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Biodegradation of Poly(vinyl alcohol) with Different Molecular Weights and Degree of Hydrolysis

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ABSTRACT

The biodegradability of poly(vinyl alcohol) (PVA) was investigated under different conditions by respirometric determinations, iodometric analysis, and molecular weight evaluation. Microbial inocula derived from the sewage sludge of municipal and paper mill wastewater treatment plants were used. A rather active PVA-degrading bacterial mixed culture was obtained from the paper mill sewage sludge. Significant biodegradation levels within quite short incubation times were obtained in liquid cultures in the presence of acclimated microbial populations. The influence of some polymer properties such as molecular weight and degree of hydrolysis on the biodegradation rate and extent was investigated in the presence of either the acclimated mixed bacterial culture or its sterile filtrate. Kinetic data relevant to PVA mineralization and to the variation of PVA concentration, molecular weight, and molecular weight distribution revealed a moderate effect of the degree of hydrolysis. The molecular weight appeared to be not a limiting factor of microbial attack. Comparison of the degradation process in the presence of either bacterial cells or their culture filtrate highlighted the ability of some microbial strains to utilize polymer chains having 5–10 kD molecular weight. This result suggests the occurrence of two PVA degradation mechanisms: a random-type attack and a terminal unzipping depolymerization process of polymer chains. Copyright © 2000 John Wiley & Sons, Ltd.

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KEYWORDS: poly(vinyl alcohol); PVA; biodegradation; iodometric titration; PVA degrading microorganisms

INTRODUCTION

Poly(vinyl alcohol) (PVA) is attracting renewed interest for the production of environmentally friendly plastic materials. Disposable PVA items (mulching films, laundry bags, etc.) can reach the environment without entering any integrated system of waste treatment. Therefore, the biodegradability of PVA and PVA-based systems is a fundamental instance to be ascertained. Basic properties of these materials are very much depending upon degree of polymerization, degree of hydrolysis, distribution of hydroxyl groups, stereoregularity and crystallinity of PVA. Thus, also the assessment of the relationship between biodegradability and polymer structure is of utmost importance.

PVA is known to be a truly biodegradable synthetic polymer since the early thirties [1] and both single microorganisms [2–6] and symbiotic mixed cultures [7] able to degrade PVA have been identified. Suzuki *et al.* [2] and Watanabe *et al.* [4] proposed two similar degradation pathways by using different *Pseudomonas* strains. In both cases, the polymer is oxidized by oxidase-type enzymatic systems with formation of carbonyl groups along the polymer chain. Activated β-diketones or α-keto groups are subsequently hydrolyzed with fission of the polymer carbon backbone [2, 4, 8–10].

The overall number of PVA-degrading micro-

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organisms is rather limited in comparison with the widespread species able to degrade aliphatic poly(ester)s, both of microbial and synthetic origin such as poly(hydroxyalkanoate)s and poly(ε-caprolactone) [11]. In addition, their presence seems to be restricted to rather peculiar environments, such as PVA-polluted textile or paper mill effluents [6]. Several *Pseudomonas* strains able to degrade PVA were isolated from soil samples [2, 4], although only limited PVA biodegradation is reported to occur in soil [12–14]. Therefore, the previously reported degradation mechanisms as well as the isolated degrading species could be not exhaustive of all microbial species and biochemical patterns involved in the mineralization of PVA.

In the present paper, the degradative behavior of different PVA samples was investigated in the presence of an acclimated microbial population. Investigations aimed at the understanding of the degradation mechanism were also carried out.

EXPERIMENTAL

Polymer Samples

Commercial PVA-based blown films (EK1 and LTS) were kindly supplied by Idroplast S.p.A. (Montecatini Terme, Italy). Polymer films (40 μm thick) were based on 88% hydrolyzed PVA.

Standard 98 (PVA98), 88 (PVA88) and 72% (PVA72) hydrolyzed PVA having respectively 20.3, 44.9, and 9.5 kD average number molecular weight (M_n), were used.

Cellulose (Sigma Cell 100) and Whatman 50 filter paper were utilized as positive controls in respirometric biodegradation tests.

Microbial Source

Microbial inocula were a sample of activated sludge from the oxidative tank of a paper mill effluent treatment plant facility (Cartiere dell'Ania S.p.A. Lucca, Italy) and a sample of the activated sludge from the wastewater treatment plant of the municipality of Pisa, Italy.

A common enrichment procedure was adopted to obtain acclimated inocula. The paper mill activated sludge sample was added in 1% by weight ratio to 100 ml of a mineral medium (MM) having the following composition: 2.2 g K_2HPO_4, 0.8 g KH_2PO_4, 0.7 g $MgSO_4{\cdot}7H_2O$, 1.0 g NH_4NO_3, 0.1 g NaCl, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.05 g yeast extract (Bacto Yeast Extract, Difco), 1000 ml distilled water, pH 7.1 ± 0.1.

PVA was added to the liquid culture at 0.1–10 g/l concentration. Cultures were then incubated at 25 °C under reciprocal shaking (120 rpm). After 3 weeks, 5 ml samples of the culture were filtered on 1.2 μm cellulose acetate filter and the filtrate was transferred into the fresh MM. Enrichment was attained by repeated sequential transfer of 1.2 μm filtered cultures into MM containing increasing PVA concentrations from 0.1 up to 10 g/l.

Biodegradation Tests

Degradation tests were carried out in the presence of mixed cultures on liquid MM supplemented with a small amount of yeast extract (15 mg/l) and trace elements solution (1 ml/l) having the following composition: 0.15 g $MnSO_4{\cdot}H_2O$, 1.3 g $CaCl_2$, 0.062 g H_3BO_3, 0.024 g $CoCl_2{\cdot}6H_2O$, 0.068 g $ZnCl_2$, 100 ml 0.1 N HCl. Polymer samples (25–50 mg) were placed as water solution or milled (0.3 mm) powder in 300 ml flasks containing 100 ml of MM. Liquid cultures were incubated at room temperature on a rotatory shaker at 120 rpm.

Respirometric mineralization tests were carried out on liquid cultures in a respirometric apparatus, by using the experimental conditions already reported elsewhere [15]. The extent of mineralization was evaluated from the amount of carbon dioxide (CO_2) evolved from the test compounds diminished of that produced by the blanks and was expressed as percent of theoretical amount of CO_2, computed from the carbon content of test compounds.

Investigation of PVA degradation with culture filtrate was performed by diluting 20 ml of filter-sterilized (Nalgene 0.22 μm cellulose acetate filter) supernatant of PVA degrading cultures with 5 ml of 21 mM phosphate buffer (pH 7.2) containing 64 mg of PVA. The resulting solutions were then incubated under magnetic stirring for 14 days at 28 °C. All solutions were filter-sterilized prior to their use.

Analyses

Quantitative determination of PVA concentration in liquid cultures was carried out by spectrophotometric analysis after addition of boric acid and iodine solutions according to the procedure described by Finley [16]

The relative viscosity of aqueous polymer solutions and PVA-supplemented liquid cultures filtered on 0.2 μm cellulose acetate filters was measured at 30 °C by a Cannon-Fenske 120 viscometer. Average degree of polymerization (DP_v) and average molecular weight (M_v) of polymers were evaluated from their relative viscosity (η_{rel}) according to the procedure reported by Finch [17]

Gel permeation chromatography (GPC) analyses were performed by a mod. 600E Waters Chromatograph equipped with mod. 410 Waters RI detector, mod 486 UV detectors, and two Ultrahydrogel linear columns connected in series. Sample elution was performed at 0.8 ml/min with 0.1 M $LiNO_3$ water solution at 45 °C. Monodisperse pullulan samples were used as standards.

RESULTS AND DISCUSSION

The biodegradation behavior of different PVA-based commercial blown films and PVA powder samples was investigated in liquid culture. Rate and extent of PVA degradation were assessed by both respirometric evaluation of polymer miner-

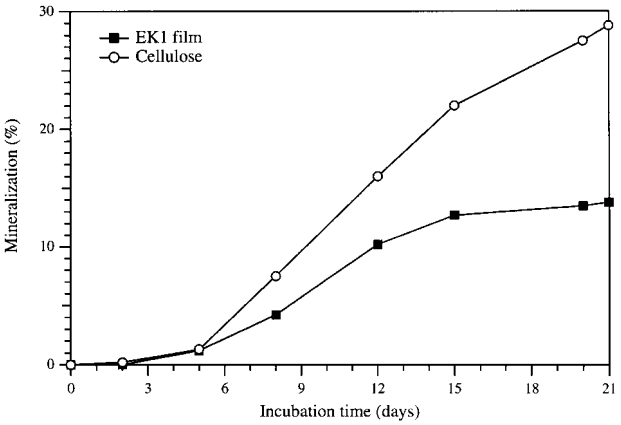

FIGURE 1. Biodegradation curves of a PVA-based commercial film and cellulose recorded in the presence of municipal sewage sludge.

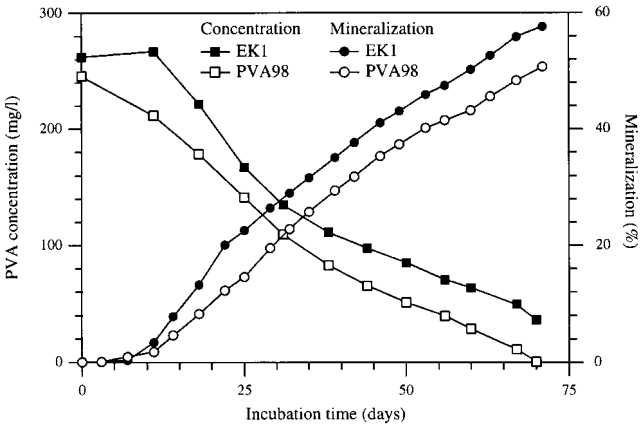

FIGURE 3. Profiles of PVA concentration and CO_2 evolution of PVA 98% and PVA-based film recorded in the presence of paper mill sewage sludge.

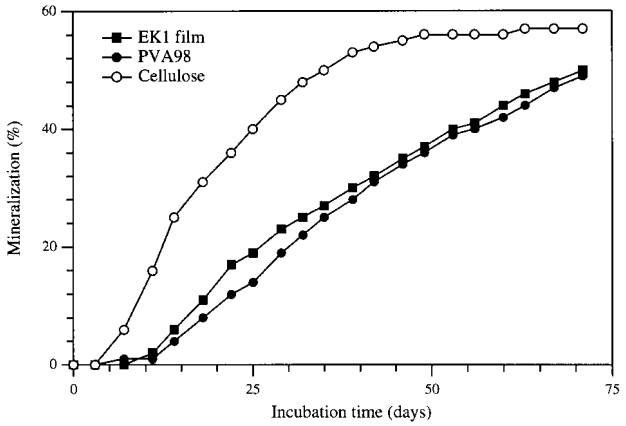

FIGURE 2. Biodegradation curves of PVA, a PVA-based commercial film, and cellulose recorded in the presence of paper mill sewage sludge.

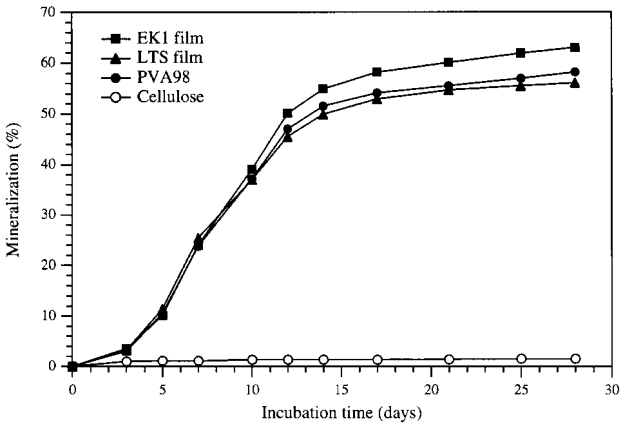

FIGURE 4. Biodegradation curves of PVA, PVA-based films, and cellulose recorded in the presence of the acclimated mixed culture.

alization and titrimetric determination of the polymer concentration in cultures inoculated with microbial populations collected from different sources.

Respirometric biodegradation tests were performed in respirometric flasks under aerobic liquid batch cultures (according to ASTM D 5338-92 and D 5209-92 tests).

Limited biodegradation values (13% after 21 days of incubation) were obtained in tests carried out on liquid cultures inoculated with municipal sewage sludge (Fig. 1). However, this result cannot be considered as conclusive due to the restricted incubation time. Indeed, a more active microbial population could be established in a longer time, as indicated by the substantial level of biodegradation of the PVA-based sample recorded after 21 days.

In the presence of the sewage sludge from a paper mill wastewater treatment plant, the extent of biodegradation of both PVA and PVA-based films reached values comparable to that of cellulose. However, this occurred only after an appreciably larger incubation time (Fig. 2). Indeed microbial strains present in the paper mill sewage sludge are particularly active because of the selective pressure exerted by the large amounts of PVA in the wastewater reaching the treatment plant of the paper factory. As expected, a close correspondence between CO_2 evolution profiles and decrease of the PVA concentration was observed (Fig. 3).

By taking into account these 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. 4, in the presence of the acclimated inoculum, rate and extent of biodegradation of PVA samples reached values much larger than those recorded in the presence of the previously tested inocula.

Acclimation of the microbial population to PVA led to substantial decrease of cellulose degrading microbial strains, as indicated by the very limited biodegradation (1.5%) of the cellulose sample.

The reported results indicate that PVA microbial attack can only occur in the presence of selected microorganisms that can be found almost exclusively in environments submitted to a continuous contamination by the polymer. In accordance, most

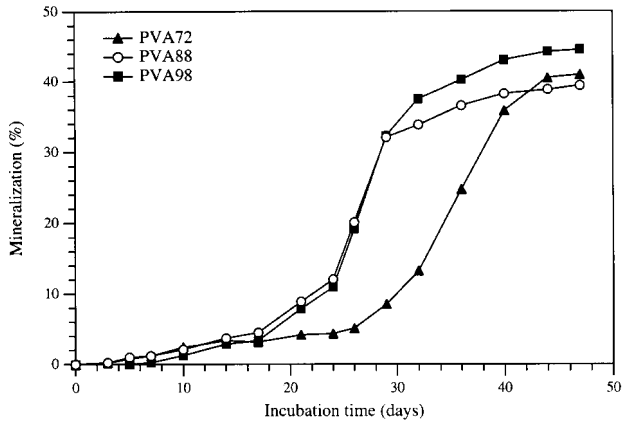

FIGURE 5. Biodegradation curves of PVA samples having different degree of hydrolysis recorded in respirometric tests.

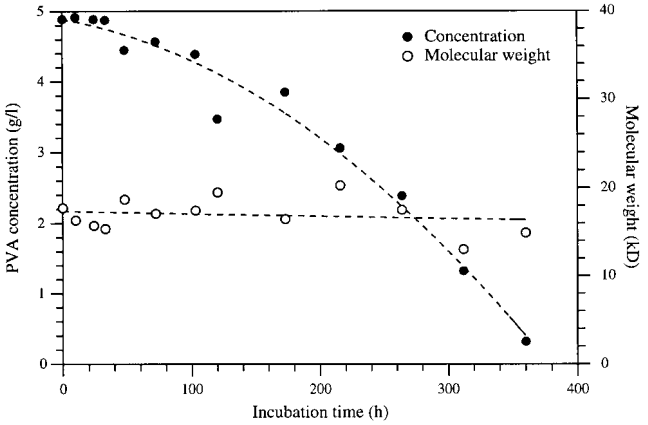

FIGURE 7. Variation of PVA concentration and viscometric average degree of polymerization (DP_v) recorded in the presence of the acclimated mixed culture.

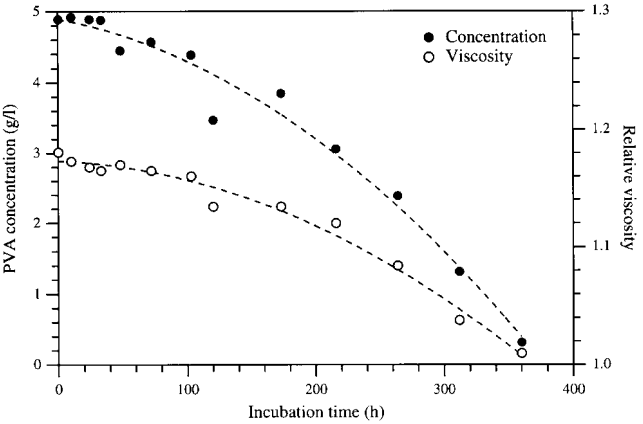

FIGURE 6. Variation of the relative viscosity and PVA concentration recorded in the presence of the acclimated mixed culture.

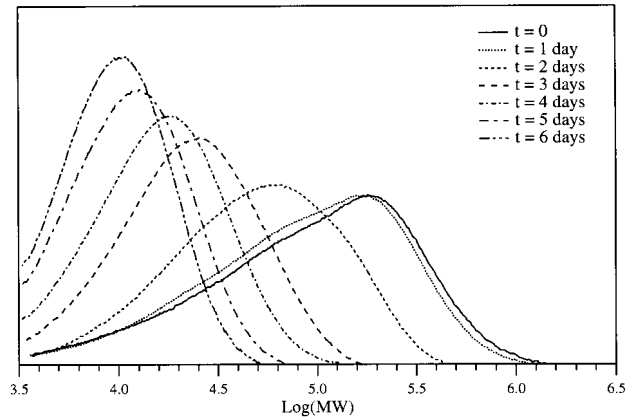

FIGURE 8. Variation of the molecular weight distribution of PVA88 recorded in the presence of the acclimated mixed culture.

of the PVA degrading microorganisms were isolated from PVA polluted environments [5, 6].

In order to investigate the degradation mechanism, PVA samples having degree of hydrolysis included between 72 and 98% were submitted to respirometric tests carried out in liquid culture in the presence of the mixed culture acclimated to PVA. After 48 days incubation, the different samples reached a degree of mineralization larger than 40% (Fig. 5). No appreciable difference in the extent of biodegradation was detected, whereas the degradation rate showed a moderate dependence on the degree of hydrolysis. This effect was particularly prominent in the case of PVA72, the most hydrophobic sample. No dependence on PVA degree of hydrolysis was observed by other authors [2] though they did not investigate samples having a degree of hydrolysis lower than 88%.

The variation of the viscometric degree of polymerization (DP_v) of PVA in liquid cultures inoculated with the enriched mixed culture was monitored during incubation time by relative viscosity measurements, as reported by Finch [17]. The variation within time of both solution relative viscosity and PVA concentration, as determined by iodometric titration (Fig. 6), allowed to evaluate the variation of the polymer average viscometric molecular weight (M_v) within time (Fig. 7). Apparently, the very slow decrease of the M_v values thus evaluated is in agreement with an unzipping-type mechanism for the degradation of polymer chains.

In order to clarify this point, the molecular weight distribution of PVA samples having different degree of hydrolysis was monitored within time by GPC in liquid cultures inoculated with the PVA acclimated mixed culture. Independent of degree of hydrolysis, the molecular weight distribution showed a progressive disappearance of the higher molecular weight fractions (Fig. 8), in accordance with a random scission of PVA chains in solution followed by assimilation by culture microorganisms. The significant decrease of PVA concentration after 5, 6, and 9 days for samples having a degree of hydrolysis of 98, 88, and 72%, respectively (Fig. 9) agrees with respirometric data.

Further attempts to clarify the degradation mechanism of PVA were carried out by using the sterile filtrate obtained from a PVA liquid culture inoculated with the acclimated mixed culture.

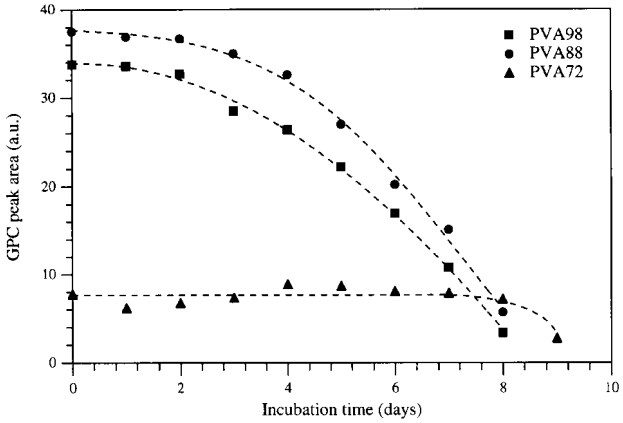

FIGURE 9. Variation of the concentration of PVA having different degree of hydrolysis recorded in the presence of the acclimated mixed culture.

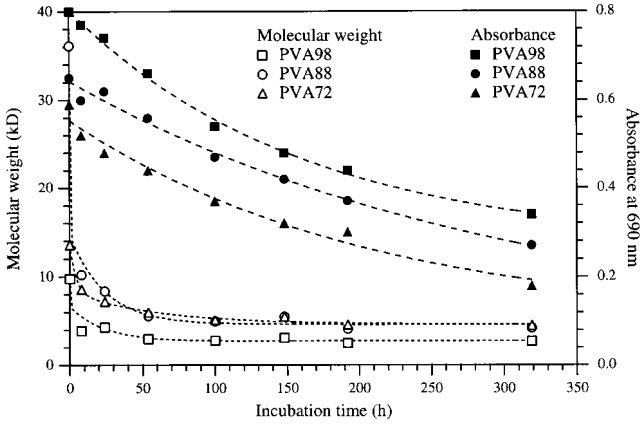

FIGURE 10. Variation of PVA average number molecular weight (M_n) and of the absorbance at 690 nm of PVA–iodine complex recorded in the presence of the sterile filtrate of the acclimated mixed culture.

These experiments were performed to assess if the PVA chain fission is effected by extracellular or endocellular enzymatic systems.

Under these conditions, the molecular weight of PVA samples having different degrees of hydrolysis quickly decreased (Fig. 10), in accordance with the presence of an extracellular enzymatic system [2, 4, 6]. Attempts to isolate and identify the enzymes are still in progress.

Comparison of the degradation process in the presence of either bacterial cells or their culture filtrate highlights the ability of some of the microbial strains present in the culture to utilize polymer chains having molecular weight as high as 5 kD or more. This behavior suggests the simultaneous occurrence of two PVA degradation mechanisms. The first one is recognizable as a random-type attack of the backbone that occurs in solution, the other one as a terminal, unzipping depolymerization process of medium-to-low molecular weight polymer chains that occurs in the microbial cells. A dual enzymatic process has been already proposed for PVA degradation [18].

Rather interestingly, the absorbance at 690 nm

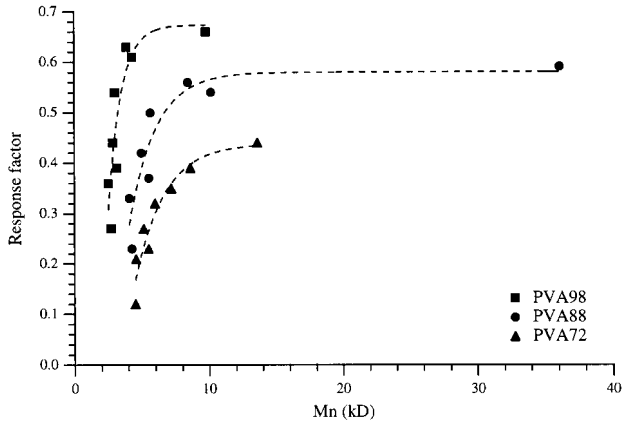

FIGURE 11. Variation of the iodometric response factor of PVA samples having different degrees of hydrolysis with the average number molecular weight (M_n).

of the iodine complex of the PVA solutions paralleled the molecular weight decrease, apparently suggesting a decrease of the PVA concentration (Fig. 10). However, this hypothesis was ruled out by the constant PVA peak intensity in the corresponding GPC chromatograms. Indeed, the PVA content should remain constant due to the absence of microbial cells in the solutions.

The observed variation of the solution absorbance at 690 nm must be therefore attributed to a variation of the binding constant of the PVA–iodine complex. A plot of the response factor, that relates the solution absorbance at 690 nm to the PVA concentration, evidenced a significant dependence on the molecular weight, particularly at lower M_n values (Fig. 11), in agreement with previous reports [19]. A large influence of the PVA degree of hydrolysis was also detected.

The apparent stability of DP_v within time previously observed in inoculated cultures must be therefore attributed to the underrating of the PVA concentration consequent to the drop of the response factor.

CONCLUSIONS

Based on the results obtained in the present investigation the following conclusive points can be highlighted:

(1) PVA degrading microorganisms seem to be mainly confined to PVA steadily contaminated environments, that is a selective pressure (acclimation) is required to differentiate active degrading microorganisms. Degradation rate and extent resulted comparable for pure PVA and PVA-based commercial films that contained 10–20% of additives. However, the degradation rate showed a moderate dependence on the degree of hydrolysis, particularly in the case of PVA72, the least hydrophilic sample. In all cases, a progressive disappearance of the higher molecular weight fractions

was observed, in accordance with a random fission of PVA chains accompanied by assimilation of the medium-low molecular weight fractions.

(2) The fast decrease of the molecular weight of PVA incubated with the sterile filtrate of PVA-degrading culture demonstrated that the polymer degradation is effected by an extracellular enzymatic system. Isolation and attempts to identify the enzymes are in progress.

(3) The absorbance at 690 nm of the iodine complex of PVA solutions evidenced a significant dependence of the iodometric response factor on both molecular weight and degree of hydrolysis. This result is of paramount relevance, as the variation of the absorbance of PVA–iodine complex is very often used to evaluate PVA degradation.

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