CO_2 evolved from each sample was calculated from the amount of barium hydroxide consumed in the reaction with the released CO_2. The carbon content of each sample was calculated and the theoretical amount of CO_2 was calculated from the carbon content of each samples. Biodegradation was expressed as percent of evolved CO_2 (corrected for the CO_2 produced in the blank experiment) with respect to the theoretical CO_2. Filter paper was used as a reference standard.

2.5.1. Non crosslinked gelatin films

A preliminary test of biodegradation of selected gelatin films was carried out using films; slightly humidified with water. Film samples VGP0, WGP2, WGP4, WGP6 and PVA as prepared according to the procedure described previously were investigated. Two strips of each film sample were used, the first was incubated between two layers of soil and the 2nd was incubated in the top of the soil, the results collected for the top samples are reported in Table 4.

Tab. 4. Behavior of some non crosslinked PVA/gelatin films incubated on forest soil.

Sample	Gelatin content (%)	Observations
VGP0	100	Growth of fungi noticed at the 4th day exposure
WGP2	10	Growth of fungi accompanied by almost total dissolution of the film at the 4th day of exposure
WGP4	20	Dissolution of the film after 24 hr exposure
WGP6	30	Dissolution of the film after 24 hr exposure
PVA	0	Dissolution of the film after 24 hr exposure

2.5.2. Crosslinked gelatin films

Film samples WGP0, WGX1, WGB2, WGBX1, WGPB10, WGPB13X, WGP14 and WGPX4 (Table 1) were selected for the submission to biodegradation trials with the aim of monitoring the effects of some fundamental structural parameters on biodegradation

Film samples listed in Table 5 were selected for the submission to biodegradation trials with the aim of studying the effect of crosslinking on biodegradation rate and extent.

Tab. 5. Selected films based on waste gelatin (WG), and crosslinked waste gelatin (X) for the biodegradation experiment.

Film	Crosslink Ratio	Weight of Sample	Visual Characteristics	
Sample	wt- p	mg	Colour	Appearance
WGX1	0.25	95	faint brown	flexible
WGX3	1.00	99	coffee-like	very -flexible
WGX5	2.50	106	brown-yellow	very -flexible
WGX7	5.00	105	coffee-like	very -flexible
WG	-	79	coffee-like	very -flexible
PVA[a]	-	137	colourless	flexible

[a] The sample used was from PVA 98% of hydroxyl content submitted prior the use to three freezing-thawing cycles.

3. Results and Discussion

Gelatin containing films were cast from solutions or dispersion containing gelatin and the other components by slow evaporation of the water at room temperature and at atmospheric pressure.

Smooth films of a constant thickness were obtained whose size depending on the composition of the film, was ranging from 0.20 mm to 0.45 mm.

Films were also obtained by a direct spraying technique on soil with the aim of testing the feasibility of a single pot application in field experiments. It was expected that, the soil once sprayed could lead to improvement of the mulching character of the films by keeping the soil warm enough for the plant cultivation. The evolution of film morphology formed on loamy soils was monitored for three weeks.

The results of sprayed film experiments showed that the films lasted for more than two weeks on the soil and the soil appeared to be conditioned and in a better state when compared with the control sample. It is worth mentioning that, during the experiment time the boots containing the sprayed films were exposed to outdoor condition that were monitored for all the duration of the experiment.

Low molar mass dialdehydes and high molecular weight dialdehydes are of the most popular crosslinking reagents, especially for proteic materials 14). Because the amino groups arising from lysine react easily at room temperature with functional groups resulting in an obvious colour change characteristic of the Schiff base linkages15-17). It was observed that, within a few minutes on treatment of the blend with a dialdehyde and casting the film, the colour changed from the red colour characteristic of our waste gelatin to colour range from pale yellow to brown. This colour change occurs only when preparing crosslinked films and it could be visually detected. The colour change is due to the formation of aldimine linkage (CH=N), between the free amino group of gelatin and the dialdehyde.

In order to control the degradation rate of the gelatin containing films, it is worth having a physical stabilization by crosslinking. The crosslinking indeed increases the persistence of the films in the environment for the required period because crosslinking reinforces gelatin structure by introducing intra- and intermolecular bonds between gelatin molecules.

3.1. Biodegradation studies

The studies were carried out by using a respirometric method viable for the determination of the amount of the CO_2 released from the samples submitted to the action of soil microflora. The first trial was run for 30 days and the % of biodegradation extent was recorded as shown in Table 6. It was noticed that, for the first week, there is no much difference between the biodegradation rate of the 0.25% crosslinked waste gelatin film (WGPX4, WGBX1, WGX1) and the corresponding non-crosslinked waste gelatin film (WGP14, WGB2, WGP0). In general for the first week, the biodegradation rate for most samples was comparable. After the first week, the degradation rates changed and in some cases the crosslinking slowed down the rate of the biodegradation (WGPX4, WGP14 and WGBX1, WGB2) and in others was even higher than that observed in analogous un-crosslinked films (WGX1, WGP0). This might be due to the small (0.25%) ratio of the crosslinking. Figure (1) shows the integral biodegradation of the films selected for the first biodegradation trial within the time.

At the end of the biodegradation trial, most of the samples disappeared except for samples from film WGB2 and WGBX1. The recovered samples (WGB2 and WGBX1) once gently cleaned from soil by small brush and dried in an oven at 37 °C were analyzed by SEM. The changes in the surface morphology after submission to the biodegradation trial were compared with the original film surface.

The SEM for films before being submitted to biodegradation, showed smooth homogenous surfaces (Fig. 2). The SEM of some recovered films after biodegradation experiment showed cracks and fractures on the surface as indicated by increased surface area of the composite due to growth of the microorganisms on the film.

Tab. 6. Biodegradation of samples based on gelatin.

	Biodegradation (%) a)								
Time (days)	Cellulose	WGBX1	WGB2	WGPX4	WGP14	WGPB13X	WGPB10	WGX1	WG
1	-0.1	1.3	1.2	1.1	1.7	0.8	1.4	1.6	1.5
2	-0.1	4.1	4.0	3.2	4.3	2.8	4.0	4.5	5.2
2.5	-0.1	5.2	5.7	4.2	5.7	3.7	5.1	5.4	7.2
3.5	0.5	8.2	8.9	6.6	8.6	6.2	8.4	9.6	11.5
4.5	1.6	12.9	16.6	11.5	13.3	11.5	14.7	22.1	19.7
5.5	2.5	20.2	22.5	17.1	18.4	17.1	19.1	29.3	29.3
6.5	3.1	22.8	25.6	21.4	21.0	19.7	21.7	37.3	33.7
7.5	5.1	26.2	30.1	25.5	24.2	22.1	24.2	42.3	36.1
8.5	6.2	28.8	32.8	29.0	26.4	23.6	26.1	46.0	38.1
11.5	8.4	32.3	37.4	33.4	30.0	25.7	29.3	50.6	43.0
12	10.7	35.3	40.5	37.0	32.4	28.5	31.4	54.8	47.0
14	14.5	38.1	43.2	40.3	35.1	31.8	33.9	60.4	51.4
16	17.6	40.4	45.5	42.5	36.5	33.4	35.6	63.8	54.0
19	21.0	43.6	48.1	43.4	39.1	34.8	37.7	67.2	56.8
22	23.5	44.6	48.9	44.9	39.7	35.5	38.5	68.5	57.1
27	25.4	45.5	49.9	45.8	39.9	36.5	39.4	69.8	57.3
30	27.5	46.3	51.1	47.1	40.7	37.7	40.7	69.9	57.6

a) Determined as $\frac{\text{Amount CO}_{2\,(\text{sample})} - \text{Amount CO}_{2\,(\text{blank})}}{\text{ThCO}_2} \cdot 100$

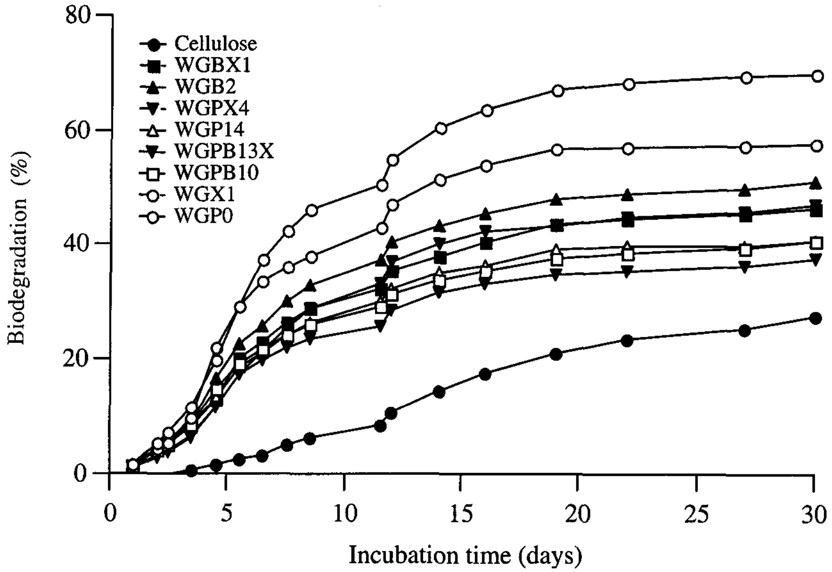

Fig. 1: Trend and overall extent of biodegradation in waste gelatin films

3.2. Effect of crosslinking on the rate of biodegradation of films based on waste gelatin

Biodegradation trials were carried out in order to investigate the effect of crosslinking on the biodegradation of the film. In the first biodegradation trials, crosslinked samples used had a crosslinking ratio of 0.25 %. At that relatively small crosslinking ratio, the effect of crosslinking on biodegradation was not specifically detectable. Therefore, in another trials we used samples with a crosslink ratio of 0.25-5.0 %.

Film samples (Table 3) and a blank were tested and compared with PVA. The biodegradation experiments related to the samples WGX3, WGX5, WGX7 were carried out in duplicate. The amount of CO_2 released from the samples was monitored by taking into account the CO_2 amount released from the soil itself. The trial was run for 39 days and the extent (%) of biodegradation was evaluated as shown in Table 7. At the end of the biodegradation trial, all the analyzed films lost their mechanical properties. From the data collected in Table 7 and from the integral profiles of the traces of CO_2 developed within the time (Fig. 3) a detrimental effect on the rate of the biodegradation and overall extent of biodegradation was detected with the increasing of the crosslinking degree.

In all cases the extent of biodegradation is larger than one order of magnitude with respect to that observed in the case of PVA 98 % degree of hydrolysis.

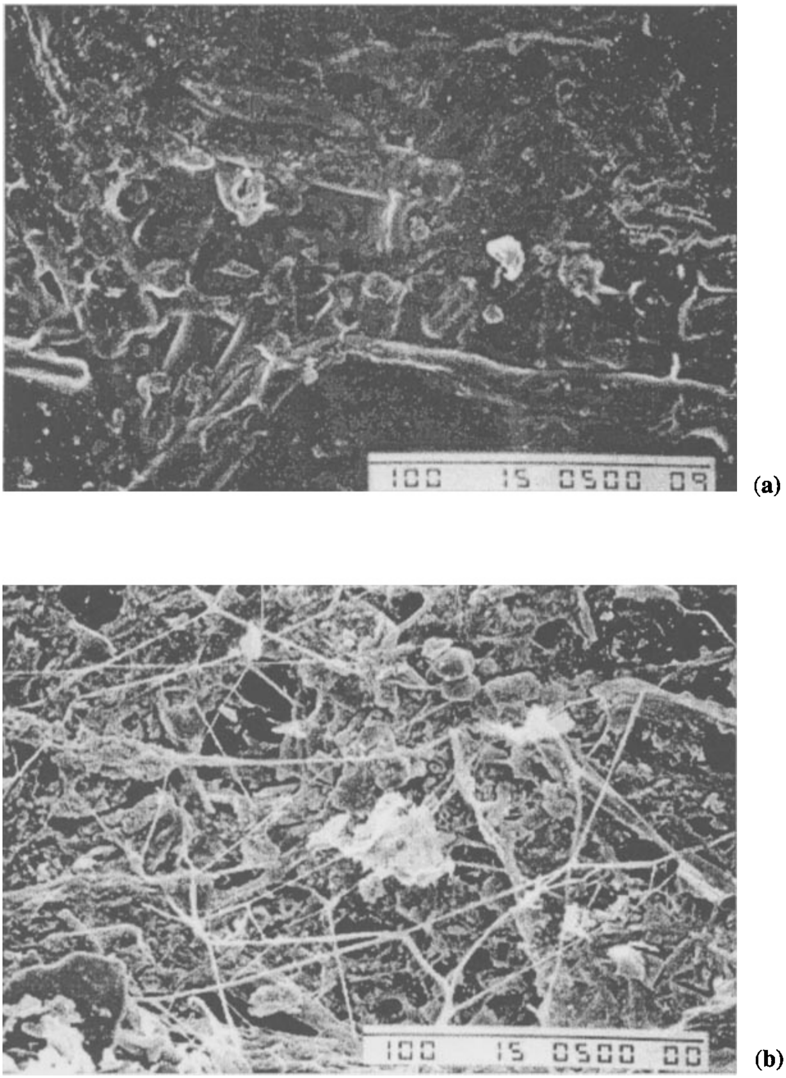

Fig. 2: Scanning electron micrograph of WGB2 film sample before (**a**) and after (**b**) microbial attack during the first biodegradation trial

Tab. 7. Biodegradation % of crosslinked and un-crosslinked waste gelatin based films as compared to PVA sample.

	Biodegradation (%) [b]					
Time (days)	PVA98 [a]	WGX1	WGX3	WGX5	WGX7	WG
1	-0.097	0.5264	0.50334	0.54162	0.2338	0
2	0.2921	4.0333	3.5225	3.10716	2.4937	3.1665
4	0.2921	11.925	7.38047	6.28947	5.377	9.2885
6	0.4868	21.92	12.6642	11.5584	10.131	18.155
8	0.404	31.175	18.7326	18.0249	15.305	26.96
10	0.7355	40.878	25.5149	24.7697	21.806	36.843
14	1.2328	54.323	39.5889	36.0963	35.081	54.286
16	1.677	61.523	46.7628	41.655	40.503	61.028
18	2.3432	68.923	55.1802	47.0662	47.613	68.973
21	2.3432	74.123	60.2498	50.1634	53.034	74.029
25	2.2322	78.123	65.415	52.6448	57.567	77.4
29	2.4543	80.723	68.0933	54.5044	60.5	80.048
32	2.5653	82.323	69.7193	55.2119	62.989	81.733
36	2.5677	84.736	71.3075	55.2766	65.135	84.639
39	2.7337	86.693	72.3872	56.817	67.008	86.455

[a] The sample used was from PVA with 98% hydroxyl content submitted prior the use to three freezing-thawing cycles .

[b] Determined as $\frac{\text{Amount CO}_{2\,(\text{sample})} - \text{Amount CO}_{2\,(\text{blank})}}{\text{ThCO}_2} \cdot 100$

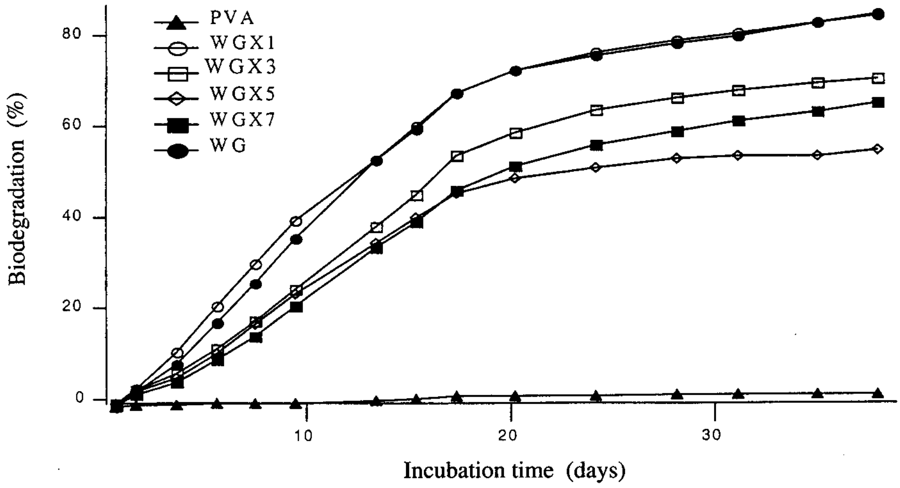

Fig. 3: Trend and overall extent of biodegradation in waste gelatin films (mean values for duplicate trials), (PVA treated with freezing & thawing)

These results indicated that the crosslinking method is an effective procedure for controlling the biodegradation rate and biodegradation extent within the time in agreement with previous finding [17-19].

Conclusions

Cohesive, flexible biodegradable films based mainly on waste gelatin were prepared either by coating of water dispersions or direct spraying. Sugar cane bagasse and PVA were used as fundamental ingredients in the formulation of composites and films respectively. The structure of the films was reinforced by incorporation of various dialdehyde crosslinking agents in the polymer blends and composites. The prepared films resulted biodegradable and the crosslinking of waste gelatin with dialdehyde was found to be an effective mean to allow them to persist in the environment for the required period of time. These biodegradable blends and composites are on the way to be used in agriculture for the fabrication of mulching films and/or soil conditioners and self fertilizers.

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