Biodegradation of PVA-based formulations

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SUMMARY: Investigation of the biodegradability of water soluble poly(vinyl alcohol) (PVA) based blown films was carried out under different lab-scale environmental conditions. In particular respirometric tests were utilized in order to evaluate the biodegradability of PVA films in composting, in modified Sturm test and in soil burial simulation tests. Several microbial inocula present in river water, mature compost, forest and farm soils as well as sewage sludge from municipal and paper mill wastewater treatments plants were utilized for the relevant tests. A mixed PVA-degrading microbial culture was obtained by a common enrichment procedure by using sewage sludge from paper mill as inoculum; this culture was tentatively utilized for the isolation of single PVA-degrading microorganisms. As a first result we can stress that significant biodegradation extent in fairly low incubation time can be obtained only in the presence of acclimated microbial populations such as those deriving from paper mill sewage sludge, in liquid cultures. Nevertheless separation of single degrading microbial species was impossible most likely due to the establishment of symbiotic or commensal interactions between the single components of the PVA-degrading mixed cultures. On the other hand, limited mineralization rates were recorded in solid cultures in the presence of soil or compost. Finally, a mechanism of degradation of polymer chains unlike random or unzipping was suggested in the presence of either PVA-degrading mixed culture and its filtrate by means of viscometric determinations of molecular weight within the time.

Introduction

Poly(vinyl alcohol) (PVA) is a well known synthetic polymer that has recently attracted renewed interest for the production of ecocompatible plastic materials. The rather peculiar PVA properties and its gradual reduction in cost account for its increasing production and end uses. In particular four main groups of PVA applications can be identified: *warp sizing*, *paper coating*, *adhesives*, and *films*. PVA-based "biodegradable" disposable items (*mulching films*, *laundry bags*, ...) can safely reach environment without incorporation into any integrated system of waste treatment. Therefore the biodegradability of PVA and PVA-based materials has to be considered as a fundamental aspect to be ascertained. Basic properties of these systems depend upon degree of polymerization, degree of hydrolysis, distribution of hydroxyl groups and stereoregularity (i.e. crystallinity) of PVA; thus also the relationship between biodegradability and polymer structure should be investigated.

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PVA is considered to be a true biodegradable synthetic polymer since the mid thirties[1]. More recently single microorganisms[2-6] and symbiotic bacterial cultures[7] able to utilize PVA as carbon source have been identified. Suzuki[2] and Watanabe[4] proposed two similar degradation pathways by using different *Pseudomonas* strains. In both cases the polymer is oxidized by oxidase-type enzymatic systems with oxygen consumption and evolution of hydrogen peroxide; the result of this enzymatic attack being the production of carbonyl groups along the polymer chain. Activated β-diketones or α-keto groups are subsequently hydrolyzed with the breakdown of the carbon backbone and hence reduction of molecular weight. Membrane-bound dehydrogenases are claimed to be involved in the initial oxidation of the polymer chains in the two members symbiotic biodegradation process[8]. It has been suggested that one of the microorganisms produce PVA-dehydrogenase as apoenzyme which is converted into the active form (holoenzyme) by the inclusion of an essential cofactor produced by the other symbiont[9]. This cofactor was identified as pyrrolequinoline quinone (PQQ)[8,10].

However the overall number of PVA-degrading microorganisms appear to be limited if compared with the widespread species capable to degrade aliphatic poly(ester)s, such as poly(hydroxyalkanoate)s (PHA's) and poly(ε-caprolactone) (PCL)[11]. Some species are found to be associated to PVA-contaminated textile or paper mill effluents[3]. Thus several *Pseudomonas* strains involved in the biochemical investigations of PVA degradation mechanisms were isolated from soil samples[2,4]. In spite of this only limited biodegradation rate and extent of the vinyl polymer in soil conditions have been reported[12-14]. Therefore the degradation mechanisms as well as the isolated degrading species previously reported should be not considered as conclusive and exhaustive of the overall of the microbial species and biochemical patterns involved in the mineralization of PVA.

In the present paper the degradative behavior of different commercial PVA-based blown films that can be used as hydrosoluble packaging was investigated in comparison with pure PVA under different test conditions. These studies led to the demand of acclimated microbial populations to achieve both significant biodegradation rate and extent of the polymer samples. Investigations aimed at the isolation of active microorganisms and understanding the degradation mechanism were also carried out.

Results and Discussion

The biodegradation behavior of different PVA-based blown films and PVA samples was investigated by different laboratory-scale tests aimed at simulating different environmental conditions. Rate and extent of biodegradation were assessed both in respirometric tests (Fig. 1) and by titrimetric evaluation[15] of the polymer concentration in liquid cultures in the presence of several microbial biocenoses collected from different environmental sources.

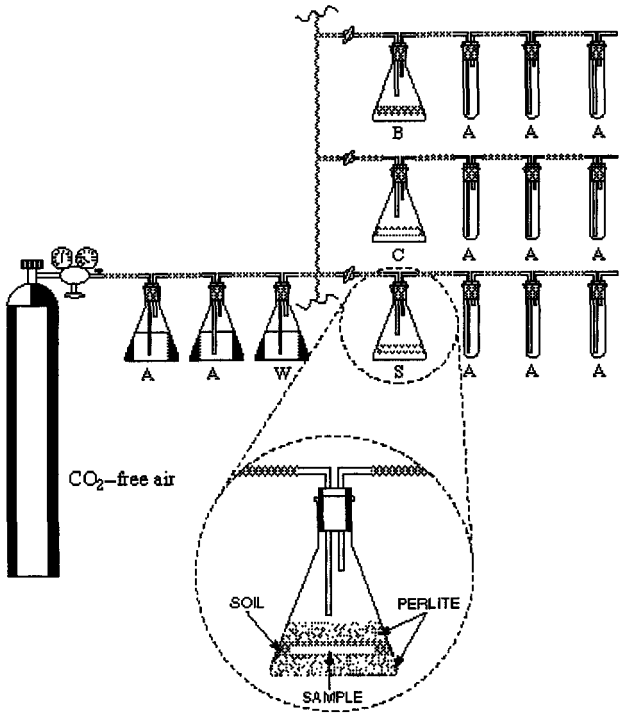

Fig. 1: Schematic representation of a respirometric apparatus (A = absorbers containing 40 ml 0.025 N $Ba(OH)_2$, B = blank, C = positive controls, S = test compounds, W = water).

Accordingly were compared the effectiveness of both incubation conditions and microbial populations in biodegradation of PVA based materials.

Respirometric tests were performed as simulated aerobic composting, aerobic biodegradation in liquid cultures and simulated soil burial in respirometric flasks[16]. Stabilized compost from urban solid waste, river water microbial biocenoses, activated sludge microflora of paper mill and municipal wastewater treatment plants, and a mixture of loamy and forest soil were utilized respectively in simulated composting, liquid cultures, and simulated soil burial biodegradation tests as microbial inocula.

In Fig. 2 are shown the results, expressed as percent of net theoretical CO_2 productions within the time, recorded in simulated composting test. Biodegradation of PVA based plastic films did not exceed 7 % in 48 days. Moreover this result was obtained only in the presence of the K20 sample. In any case only small differences can be detected between PVA based films and a high density poly(ethylene) (HDPE) film sample utilized as negative control, indicating that under these conditions the polymer samples undergo an extremely limited microbial

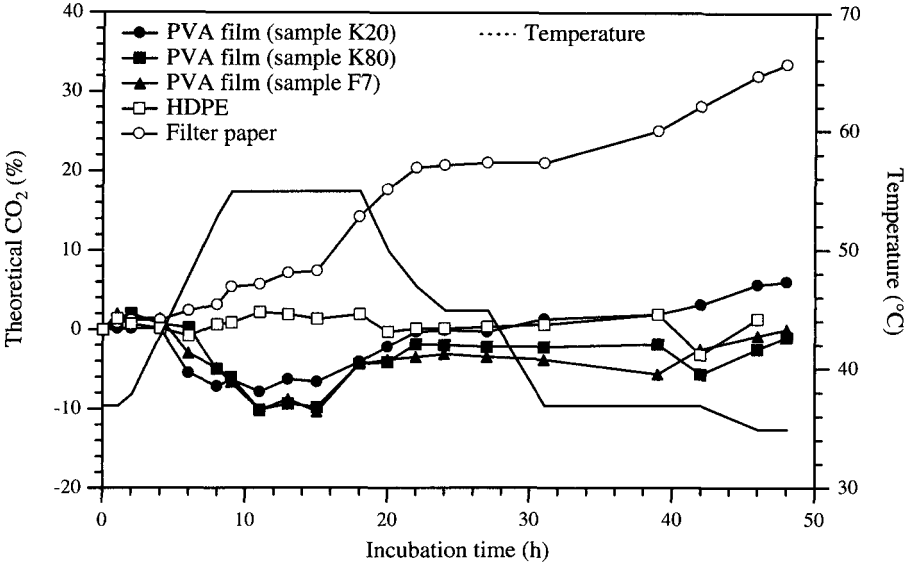

Fig. 2: Biodegradation curves of PVA-based films, filter paper (positive control) and HDPE (negative control) in simulated aerobic composting tests.

attack. Moreover the large CO_2 production recorded in the blank run (about 77 % of that of filter paper) could affect the accuracy of this test, as indicated by the fairly low extent of biodegradation of filter paper utilized as a positive control. A rather interesting feature observed during the incubation time was the lower CO_2 production, as compared to the blank, in cultures supplemented with PVA based plastic films in the correspondence of the thermophilic step of the simulated composting procedure. This phenomenon can be tentatively attributed to a toxic effect possibly exerted by the polymer samples, that were completely disperse in the compost bulk as a gel material, on the thermophilic microflora.

Also in simulated soil burial respirometric tests PVA based films underwent a limited (8-9 %) biodegradation in 74 days (Fig. 3); however in this case the CO_2 production (about 27 % of that of filter paper) recorded in blank runs allowed for more reliable results. This was possible because the amount of soil introduced in any runs was limited by the specific adopted incubation conditions that provide a solid substrate constituted in the largest volume by inert mineral bulk (perlite). Moreover, rate and extent of biodegradation apparently were not affected by the polymer concentration or its physical state, as indicated by the almost identical results obtained in the presence of different amounts of polymer samples, as either film or powder. In another experiments, carried out in analogous soil burial simulation conditions, mineralization rate and extent of PVA88 samples resulted still limited as 7 % in a

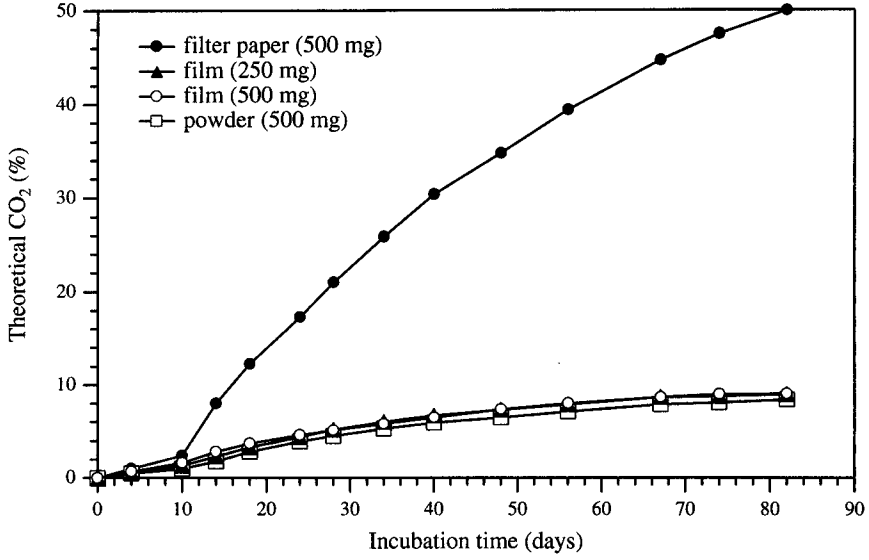

Fig. 3: Biodegradation curves of a PVA-based film (sample EK1) and filter paper in soil burial respirometric tests.

very prolonged incubation time (160 days), whereas other synthetic polymers utilized as positive controls still undergo to a significant mineralization.

Limited biodegradation values of PVA based films (13 % after 21 days of incubation) were also obtained in aerobic biodegradation test carried out in liquid cultures inoculated with municipal sewage sludge (Fig. 4). However due to the short incubation time the result obtained can not be considered as conclusive because a more active microbial population could be established within a prolonged duration of the experiment as revealed by the significant level of biodegradation of the PVA based sample.

In the presence of sewage sludge from a paper mill wastewater treatment plant, the biodegradation extent of PVA and PVA based films reached values comparable to that of cellulose, even if this occurred only after a appreciably larger incubation time (Fig. 5).

This last result can be explained by considering that microbial strains present in the paper mill sewage sludge are particularly active as a consequence of the selective pressure exerted by the large amounts of PVA in the wastewater reaching the treatment facilities of the paper factories.

According to the CO_2 evolution profiles (Fig. 6), a significant decrease of the PVA concentration was also monitored in liquid cultures inoculated with paper mill sewage sludge samples.

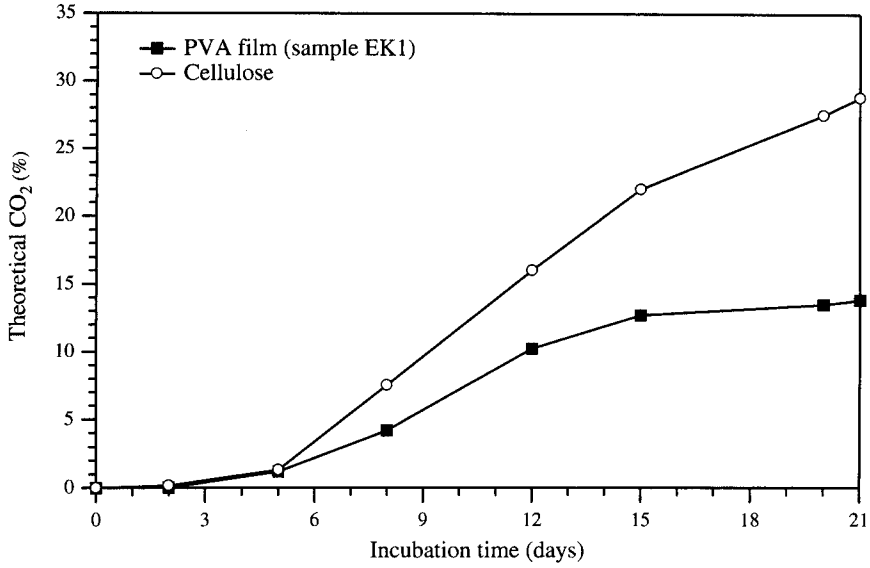

Fig. 4: Biodegradation curves of a PVA-based film and cellulose in the presence of municipal sewage sludge.

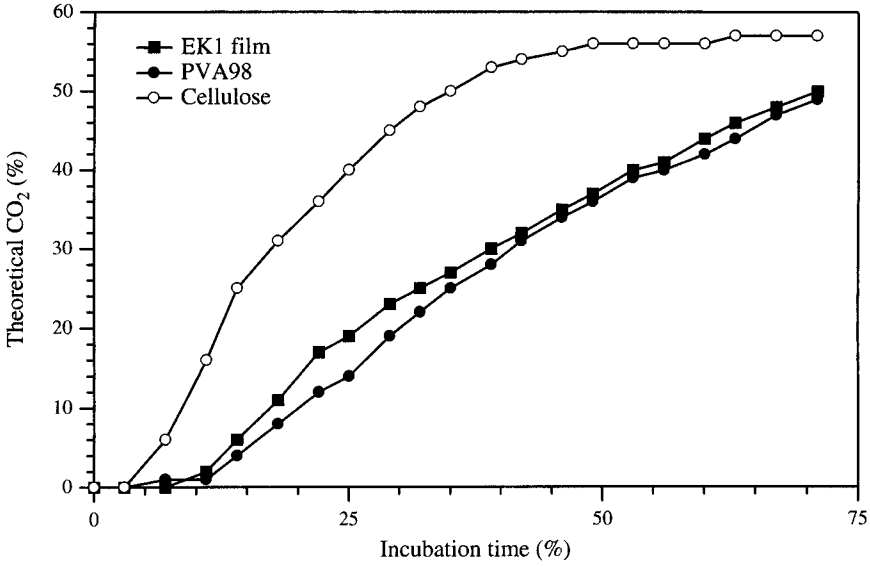

Fig. 5: Biodegradation curves of PVA, PVA-based film and cellulose in the presence of paper mill sewage sludge.

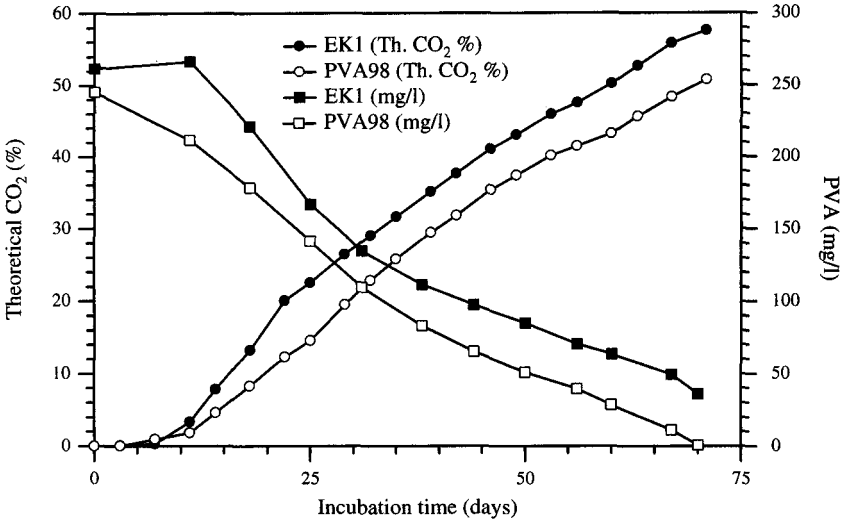

Fig. 6: Profiles of PVA concentration and CO_2 evolution of PVA and PVA-based film in the presence of paper mill sewage sludge.

By taking into account the above reported results, paper mill sewage sludge was enriched in liquid cultures in the presence of 250 mg/l PVA as sole carbon and energy sources and utilized, after repeated sequential transfers, as an acclimated inoculum in respirometric tests. As shown in Fig. 7, rate and extent of biodegradation of PVA samples attained in the presence of the acclimated inoculum were larger than those recorded in the presence of the previously tested inocula. Moreover the acclimation of the microbial population to PVA led to the failure of cellulose assimilation by the same microorganisms, as revealed by the very limited biodegradation (1.5 %) of the cellulose sample.

The reported results indicate that the microbial attack of PVA is strictly related to the presence of selected microorganisms which can be found almost exclusively in environments continuously contaminated by PVA; moreover the selective pressure exerted by the procedure utilized to obtain the acclimated culture tends to increase the populations of PVA degrading microorganisms.

According to these findings several degrading microorganisms previously isolated by different authors derived mainly from PVA polluted environments[5,6]. However, the ability to break the high energy C-C bond in the synthetic polymer, at the present seems to be restricted to a limited number of microorganisms, as revealed by the fairly low number of reports dealing on the isolation and characterization of microbial species involved in the biodegradation of PVA.

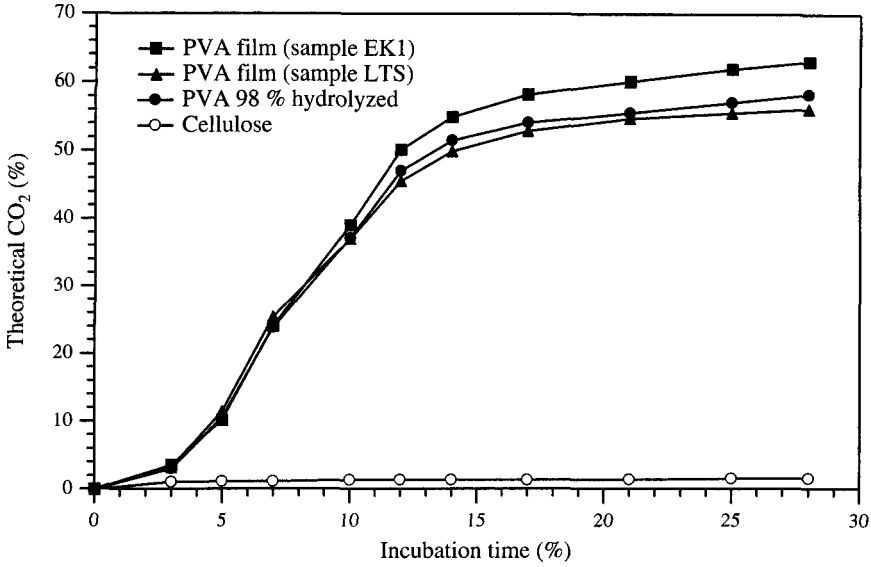

Fig. 7: Biodegradation curves of PVA, PVA-based films and cellulose in the presence of acclimated mixed culture.

An acclimated mixed culture was also utilized in order to isolate single PVA-degrading microorganisms. The mixed culture was diluted and plated over several different agarized media, such as nutrient agar, MM medium with or without yeast extract and MM medium solidified with silica gel as solidifying agent, each containing 250 ppm of PVA as the largest source of carbon. In order to avoid possible interferences by oligomers, additives and low molecular weight impurities, the polymer sample utilized for the isolation of single active microbial strain was dialyzed over a 6 kD cut-off membrane prior its use. All the microbial colonies developed on the solidified media were tested for their ability to degrade PVA by the reaction with iodine, both directly on the plate and in liquid cultures inoculated with any isolated colony. In some cases positive results (haloes formation around the microbial colonies) were obtained on the plate; however a significant reduction of the polymer concentration was never observed in axenic liquid cultures, indicating that none of the isolated microbial colonies was able to degrade PVA once transferred in liquid culture.

On the basis of previous reports dealing to the symbiotic utilization of PVA[7], the mixed culture was reconstituted in liquid media by mixing the isolated colonies in order to verify the presence of a real symbiosis or commensal interactions; nevertheless significant PVA degradation was still not observed. This result could be attributed to a specific physiological or biochemical requirement by the PVA-degrading microorganisms. It is also possible that

the PVA-degrading microorganisms represent a minor component of the overall mixed microbial population of the active culture; their isolation can not be therefore achieved by seeding serial higher dilutions of the liquid cultures on agar plates. This feature may be partially supported by the failure to isolate the active strains also on nutrient agar plates. Moreover, by streaking directly on MM agar plates the mixed active culture without any dilution, the development was observed of a large heterogeneous microbial colony which was able to degrade PVA once transferred in liquid culture. This latter result seems to confirm the presence of PVA-degrading strains as a minor component of the overall mixed degrading population; in other words we may infer the presence of microorganisms that, to some extent, start the degradation of the synthetic polymer and after this is achieved several "saprophytic" species can exhibit a largest growth at the expense of the degraded polymer up to its complete mineralization.

Attempts to clarify the degradation mechanism of PVA were carried out by using the enriched mixed culture. In particular, PVA degree of polymerization (DP_n) was evaluated from the relative viscosity of samples withdrawn from the culture at different incubation times, as reported by Finch[17]. Results are shown in Figs 8 and 9, in which relative viscosity and DPn are compared with the PVA concentration profile within the time. Similar results were obtained in experiments carried out in phosphate buffer in the presence of the mixed culture sterile filtrate that degrade PVA and PVA-based formulation (Figs 10 and 11).

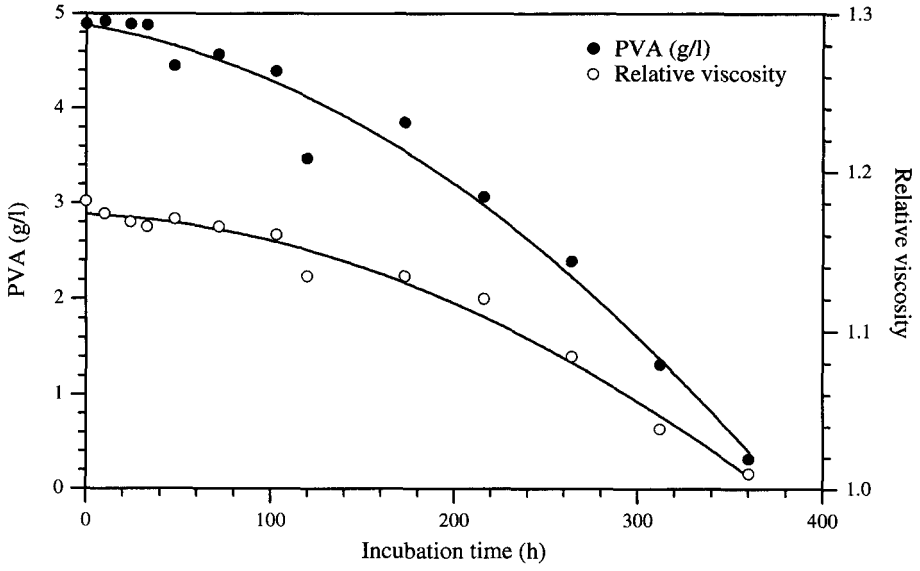

Fig. 8: Variation of the relative viscosity and PVA concentration in biodegradation tests carried out in the presence of acclimated mixed culture.

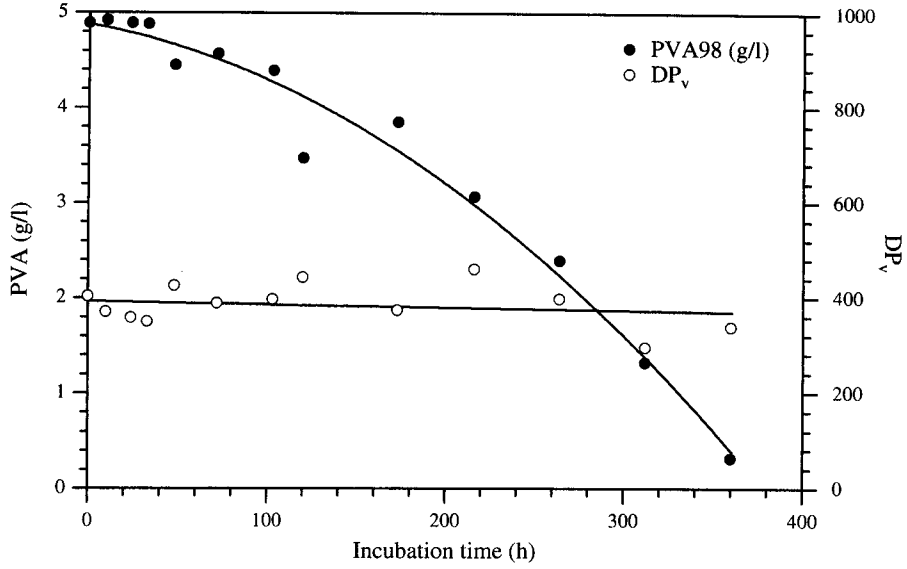

Fig. 9: Variation of PVA concentration and DPn in biodegradation tests carried out in the presence of acclimated mixed culture.

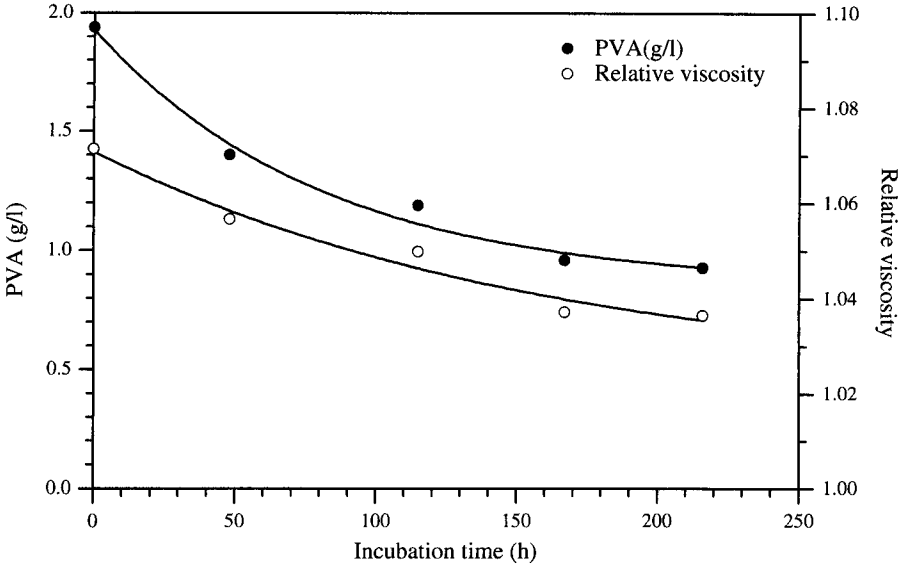

Fig. 10: Variation of relative viscosity and PVA concentration in degradation tests carried out in the presence of the filtrate of acclimated mixed cultures.

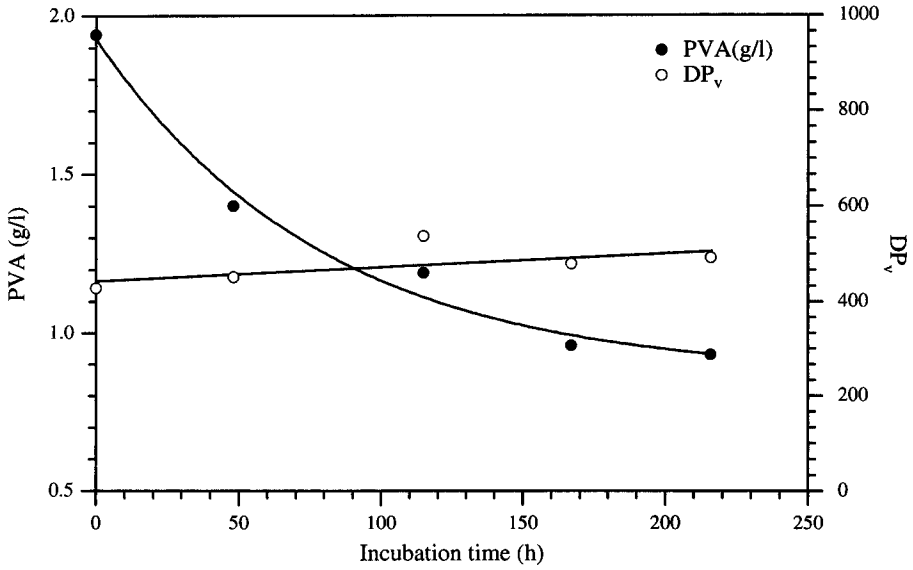

Fig. 11: Variation of PVA concentration and DPn in degradation tests carried out in the presence of the filtrate of acclimated mixed cultures.

Within the limits of the experimental deviations, these results seem to suggest that the initial "activation" of a single polymer chain by microbial attack is followed by the complete assimilation of the same polymer chain. In fact a random degradation mechanism mediated by endogenous enzymatic systems attack should lead to a large molecular weight decrease before the occurrence of an appreciable reduction of the polymer concentrations. On the other hand, the molecular weight decrease should parallel the biodegradation if an unzipping mechanism is active. However, a more extensive investigation is needed in order to confirm these preliminary results, by using a more accurate viscometric measurements combined with GPC analysis.

The appreciable PVA degradation observed in the presence of a sterile culture filtrate of the mixed PVA-degrading culture seems to demonstrate that an extracellular degrading enzyme is present in the mixed active culture.

Conclusions

On the basis of the results obtained in the present investigation the following conclusive points can be highlighted.

PVA degrading microorganisms seems to be confined mainly into PVA contaminated environments; in other words a selective pressure (acclimation) is required to isolate single

active microorganisms or mixed degrading microbial cultures. PVA-degrading single microorganisms were not isolated in pure cultures despite the several isolating techniques utilized. This could be attributed in first instance, to close symbiotic conditions or to a particular dynamic of microbial populations in the degrading mixed culture so that the degrading species reached an overall cell density much lower of other species which may utilize the degradation intermediates as a commensal.

Investigations carried out in the presence of culture filtrate of PVA-degrading mixed culture allowed to propose the presence of an active extracellular enzymatic system. Moreover, within the limits of the experimental errors, the data of relative viscosity and relevant degree of polymerization compared with the polymer concentration in PVA solutions supplemented with mixed active culture filtrate may account for a degradation mechanism that promote the overall dissipation of single polymer chains once this has been "activated". Upon further confirmation of these preliminary evidence one may claim an oxidative "unzipping-type-mechanism" as occurring in the enzymatic biodegradation of PVA. GPC analysis combined to viscometric measurements should provide further insights for substantiating the proposed mechanism.

Finally the PVA degradation was rather limited under simulated composting and soil burial conditions. However in the first case both the large amount of polymer samples and the high level of carbon dioxide production from the blank negatively affected the results confidence. On the other hand under soil burial conditions the same low, but reliable, biodegradation of PVA could be attributed either to the absence of degrading microorganisms in the soil microflora or to the polymer-soil interactions hindering the first enzymatic attack to hydroxyl groups. This latter issue has to be confirmed by further investigations also because of the increasing interest in the applications of PVA films as biodegradable mulching and soil conditioning films.

Acknowledgments

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