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A COMPLEX WAY OF ASSESSING BIODEGRADABILITY OF POLYMER FILMS – A PRACTICAL APPROACH

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Commercially available starch-based biodegradable films were tested for the assessment of biodegradability in accordance with standardised and non-standardised methods providing possibilities to directly monitor microbial activity. A method originating from the measurement process of the biological activity of soils was modified and applied according to the recommendation of international standards to test polymers under controlled composting conditions. Based on the results of our experiments, it is not possible to clearly assess the biodegradability of films based exclusively on the measurements of the produced CO_2 during the degradation process. Additional measurement on the microbial activity in the nearest milieu of the samples, as well as the microscopic investigation of the samples together with the CO_2 production provide a complex information on biodegradability.

Keywords: biodegradation, starch-based films, controlled composting conditions

In order to reduce the wastes originating from the packaging materials using non-renewable resources as well as to increase the use of renewable raw materials, development of biodegradable packaging materials is going on worldwide. The application possibilities of biodegradable materials for food packaging are also being studied (STRANTZ & ZOTTOLA, 1992; KESZLER et al., 2000). In developing biodegradable packaging materials the testing of compostability and biodegradability by standard, internationally accepted methods is of the utmost importance.

Several test methods have been standardised for the measurement and assessment of biodegradation of polymers by national and international standardisation committees and organisations (ISO, CEN, ASTM, DIN, etc.) during the last 20 years. However, it is interesting that only two of the international standards (OECD, 1983; ISO, 1999) recommend the biodegradability test of polymers under composting conditions albeit candidate biodegradable polymers are designed not only biodegradable but compostable as well; all the other international standards propose the use of a special aquatic medium (ISO, 1997; PAGGA, 1997). In the case of national standards, ASTM (1992) and DIN (1998) determine the biodegradability under aerobically controlled composting

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conditions and are based on organic carbon conversion into CO_2 (ASTM, 1992; DIN, 1998). Actually, two drawbacks of the experiments carried out with the application of compost or soil are usually highlighted: (i) compost possesses relatively high background production of CO_2 capable to mask the amount of CO_2 resulting from the degradation of the polymer to test, (ii) it is difficult to recover the polymers left after the test from the compost and to prepare a carbon balance. Aquatic tests make both processes easy to carry out. On the other hand, a great disadvantage of aquatic tests is the fact that they do not reflect the real composting environments where high microbial activity is provided by not only bacteria but also fungi and actinomycetes, especially in case of higher temperatures (50–60 °C). Therefore aquatic tests usually show lower degradation potential for a polymer than composting tests do. In this case either the duration of aquatic tests should be increased even up to 6 months or a carbon balance should be made along with the determination of BOD and CO_2 evolvement in order to get a more correct potential value (PAGGA, 1999; PAGGA et al., 2001). The measurement of the evolving CO_2 is not adequate to unequivocally determine the amount of degraded polymers because microorganisms use the carbon from the degradation of polymers not only for the production of CO_2 but to synthesise oligomers and new biomass. That is why the determination of dissolved organic carbon (DOC) should be assessed according to several standpoints (STROTMANN et al., 1995; CALMON-DECRIAUD et al., 1998) to prepare the balance of carbon and to check if there are no unexpected losses.

At the same time, the effectiveness of composting tests cannot be questioned, so they have been continuously developed to set out new techniques such as tests based on the use of vermiculite, a clay mineral which can be activated with an appropriate microbial population and used as a solid matrix in place of mature compost in the controlled composting test (STARNECKER & MENNER, 1996; BELLIA et al., 1999). As the polymers, either imported or to be produced later in Hungary also, are intended to be composted, a method originating from the measurement process of the biological activity of soils was modified and applied to test the polymers under controlled composting conditions. The measuring set-up was firstly used for the analysis of the imported films that are similar to some biodegradable films previously tested by other laboratories (BASTIOLI, 1998; KIM et al., 2000). The purpose of experiments was to test the different tests and to work out the appropriate way to assess biodegradability.

1. Materials and methods

1.1. Compost

The mature compost was obtained from a local composting plant treating vegetable solid waste originating from urban parks of Budapest, Hungary. The compost had the other following characteristics: pH 7.4; total dry solids (t = 105 °C): 45%; volatile

solids (t = 550 °C): 33% of the dry solids. Before the experiments the compost was sieved through a screen of 5 mm to obtain a homogeneous material of sufficient porosity and its water content was set to 55% by adding distilled water.

1.2. Compost extract

The compost extract was obtained by the process recommended by DIN (1998).

1.3. Test materials

Three different types of starch-based Mater-Bi® (Novamont, Italy) films (the carbon contents of sample 'A', 'B' and 'C' were 69%, 59.8% and 60.4%, respectively) with the same thickness (20 μm) were tested. The films were cut to 5 mm×5 mm or 20 mm×20 mm sized samples depending on the test methods applied. Microcristalline cellulose (Avicel; Fluka, Buchs, Switzerland; C content: 42.5%) was used as positive reference material together with 2 g of samples embedded in compost and placed in aquatic media.

1.4. Experimental methods

1.4.1. Determination of weight loss in compost. Films (20 mm×20 mm) were separately embedded in 80 g of compost. Hundred ml beakers with the compost and the sample were weighed and covered with aluminium films with small holes for aeration and incubated at 37 °C. Water loss caused by evaporation was compensated by adding water according to the weight loss determined. At the end of the 3rd and 6th weeks the samples were removed, cleaned with water followed by (i) the determination of residual total dry solids content at 105 °C, 24 h; (ii) visual observation of the sample surface by macroscopy and microscopy. All measurements were done in 4 replicates.

1.4.2. Determination of weight loss in aquatic media. Films (20 mm×20 mm) were placed in an aquatic medium recommended by the German Standard DIN (1998). For practical reasons, 150 ml of aquatic media were placed in 300 ml Erlenmeyer flasks. The flasks were inoculated with compost extract, closed and covered with aluminium films to avoid photodegradation. Incubation was carried out at ambient temperature (20–25 °C). The change in the Colony Forming Units (CFU) was weekly detected over a 6-week period on TGE and Malt Extract Agar plates (Merck). At the end of the 3rd and 6th weeks the samples were removed and the residual total dry solids content was determined at 105 °C, 24 h.

1.4.3. Determination of the effects of leaching. Films were studied in the same way as it has been described in section 1.4.2 except that aquatic media were not inoculated with compost extract.

1.4.4. Determination of the CO_2 production in an aerated measurement set-up. In our study a modified version of the method described by SZEGI (1978) was applied, which is described as follows. A drain layer of 20 mm width made of glass beads (∅4 mm) was formed on the bottom of 250 ml Erlenmeyer flasks (incubator flasks).

This layer was covered with a discoid piece of plastic screen followed by the mixture of compost (80 g) and the film samples to analyse were cut to quadrate of 5 mm edge length or positive reference material (2 g Avicel) (Fig. 1/a). The sample/compost ratio (2 g/80 g) was set according to the experiment described by SZÁRAZ and BECZNER (2002). Two glass tubes (∅ 5 mm) were inserted into the borings of the rubber cork. The end of the longer tube was covered with plastic drape to avoid being blocked by compost grains. Two pieces of 40 ml gas-washer tubes containing 20–20 ml of 0.5 mol l^{-1} NaOH (analytical reagent grade; Reanal, Budapest, Hungary) were connected in-line to the incubator flask. The schematic diagram of the complete set-up is shown in Fig. 1/b. Aeration was done with CO_2-free air with the volume flow rate set to 50 ml per min with the help of Cole-Palmer rotameters (Niles, IL, USA) in each measuring line. The incubator flasks were placed in a thermostat set to 37 °C. The quantity of CO_2 produced was determined by titration of the excess NaOH by a standard solution of hydrochloric acid (HCl, 1 mol l^{-1}, Reanal) with the means of an automatic titration device (TIM900 Titration Manager; Radiometer, Copenhagen, Denmark) twice a week for a period of 45 days. The theoretical amount of carbon dioxide ($ThCO_2$) and the degree of biodegradability (D_t in % of $ThCO_2$) of the test material were calculated according to DIN (1998). This experiment was carried out in two replicates using three subsamples of the samples.

1.4.5. Statistical evaluation. Statistical evaluation of the data and of the curves was carried out with the help of SPSS 10.0 for Windows statistical software (SPSS Inc., Chicago, IL, USA) applying two-sample *t*-method and non-linear regression, respectively. Confidence intervals were set to 95% all over the evaluation.

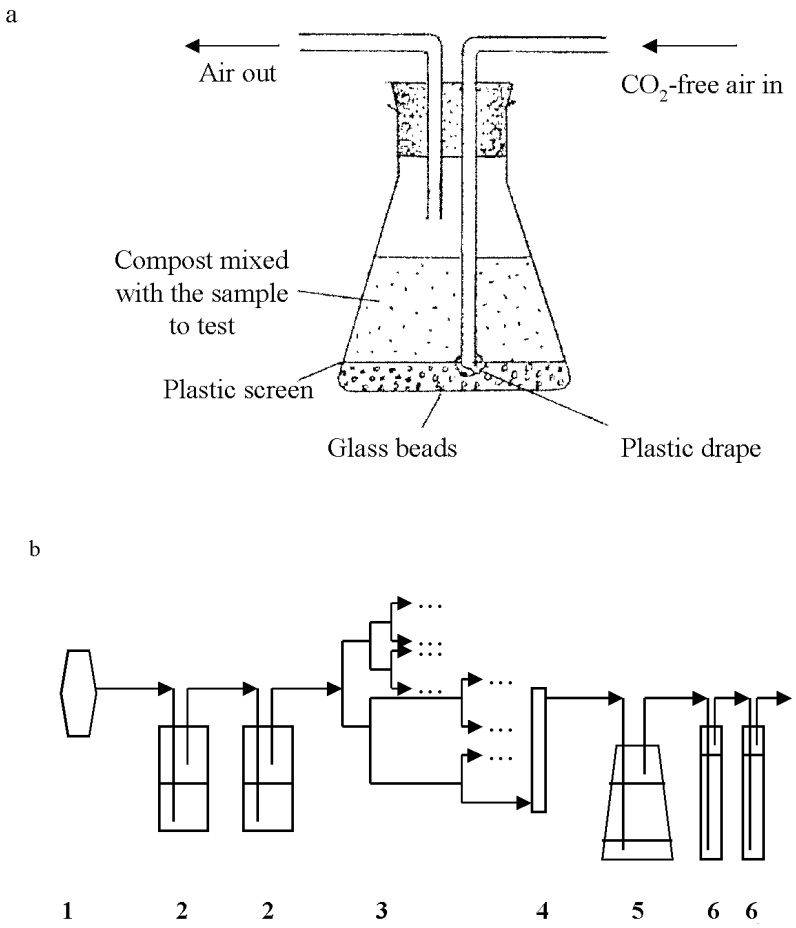


Fig. 1. Schematic figure of the incubator flask (a) and of the controlled composting test; (b) 1: air pump; 2: in-line connected flasks filled with 2 mol l^{-1} NaOH in order to remove CO_2 from the compressed air flow; 3: 8-way branching; 4: air flow controlling rotameter; 5: incubator flask; 6: in-line connected gas-washers to trap the produced CO_2

2. Results and discussion

2.1. Determination of weight loss in compost

The values of the actual total dry solids contents after 3 and 6 weeks of incubation are presented in Table 1. Sample 'C' showed the highest weight loss (≈23%) compared to the other two samples (≈10–12%). During the first 3 weeks of the experiment only sample 'C' showed considerable weight loss (≈6.5%), while samples 'A' and 'B' showed considerable weight losses only during the second half of the experiment, the difference being non-significant after 3 weeks because of high standard deviation values. The data at the end of the experiment were significantly different from those measured after 3 weeks for samples 'A' and 'C'. The differences were significant between the start-time data and those obtained after 6 weeks for all samples (Table 2).

Table 1. The values of the actual total dry solids contents (%) of the 3 studied samples in the experiments carried out in compost, inoculated aquatic media and non-inoculated aquatic media after 3 and 6 weeks

	Sample 'A'			Sample 'B'			Sample 'C'		
	Compost	Aquatic media + inoculum	Aquatic media	Compost	Aquatic media + inoculum	Aquatic media	Compost	Aquatic media + inoculum	Aquatic media
After 3 weeks	101.3±1.2	101.4±1.3	100.5±4.4	98.0±5.5	99.2±1.5	98.6±2.6	93.5±5.6	98.4±2.7	96.4±2.2
After 6 weeks	87.7±2.3	86.9±4.6	88.1±3.0	90.2±1.2	86.8±0.6	90.1±2.3	76.8±2.9	86.6±0.5	89.5±3.2

The values presented are related to the original dry solids. Standard deviation values are based on four replicates

Table 2. Statistical evaluation of the data of total dry solids content from the experiments carried out in compost, inoculated aquatic media and non-inoculated aquatic media after 3 and 6 weeks of the experiment

		In compost		Inoculated aquatic media		Non-inoculated aquatic media	
		Start time	After 3 weeks	Start time	After 3 weeks	Start time	After 3 weeks
Sample 'A'	after 3 weeks	19%	–	21%	–	87%	–
	after 6 weeks	0.17%	0.05%	1.1%	0.94%	0.46%	2.5%
Sample 'B'	after 3 weeks	52%	–	40%	–	39%	–
	after 6 weeks	0.02%	6.9%	0.42%	0.25%	0.06%	0.67%
Sample 'C'	after 3 weeks	11%	–	69%	–	6.8%	–
	after 6 weeks	0.07%	0.62%	0.02%	0.07%	0.81%	1.4%

The values expressed in % represent the probability of identical results. Data in bold being under 5% probability represent significant difference between the expected value of total dry solids content of the given sample

2.2. Determination of weight loss in aquatic media

In aquatic media inoculated with compost extract the weight loss was similar (≈13%) for all samples (Table 1), showing significant differences only between the final (after 6 weeks) and middle-time (after 3 weeks) results. The results obtained in compost and in aquatic media were the same for samples 'A' and 'B' (Table 1).

2.3. Determination of the effects of leaching

A 10–12% decrease in the total dry solids content was observed for all samples, mostly arising in the second half of the period, similarly to the weight losses in compost or in aquatic media (Table 1). The results of these leaching effects lead to the conclusion that the decrease in the total dry solids content in aquatic media is basically generated by physical dissolution and not by degradation processes, which can be confirmed by statistical evaluation as well (Table 3).

Table 3. Statistical evaluation based on the data of the total dry solids content resulting from the experiments in inoculated and in non-inoculated aquatic media

	After 3 weeks	After 6 weeks
Sample 'A'	75%	68%
Sample 'B'	71%	28%
Sample 'C'	31%	23%

The values expressed in % represent the probability of identity between the correspondent results

2.4. Determination of CFU in the aquatic media

The CFU values of the aquatic media after inoculation with compost extract were as follows: 10^4 CFU cm^{-3} for bacteria, 10^2 CFU cm^{-3} for actinomycetes and 3×10^2 CFU cm^{-3} for yeasts. Figure 2/a presents the growth curve recorded during the experimental period of 45 days. This figure shows the bacterial CFU only because neither fungi nor yeasts were detectable from the 3rd week in the aquatic media. The bacterial CFU increased only by two orders of magnitude in these media and reached a plateau in the case of all samples on days 10–12, showing an almost identical growth pattern. With the inoculum not only the microorganisms but also nutrients for them got over to the aquatic medium from the compost, which explains the considerable microbial activity in the blank (aquatic media without sample). Therefore, growth curves corrected with the CFU values of the blank were calculated representing the actual microbial growth in the presence of the biodegradable samples (Fig. 2/b). The corrected curves show bacterial growth of about only one order of magnitude in the first 2 weeks of the experiment, then declined and from the 3rd week the microbial population increased continuously, possibly due to the adaptation of microbes to the nutrients slowly released from the samples. This phenomenon coincides with the observation of detecting a decrease of the total dry solids contents after the 3rd week of

incubation in the inoculated aquatic media. On the other hand, the increasing phase warns to continue the recording of microbial growth over 6 weeks in order to precisely monitor the weight loss resulting from microbial activity. The behaviour of sample 'A' is slightly different from the others as its bacterial CFU decreased less than that of the others, probably because of the greater availability of nutrients from the sample.

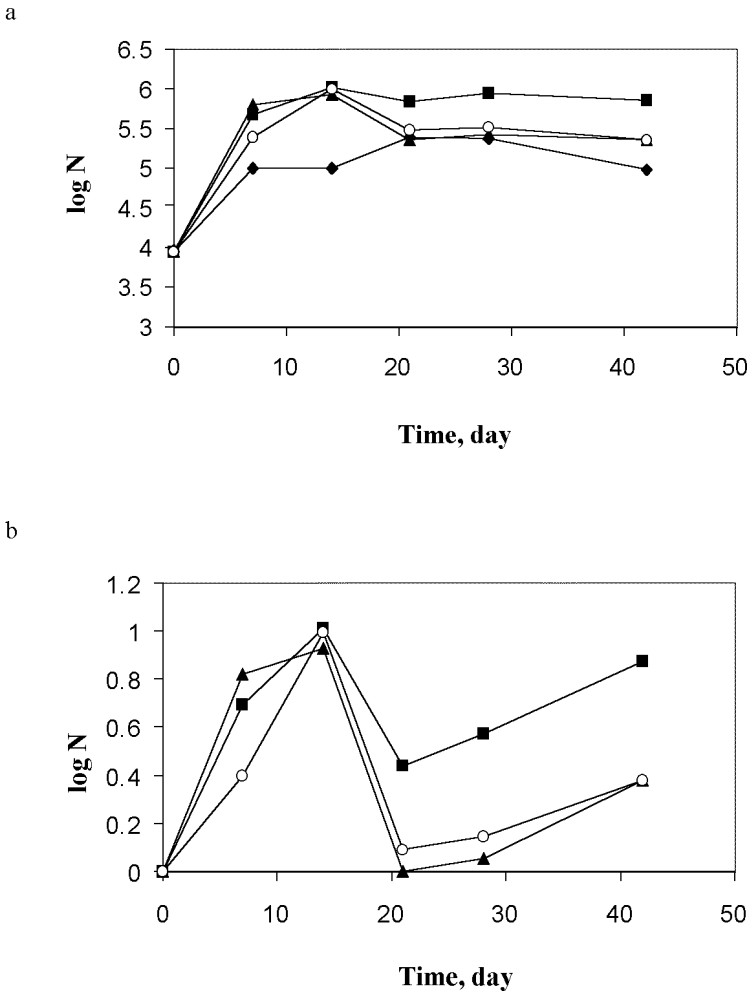


Fig. 2. Growth curves in aquatic media. Uncorrected curves of the samples and the blank (a); and blank-corrected growth curves (b). ■: sample 'A'; ▲: sample 'B'; ○: sample 'C'; ◆: blank

2.5. Visual observation

Visual observations by macroscopy and with the help of an optical microscope played an important role in the assessment of biodegradability of the samples. After 3 weeks of experiment, yellowish-pinkish spotted surface was observed in the case of all the films embedded in compost; these spots got larger and darker by the sixth week. In aquatic media only sample 'A' showed a slightly pink discolouration of the surface. Holes in the samples detectable by macroscopy were observed exclusively for sample 'A' appearing after 3 weeks and all specimens of this sample became totally ragged and fenestrated by the sixth week. Figures 3–5 show the surface of the samples investigated by light microscope before and after being embedded in compost for six weeks. On one hand, these pictures demonstrate the best degradability of sample 'A' being the specimen thoroughly webbed by hyphae compared to the others. On the other hand, they visualise the mechanism of the degradation process: spots arising from leaching presumably generate small holes, thus creating relatively larger surface for the microbial activity.

2.6. The assessment of biodegradation based on CO_2 production

Figure 6/a shows the time course of microbial CO_2 formation, while Fig. 6/b shows the time course of biodegradation of 4 materials (3 film samples and Avicel) in controlled composting conditions. As it can be seen in Fig. 6/a, an increased microbial activity is recorded already one week after the embedding in the case of microcrystalline cellulose used as positive reference material, while for the films the degradation is delayed by a week. There is only a slight difference in the CO_2 production of samples 'A' and 'C' in spite of that the carbon content of 'A' is about 10% higher than that of 'C'. This difference was not found significant by statistical evaluation; however the degradation curve of sample 'B' was significantly different from those of the other film samples.

When comparing the degradation of the 3 films (see Fig. 6/b), the highest amount of CO_2 is produced during the degradation of sample 'B'; however, this amount turns up quite low (about 20%) compared to the theoretical amount of CO_2 ($ThCO_2$) expectable after total oxidation of the added test or reference material. Usually, 60% degradation is required in practice. The CO_2 production and the degree of biodegradation (D_t in % of $ThCO_2$) of Avicel were adequate, being far over the degradation rate of all the films. At the same time, degradation parameters presented in Fig. 6/b are below those presented previously by PAGGA and co-workers (1995) and BELLIA and co-workers (1999) where Avicel showed 60% degradation after about 40 days, while in our study the degree of degradation of Avicel did not reach this limit.

a)

b)

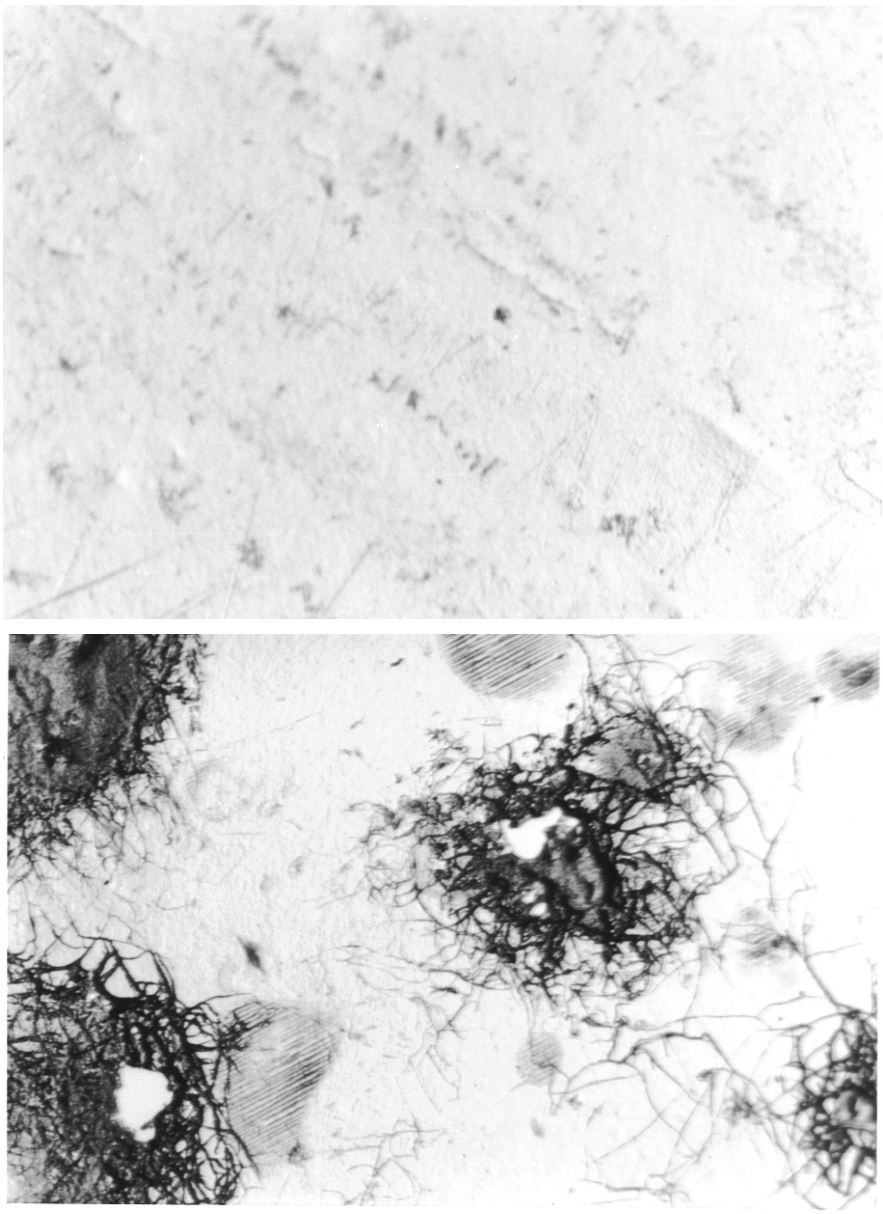
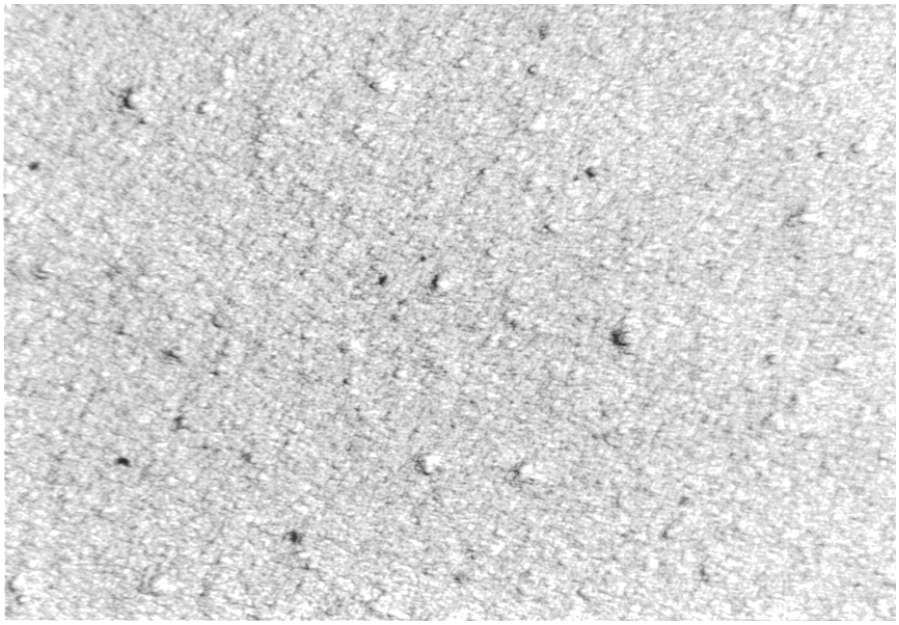


Fig. 3. Surface of sample 'A' untreated (a) and in compost for 6 weeks (b). Magnification: ×400

a)



b)

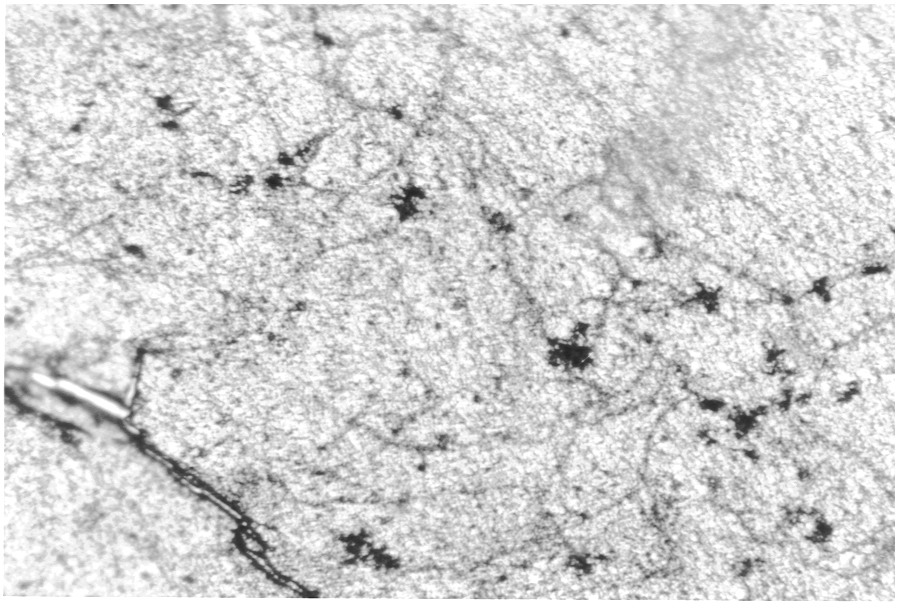


Fig. 4. Surface of sample 'B' untreated (a) and in compost for 6 weeks (b). Magnification: ×400

a)

b)

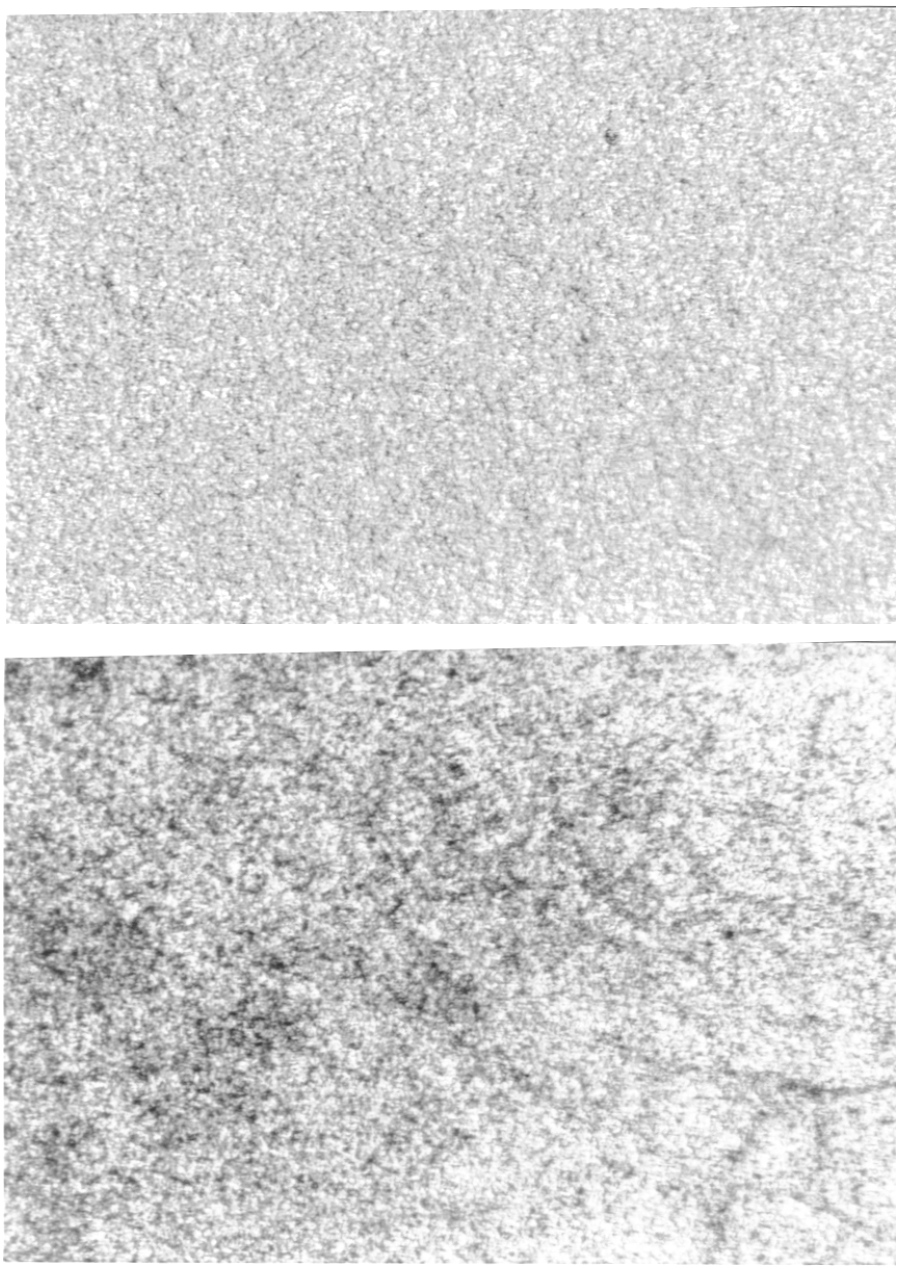


Fig. 5. Surface of sample 'C' untreated (a) and in compost for 6 weeks (b). Magnification: ×400

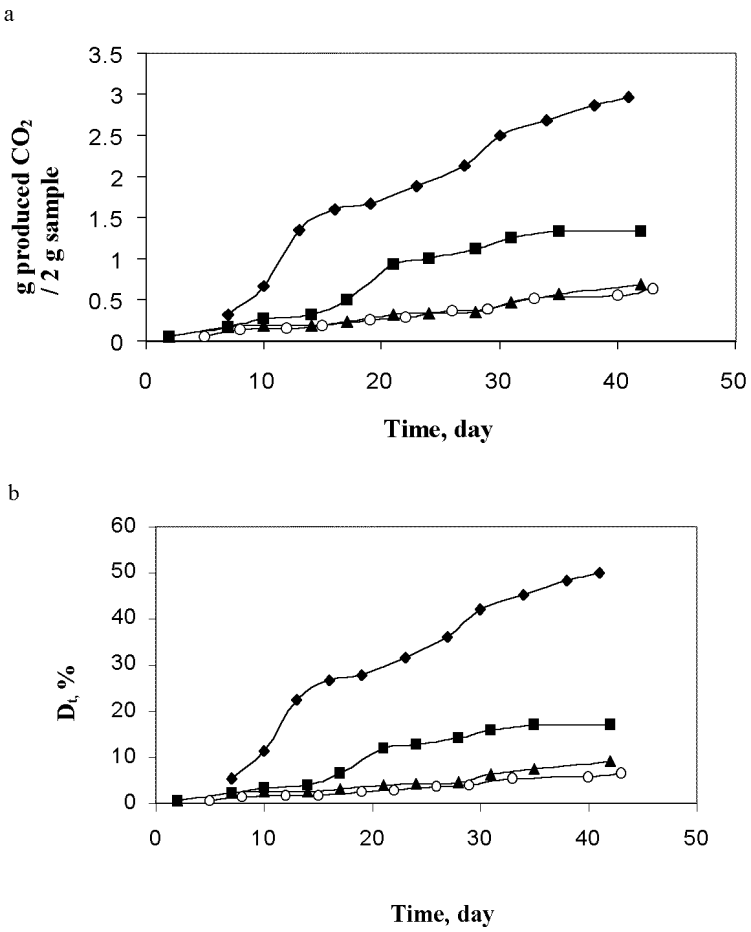


Fig. 6. The blank-corrected CO_2 production (a) and the degree of degradation of the samples (b). ○: Sample 'A'; ■: sample 'B'; ▲: sample 'C'; ◆: Avicel

This might be explained by the shortness of the experiment, which can be demonstrated by the fact that the curve of Avicel did not reach the plateau. In the case of the films tested the plateau phase was reached, but only 10–20% of the degradation was achieved on the basis of CO_2 production. We have tested the samples as films (except Avicel), though commonly they are tested in powdered form. We believe that testing in film form the situation is closer to reality.

3. Conclusion

The continuous development of candidate biodegradable polymers requires the working-out of degradation tests that are compatible with international regulations by assessing biodegradability on the basis of the amount of produced CO_2 to provide convincing information for end-users.

It has been shown that although there is a possibility to make an order of biodegradability of really similar starch-based films on the basis of their weight losses detected in aquatic medium or after embedding them in compost, but the results do not agree with those of other tests. Even the results arising from microscopic observations contradict the former order by highlighting another film to be better at degradation. Leaching tests indicate physical dissolution rather than biodegradation. Weight loss and microbial activity measurements lead to the conclusion that an interval of 45 days is not sufficiently long enough for testing materials in film form. Nutrients available for microorganisms are carried over from the compost to the aquatic media when preparing inoculum, so the microbes are not forced to assimilate the components of the films at the beginning of the experiment and microorganisms decayed may also serve as nutrients for a while.

The recording of CO_2 production made a different order among the films and unequivocally confirmed the better biodegradability of sample 'B'. Our system set-up provided adequate CO_2 production data for the positive reference material Avicel. However, manual operation goes together with special inaccuracies (i.e. it is not possible to trap all the CO_2 produced during the degradation) but working with a relatively low amount of compost helps to eliminate huge CO_2 losses. Furthermore, two gas-washers connected in-line provided sufficient efficiency in trapping CO_2, making unnecessary to build in more gas-washers. CALMON-DECRIAUD and co-workers (2000) compared the degradation curves of sodium acetate recorded by both manual titration and with the help of two automatic CO_2 measuring systems. Their results are consonant with the results of our study indicating that manual methods – especially when complemented with a different technique e.g. visual observation – can be considered applicable to assess biodegradation on a practical basis.

Based on the results of our experiments it is not possible to clearly assess the biodegradability of films based exclusively on the measurements of the produced CO_2. Therefore, there is a need for carrying out more complex experiments providing information on the microbial activity in the nearest milieu of the sample.

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