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Biodegradation of poly (vinyl alcohol) based materials

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Abstract

Poly(vinyl alcohol) (PVA) is recognized as one of the very few vinyl polymers soluble in water also susceptible of ultimate biodegradation in the presence of suitably acclimated microorganisms. Accordingly, increasing attention is devoted to the preparation of environmentally compatible PVA-based materials for a wide range of applications. The present article is aimed at providing a survey of the available information on the environmental fate of PVA and PVA-based materials. Literature data and recent advances on the biochemistry and microbial physiology of PVA biodegradation and on the influence of environmental conditions are discussed along with the biodegradation processes of other water-soluble materials. The biodegradation behaviors of several PVA-based materials including blends, composites and copolymers are also reviewed and discussed.

Keywords: Water-soluble; Poly(vinyl alcohol); Poly(vinyl acetate); Biodegradation; Blends; Composites

Contents

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1. Introduction

1.1. Generalities on water-soluble biodegradable polymeric materials

Water-soluble polymeric materials constitute a diverse class of macromolecules, and may be classified according to their source:

1. *Natural origin*, better known as biopolymers (polysaccharides, proteins, polypeptides polynucleotides, polyphosphates, and polysilicates), that direct and modulate the complex functional processes fundamental to living organisms in terrestial and aquatic environments, and that may also assume key roles as irreplaceable structural materials in manufactured products.
2. *Semisynthetic origin*, comprising chemically modified natural polymers (formerly known as artificial polymers). Most natural polymers must be submitted to temporary or permanent structural and/or functional group manipulations in order to allow processing and conversion to useful items.
3. *Synthetic origin*, based on feed-stocks derived from fossil fuel and renewable resources. The latter are gaining increased attention for industrial development, driven by the principles of sustainability.

Independent of their origin, thanks to their solubility–swellability in water, an inexpensive and harmless solvent, these polymeric materials cover a wide range of applications in different industrial–commercial segments. Among others, applications include food, textiles, leather, coatings, paper, healthcare, oil recovery, waste water, treatment biomedical, and pharmaceutical fields. These represent an enormous commercial impact, and interestingly have minimal environmental concern. The specific structural characteristics of the solvated macromolecular backbone, which derive from the nature connectivity, and sequencing (primary structure) of monomeric units, as well as the configurational and conformational assembly of macromolecules in single (secondary structure) and multiple arrays (tertiary–quaternary structure), affect the solution properties and ultimately

Table 1
Categorization of water-soluble/water-swellable synthetic polymers

Non ionic	Cationic	Anionic
Poly(acrylamide)	Poly(diallyldimethyl ammonium chloride)	Poly(acrylic acid)
Poly(*N*-isopropylacrylamide)	Poly(diallyldiethyl ammonium chloride)	Poly(methacrylic acid)
Poly(2-hydroxyethyl methacrylate)	Poly(diethylamino ethyl methacrylate)	Poly(maleic acid)
Poly(*N*-vinyl pyrrolidinone)	Poly(dimethamino ethyl methacrylate)	Poly(fumaric acid)
Poly(ethylene glycol)	Poly(methacryloyloxyethyltri methylammonium sulfate)	Poly(vinylsulfonic acid)
Poly(ethylene oxide)	Poly(methacryloyloxyethyl trimetyl ammonium bromide)	Poly(4-vinylbenzoic acid)
Poly(vinyl alcohol)	Poly[(3-methacrylamide)propyl trimethyl] ammonium chloride	Poly[*N*-(2-methylpropyl sulfonic)acrylamide] Poly[*N*-(2-methylpropyl carbonyloxy)acrylamide]

the performance of water-soluble polymeric materials.

The relative balance between the functional groups (structure, relative concentration, and position in the repeating unit) and the overall hydrocarbon (hydrophobic) content of the repeating units, as well as their arrangement in homopolymer or copolymer structures represent a key aspect in affecting water solubility.

The functional groups present in the building blocks of water-soluble polymers can be neutral (non-ionic polymers) or charged (anionic, cationic, zwitter ionic, amphoteric polymers).

Typical examples of the various classes of water-soluble/swellable to synthetic polymers are listed in Table 1.

Amphoteric polymers not included in Table 1 comprise

(a) Single monomeric units bearing opposite charges (polybetains).
(b) Monomeric units of different structure with anionic and cationic character, respectively, (polyampholytes).
(c) Macromolecular chains with cationic and anionic repeating units (template polymers).

This contribution is mainly focused on the biodegradation of poly(vinyl alcohol) and poly(vinyl alcohol)-based materials under different environmental conditions. These materials constitute a major area in the field of water-soluble/swellable polymeric materials of synthetic origin. Further comment on naturally occurring water-soluble polymers or on their derivative counterparts are not included in these introductory remarks, aside from the listing of the major classes of natural polymers, and their major application fields, in Table 2. The reader may refer to books and monographic chapters cited in Refs. [1–6] for a more comprehensive discussion of a specific topic.

1.2. Biodegradation of water-soluble polymeric materials

Among the various synthetic/semisynthetic polymeric materials, water-soluble polymers are one of the major organic pollution sources in streams, river, and marine ecosystems. In fact, once dispersed in aqueous systems, they can directly interfere with the life cycle of aquatic organisms due to their direct toxicity, and by limiting and altering gas exchanges with often serious alterations to the complex aquatic ecosystems. Investigation of the environmental fate of these materials deserves a considerable level of attention. In particular, the potential tracking and removal of these materials from the environment using natural, biological

Table 2
Water-soluble, water-swellable natural and artificial polymers

Natural and artificial polymers			
Organic		Inorganic	
Class	Field of applications	Class	Field of applications
Polynucleotides (DNA, RNAs)	Replication processes Protein synthesis	Polyphosphates	Detergency Bioengineering
Proteins/peptides	Transport functions Enzymatic activity Gene expression Hormonal regulation Detergency Biotech processes Biomedicine	Polysilicates	Glasses Glass fibers
Polysaccharides	Paper industry Textile industry Water treatment Packaging Coating segment Biomedicine Pharmacy		

processes mediated by microorganisms and their enzymes (e.g. biodegradation) becomes fundamentally important, as does studying the nature and biological activity of intermediate metabolites and the final products deriving from the biodegradation processes.

Investigation of the potential biodegradability of water-soluble polymers should be performed in aqueous media, based on their most probable post consumer disposal environment. Therefore, degradation tests are carried out under conditions aimed at mimicking the natural environments of uncontrolled disposal as well as activated sludge systems.

Many water-soluble polymers, such as PVA, are widely used in the preparation of plastic items and as additives in the paper, wood, tannery, paint, textile, and agro industries. When used in this fashion, these polymers can also be disposed of in terrestrial ecosystems. Consequently, the ultimate fate of many water-soluble polymers must also be investigated under conditions aimed at reproducing natural soils and compost environments.

The most important water-soluble polymers of potential environmental concern are collected in Table 3.

1.2.1. Water-soluble polymers with hydrocarbon backbone

Examples in this class of water-soluble polymeric materials include poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMA), poly(acrylamide) (PAAm) and poly(vinylpyrrolidone) (PVP).

Table 3
Major water-soluble polymeric materials of potential environmental concern

Class	Type	Acronym
Poly(carboxylate)s	poly(malic acid)	PMLA
	Poly(metacrylic acid)	PMA
	Poly(aspartic acid)	PAsA
Poly(acrylics)	Poly(acrylic acid)	PAA
	Poly(acrylamide)	PAAm
Poly(ether)s	Poly(ethylene glycol)	PEG
	Poly(propylene glycol)	PPG
Poly(glutamic acid)		PGlA
Poly(hydroxylate)s	Poly(vinyl alcohol)	PVA

1.2.1.1. Poly(acrylic acid) and poly(methacrylic acid). A typical example is poly(acrylic acid) (PAA), largely used in the form of a sodium salt as a builder in the formulation of detergents for industrial and domestic applications. In recent years, a wide variety of homopolymers and copolymers containing carboxyl moieties in the side chain were synthesized in order to replace the euthrophycation-inducing poly(phosphate)s used in many detergency products [7].

Both homopolymers and copolymers of acrylic acid exhibit a high resistance to biological degradation. Consequently, these polymers can effectively accumulate in aquatic ecosystems [8], although they are considered substantially non-toxic. Nevertheless, Rittman et al. [9] reported the biological attack of high molecular weight PAA by methanogenic (e.g. anaerobic) bacteria, whereas Matsumura et al. [10] and Haiyashi et al. [11] described the microbial assimilation of PAA oligomeric fractions.

More recently, a biodegradation mechanism for PAA oligomers was proposed as based on the structure of the degradation products deriving from attack by selected microorganisms [12] on the low molar mass model compound 1,3,5-tricarboxylpentane. An oxidative exocleavage process starting from the terminal units with the release of carbon dioxide and acetic acid was suggested [12]. Analogous investigations on the potential biodegradation of oligomeric fractions of poly(methacrylic acid) evidenced the ineffectiveness of the enzymatic reactions occurring for PAA oligomers. Very likely, this behavior is connected to the steric hindrance of methyl groups that inhibit the initial oxidation step leading to double bond formation [11].

In any case, the biodegradation of PAA seems to be restricted to polymer fractions having molecular weight less than 3 kD, as suggested by experiments recording the mineralization of acrylic acid oligomers and polymers in the presence of sludge formerly acclimated by continuous exposure to low molecular weight PAA [13]. Indeed, efficient mineralization in a reasonable timeframe was recorded only for PAA with molecular weights lower than 1000 D. These findings are in accordance with the generally observed degradation rate of water-soluble synthetic polymers having a hydrocarbon backbone, whose biological attack is very limited until their molecular weight drops below 1000 D. This general trend holds true, with the noticeable exception of PVA, for many water-soluble materials, including poly(*N*-vinyl-2-pyrrolidinone), poly(acrylamide)s, and several other poly(carboxylate)s [14].

In order to overcome this limitation, the introduction of functional groups susceptible of specific enzymatic attack constitutes an effective strategy to improve the environmental compatibility of many water-soluble materials. For instance, significant biodegradation levels were recorded for modified poly(carboxylate)s containing ether or ester linkages in the main chain, such as poly(sodium epoxysuccinate) (PES) and poly(sodium glycidate) (PG) [10,15]. In particular, a biodegradation mechanism based on the sequential release of sodium hydrogen carbonate leaving a poly(ether) backbone susceptible of further biodegradation was proposed for PES [10].

1.2.2. Water-soluble polymers containing heteroatoms in the main chain

1.2.2.1. Poly(aspartic acid). Poly(aspartic acid) (PAsA) has recently attracted a great deal of attention as a replacement for the poorly biodegradable poly(carboxylate) builders in detergents and in the wastewater treatment of paper and paint industries.

PAsA, as well as other poly(aminoacid)s, are currently synthesized by polycondensation of the appropriate *N*-carboxyanhydrides, PAsA can also be obtained by the base-catalyzed hydrolysis of poly(succinimide) produced by thermal condensation of L-aspartic acid [16]. Three functional groups allow the monomeric units of PAsA to be chained together by α- or β-peptide link, as well as by imide-like bonds.

The synthetic procedure used to produce this water-soluble polymer influences both the biodegradation rate and the extent of PAsA in the presence of activated sludge microorganisms [17,18]. The precise biodegradation pathway of PAsA is not yet fully established, however, the responsibility of particular exo or endo peptidases has been suggested [18]. The activity of these enzymes is expected to be unique because PAsA does not possess a typical protein-like structure. However, investigations into the biodegradability of PAsA show similarity to that of synthetic polycondensation polymers. The presence of functional hetero linkages in PAsA's backbone,

resembling those occurring in natural polymers, seems to allow for a relatively high propensity to biological attacks, much like the synthetic polycondensation polymers. The same consideration can hold true for the superior homologue poly(glutamic acid).

1.2.2.2. Poly(ethylene glycol)/poly(ethylene oxide). Traditionally, poly(ether)s constitute one of the main classes of water-soluble synthetic polymers. Ether bonds, whose occurrence in nature is restricted almost exclusively to the structural units of lignin, link the aliphatic units of these materials.

Since the first investigations by Fincher and Payne [19], several studies were carried out on the environmental fate of poly(ethylene glycol) (PEG), the most synthesized and utilized poly(ether). In this connection, several microorganisms able to use PEG as their sole carbon source were isolated from soil [20], water, and wastewater treatment plants [21,22]. Surprisingly the main PEG-degrading microorganisms are Gram-negative aerobic bacteria, although in nature the cleavage of lignin alkyl ethers is mediated predominantly by fungal or actynomycete species.

It was repeatedly ascertained that PEG molecular weight strongly affects its biodegradation propensity. In most cases microbial species capable of assimilating only PEG with molecular weights lower than 1000 were isolated [19,23,24], whereas high molecular weight (10 kD) PEG samples were shown to be substantially recalcitrant to biological attack [22,25]. However, significant biodegradation of samples with molecular weights up to 20 kD have also been reported [26].

The biochemistry of PEG degradation has been extensively investigated. In particular, different PEG-degrading enzymes were found to be associated to the cell membrane, most likely exposed to the periplasmic space of the cell wall of Gram-negative bacteria [21,27]. The intracellular location of PEG-degrading enzymes may explain the biodegradation recalcitrance of high molecular weight PEGs, whose accessibility to the enzyme active sites is hindered by their long random-coiled chains [28].

The cleavage of PEG ether links occurs through oxidative or non-oxidative pathways. The oxidative degradation of PEG was elegantly established by Kawai and Yamanaka [26,33]. Three different membrane-bound enzymes were recognized as *PEG-dehydrogenase*, *PEG-aldehyde-dehydrogenase* and *PEG-carboxylate-dehydrogenase*, respectively. It was suggested that these enzymes act sequentially to produce terminal carbonyl and carboxyl groups from the terminal units of poly(ether) chains, followed by the release of C2 units as glioxylic acid. In the latter case, the formation of acetaldehyde via the enzymatic cleavage of a hemiacetal group created through the sequential migration of the terminal hydroxyl group to the α-position was proposed [29–32].

Independent of the nature of the PEG biodegradation mechanism, it was clearly established that the cleavage of macromolecules proceeds through the sequential attack of terminal units via an exo-type mechanism. This mechanism is supported by the inability of PEG-degrading enzymes to attack alkyl-substituted terminal hydroxyl groups [24,27]. Further investigations, demonstrated that PEG monomethyl ether with M_w 550 is quickly degraded by a *Pseudomonas* strain isolated from river water [34], whereas PEG dimethyl ether with M_w 1000 was not degraded at all, in spite of the similar low molecular weight. These results clearly confirm that the presence of at least one free hydroxyl end group is required for the enzymatic attack of PEG.

1.2.2.3. Poly(propylene glycol). Poly(propylene glycol) (PPG), the other poly(ether) of widespread use, is less water-soluble than PEG and it is currently used in the formulation of lubricants, in the cosmetic industry, in the production of inks, and paints, and in the synthesis of non-ionic surfactants. The water solubility of PPG is heavily affected by its molecular weight, the upper limit being a molecular weight of about 1000. It was suggested that the biochemistry of PPG undergoing microbial attack is similar to the oxidative biodegradation of PEG and it is mediated by a specific dehydrogenase associated to the cell membrane of Gram-negative bacteria [35].

Generally, the biodegradation propensity of the most utilized water-soluble polymeric materials appears to be affected by different structural parameters, such as the molecular weight and nature of the main chain. In fact, it has been established that molecular weight, and consequently molecular dimensions, plays a crucial role in affecting the potential biodegradation of fully hydrocarbon

backbone polymers, namely poly(carboxylate)s, thus demonstrating the occurrence of significant microbial attack only in the presence of oligomeric fractions at fairly low degree of polymerization (DPn 5–10).

However, molecular weight does not seem an insurmountable limiting factor for the biodegradation of many water-soluble polymers containing heteroatoms in the backbone, such as poly(ether)s (mainly PEG) or other functional polymers like polyaspartic and polyglutamic acids, at least for fractions up to 20 kD. In this last case, the hydrolytic cleavage of functional groups along the macromolecule may represent the initial step of the biological attack. However, enzymatic attack of terminal units, followed by an unzipping-type degradation process, constitutes a common feature in the biodegradation pathway of many water-soluble polymers having either a hydrocarbon backbone or containing heteroatoms in the main chain. This point further underscores the limiting effect induced by the macromolecular dimensions in the presence of intracellular degrading enzymes.

1.3. Production of poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is produced on the order of several hundred kton/yr all over the world (Table 4), making it the largest volume water-soluble polymer produced today. PVA is often a convenient product in integrated production cycles of petrochemical industries.

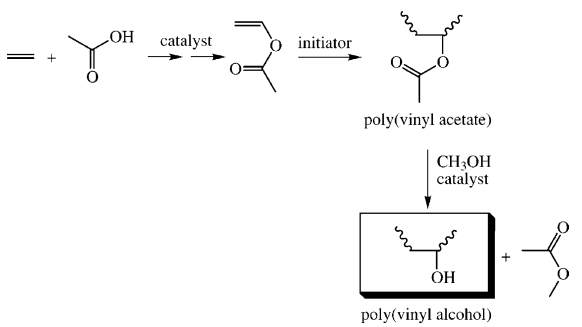
PVA is not produced by direct polymerization of the corresponding monomer, since vinyl alcohol tends to convert spontaneously into the enol form of acetaldehyde, driven by thermodynamic reasons and with extremely limited kinetic control [36]. PVA is attained instead from the parent homopolymer poly(vinyl acetate) (PVAc).

The polymerization of vinyl acetate occurs via a free-radical mechanism, usually in an alcoholic solution (methanol, ethanol) [37] although for some specific applications a suspension polymerization technique can be used [38].

PVA is produced on an industrial scale by hydrolysis (methanolysis) of PVAc, often in a one pot reactor. Different grades of PVA are obtained depending upon the degree of hydrolysis (HD). Polymerization reactions can be carried out in batch or in continuous processes, the latter being used most for large-scale productions. In the continuous industrial process, the free-radical polymerization of vinyl acetate is followed by alkaline alcoholysis of PVAc (Scheme 1). The molecular weight of PVAc is usually

Table 4
World production of PVA

Producers	Trade Mark
Clariant GmbH, Germany www.cepd.clarinet.com	Mowiol
Erkol S.A., Spain www.erkol.com	Erkol
Novacky, Slovakia www.nchz.sk	Sloviol
Vinavil SpA, Italy www.mapei.it/it /vinavil/home.htm	Polyvinol
DuPont, USA www.dupont.com/ industrial-polymers/elvanol/ index.html	Elvanol
Celanep, USA www.celanesechemicals.com	Cevol
Air Products, USA	Airvol
Kuraray Co. Ltd, Japan www.kuraray.co.jp/en	Kuraray Poval
Unitika Ltd, Japan www.unitika.co.jp/e/ home_e2.htm	Unitika Poval
Nippon Gohsei—The Nippon Synthetic Chemical Industry Co. Ltd, Japan www.nippongohsei.com/ gohsenol/index.htm	Gohsenol
Ghang Chun, Taiwan	–
Hap Heng, Hong Kong, China	Hapol



Scheme 1. Schematic representation of reaction sequence used in the industrial production of PVA.

controlled by establishing the appropriate residence time in the polymerization reactor, vinyl acetate feed rate, solvent (methanol) amount, radical initiator concentration, and polymerization temperature. The hydrolysis degree of PVAc is also controlled by residence time, catalyst (base) concentration, and temperature.

PVA grades with HDs ranging between 70 and 99% are commercially available for applications that are somewhat bound to the degree of polymerization, melting point, and rate of dissolution in water.

1.4. Applications of poly(vinyl alcohol)

Plastic items based on PVA are mainly obtained using casting techniques. However, the increasing interest in the production of disposable plastic articles based on PVA, and in particular PVA films, stimulated the development of melt processing technology in order to overcome the less cost-effective casting technologies. The main difficulty in PVA thermal extrusion processing is the close proximity of its melting point and decomposition temperature.

The thermal degradation of PVA usually starts at about 150 °C or above, depending upon the PVA grade (HD and pH). The degradation process gives rise to the release of water from the polymer matrix, accompanied by the formation of volatile degradation products, such as acetic acid in partially acetylated samples. In turn, the acid can catalyze further degradation and polymer discoloration due to the formation of polyene structures [39–41]. The release of water molecules from the polymer chains is subjected to a mass action equilibrium, which can be shifted toward the thermoplastic character of PVA by the addition of water and the application of pressure [42]. Consequently, the thermoplastic processing of PVA requires its plasticization with relatively large amounts of water and organic plasticizers before extrusion. Several suitable PVA plasticizers capable of enhancing its thermal stability, such as glycerol, ethylene glycol and its dimer and trimer, amine alcohols, and polyvalent hydroxyl compounds have been proposed and utilized in industrial processes [43].

For several applications, PVA films can be obtained by an inexpensive melt extrusion of mixtures of PVA, solid polyols, water and other plasticizers [44], followed by a melt blow extrusion procedure. This manufacturing process does not require special conditions; both impulse welding and hot bar techniques as well as other traditional welding techniques can be used. Varying the hydrolysis degree of the PVA and the extrusion conditions can produce films with temperature-dependent water solubility.

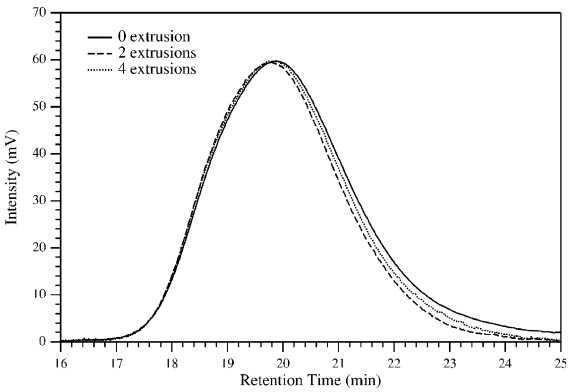


Fig. 1. SEC chromatograms of a PVA-based blown film recycled throughout four-fold extrusions [45].

From an industrial standpoint, this technology allows for effective recycling of the scraps generated during film extrusion. No appreciable variation of molecular weight and molecular weight distribution was detected in four-fold reprocessing of the same PVA batch (Fig. 1) [45].

2. Biodegradation of poly(vinyl alcohol)

2.1. General concepts on mechanisms

The environmental fate of PVA, one of the very few vinyl polymers soluble in water, was investigated due mainly to its large utilization in textile and paper industries, which generate considerable amounts of PVA-containing wastewaters. In 1936, it was observed that PVA sustained ultimate biodegradation when submitted to the action of *Fusarium lini*, a phytopathogenic fungus, producing carbon dioxide and water as a result of extracellular attack by a 'dehydratase' [46].

Studies carried out in the presence of domestic or non-acclimated sewage sludge highlighted that

the long-term acclimation of the microbial populations is a stringent parameter for an efficient removal of the polymer [47,48]. In fact, negligible biodegradation was recorded in the presence of non-acclimated domestic sludge microorganisms. Respirometric assays demonstrated that PVA could be completely mineralized in the presence of suitable microorganisms under appropriate incubation conditions [49].

It was later observed that specific microbial strains were able to induce a rapid decrease of the viscosity of aqueous culture media containing PVA. It was therefore supposed that at least the initial microbial attack should be consistent with a random endocleavage of the polymer chains. At the same time, the occurrence of oxidative reactions of the tertiary carbon atoms of PVA chain, leading to the formation of hydrolyzable β-hydroxylketone and 1,3-diketone groups along the polymer backbone, was established. These reactions were catalyzed by specific oxidases and dehydrogenases that were isolated mainly as extracellular proteins from the culture supernatant of different bacterial species. Accordingly, it was suggested that the initial attack of PVA chains occurred extracellularly in water solution [50,51].

The first microorganisms capable of assimilating PVA as their sole carbon source were isolated from soil samples and identified as *Pseudomonas* species [50–52]. Further studies evidenced that a similar endocleavage mechanism was active in the degradation of PVA by different bacterial species. Along with the various *Pseudomonas* species, other PVA degrading aerobic bacteria, such as *Alcaligenes* and *Bacillus*, were isolated from PVA-contaminated environments.

2.2. Biochemistry and physiology of poly(vinyl alcohol) biodegradation

2.2.1. Biodegradation of poly(vinyl alcohol) by single bacterial strains

Detailed investigations on the biochemical pathways of PVA degradation by microorganisms started in the early 1970s. Watanabe and co-workers [50,52,53] carried out one of the key studies aimed at the interpretation of the enzymatic reactions in the degradation of high molecular weight PVA. An extracellular PVA-degrading enzyme was purified from the culture supernatant of a *Pseudomonas* strain isolated from soil. A single 30 kD colored protein was found to reduce dramatically the viscosity of PVA buffered solutions. This enzyme was recognized to be a *secondary alcohol oxidase* (SAO), based on its O_2 consumption and H_2O_2 production during the reaction, its colored nature, and its ability to degrade some low molecular weight secondary alcohols [53].

The wide distribution of low molecular weight PVA degradation products also suggested the random cleavage of C–C bonds along the polymer chains. This cleavage formed carboxylic acids indicated by a pH drop in the reaction mixture. The colored nature of the enzymatic protein suggested the presence of a co-factor, closely associated to the apoprotein. This co-factor was found to be non-metallic, and showed inactivity towards 1,2-glycol bonds. Finally, a molecular weight of the substrate corresponding at least to 4-heptanol was required. These results indicated that the purified SAO was highly specific for PVA 1,3-hydroxyl groups, and that a definite molecular arrangement adjacent to the substrate was needed for an efficient exploitation of the enzyme catalytic activity.

Other studies suggested that a second enzyme performed the chain cleavage of oxidized PVA in the culture broth of the isolated *Pseudomonas* species [54]. In these studies, the carbon chain of PVA was cleaved by two consecutive reactions. The first step was catalyzed by SAO, leading to the formation of β-hydroxyketone groups that were then cleaved by a specific hydrolase with the formation of carboxyl- and methyl-terminated degradation products.

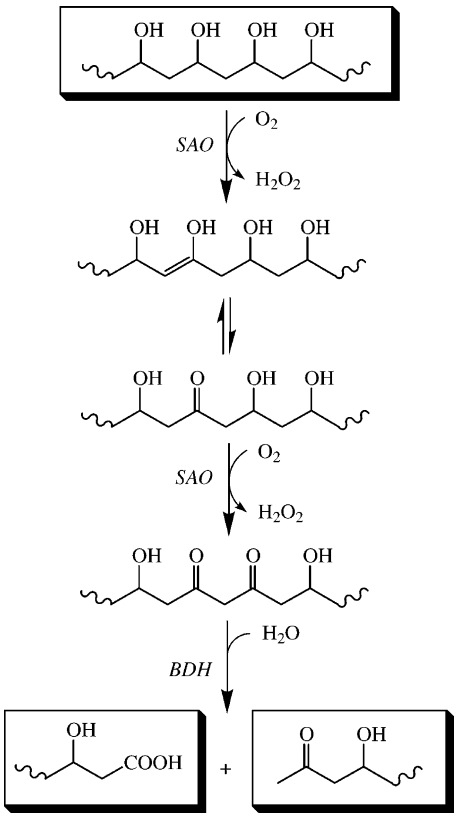
Isolation of enzymes able to degrade oxidized PVA from the culture broth of three different soil bacteria, including *Pseudomonas* species and *Brevibacterium incertum*, confirmed the active participation of a hydrolase in the PVA biodegradation [55]. The hydrolytic reaction did not required oxygen or other electron acceptors, and promoted a fast drop in the viscosity of SAO-oxidized PVA solutions, with the reduction of pH and an increase of the content of carboxylic acid compounds.

SAO from *Pseudomonas* species catalyzed the oxidation of vinyl alcohol oligomers with molecular weights in the 220–1500 range, as well as several aliphatic β-ketols, such as 5-hydroxy-3-pentanone, 4-hydroxy-2-nonanone, 3-hydroxy-5-nonanone, 6-hydroxy-4-nonanone, 7-hydroxy-5-dodecanone, and

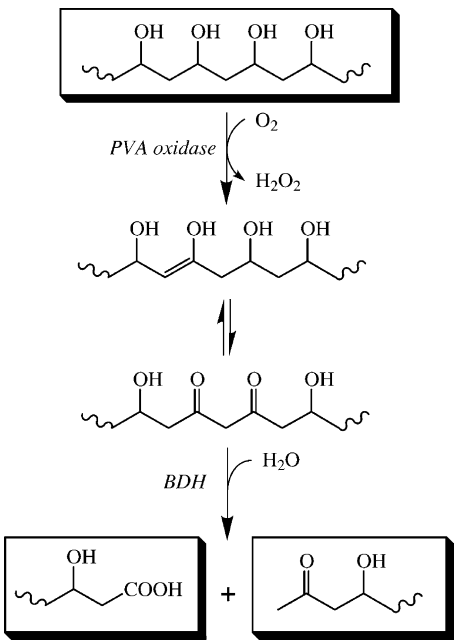
8-hydroxy-6-tridecanone [52]. The resulting β-diketones underwent hydrolytic cleavage by a specific *β-diketone hydrolase* produced and excreted in the culture supernatant by the same bacterium. This enzyme did not cleave cyclic β-diketones or β-diketone moieties next to carboxylic groups, such as those found in acetopyruvate, nor did it hydrolyze monoketones or β-ketols. Moreover, a β-diketone chain length of more than five carbon atoms was required for a significant activity of the hydrolase.

In particular, 4,6-nonanediol, a low molecular weight model compound of PVA, was oxidized to 6-hydroxy-4-nonanone, and then further oxidized to the corresponding β-diketone. The β-diketone was hydrolyzed to 2-pentanone and *n*-butyric acid by the β-diketone hydrolase, as evidenced by glc-mass spectrometry [52]. When the carbon chains at each side of the β-diketone structure had different lengths, hydrolytic cleavage took place at the shorter side, thus producing a methyl ketone and a carboxylic acid from the longer and the shorter segment, respectively [52]. Based on these results, a PVA biodegradation pathway mediated by the two enzymes produced by the isolated *Pseudomonas* species was suggested (Scheme 2).

Along with the studies of Watanabe and co-workers, Suzuki's group carried out an extensive investigation on PVA biodegradation. A *Pseudomonas borealis* O3 strain able to grow using PVA as its sole carbon source was isolated from soil samples [51]. The bacterial cells secreted an inducible PVA-degrading enzymatic system, whose oxidase nature was substantiated by both the oxygen demand and the concomitant H_2O_2 production promoted by the isolated proteins in the presence of the vinyl polymer. An endo-type random cleavage of PVA chains was also envisaged [51]. The characterization of enzymatic degradation products led to the identification of carboxyl and methyl-ketone groups in the metabolites [56]. A PVA degradation mechanism operating through the formation of 1,3-diketones was proposed based on the equivalence between the number of carboxyl groups and methyl ketones (Scheme 3).



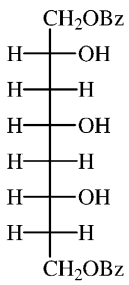
Scheme 2. Biodegradation pathway of PVA as mediated by secondary alcohol oxidase (SAO) and β-diketone hydrolase (BDH) [52].



Scheme 3. Biodegradation pathway of PVA as mediated by a specific PVA-oxidase and β-diketone hydrolase (BDH) [56].

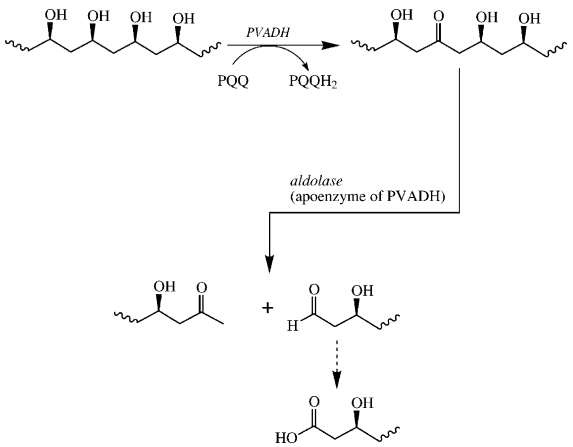
The chemically unstable 1,3-diketone moieties were supposed also to be spontaneously hydrolyzed without mediation by a specific hydrolase, yielding a carboxylic acid and a methyl ketone as terminal groups on the PVA-cleaved chains [56]. This mechanism turned out to be quite similar to that proposed by Watanabe et al. [52].

Further evidence of the biochemical route leading to the chain cleavage of oxidized PVA was recently reported by Matsumura et al. [57]. Degradation studies were carried out by using *Alcaligenes faecalis* strain KK314, previously isolated from the enrichment of river water samples [58]. A specific *PVA-dehydrogenase* (PVADH) was isolated and purified from cell-free extracts using an isotactic-type vinyl alcohol trimer as a model substrate with pyrroloquinoline quinone (PQQ) and $CaCl_2$ as essential co-factors. The isolated enzyme catalyzed the formation of β-hydroxyketone moieties along the polymer chains, without any further oxidation to β-diketone. The same authors isolated a specific β-hydroxyketone cleaving enzyme from the cell-free culture extract by using (2S, 4S, 6S)-1,8-bis(benzyloxy)-2,4,6-octanetriol as a screening substrate.



2S,4S,6S-1,8-bis(benzyloxy)-2,4,6-octanetriol

The model compound was cleaved by the enzyme to give a methyl ketone and an aldehyde that readily dehydrogenated to the corresponding carboxylic acid via an aldolase-type reaction, regardless of PQQ and $CaCl_2$ addition [57]. However, the enzymatic protein that was supposed to perform the scission of β-hydroxyketones was also found to catalyze the dehydrogenation of PVA in the presence of PQQ and $CaCl_2$. Identical molecular weights and gel filtration profiles were recorded for the two isolated enzymes, thus suggesting that they were created by a single protein containing both dehydrogenase and aldolase active sites. In conclusion, the isolated protein was the PVADH apo-enzyme, which requires PQQ as coenzyme [57]. The PVA biodegradation mechanism reported in Scheme 4 was proposed from these results [57].



Scheme 4. Biodegradation pathway of PVA as mediated by PVA-dehydrogenase from *Alcaligenes faecalis* KK314 by an aldolase-type reaction [57].

Based on previous studies regarding the isolation of separated proteins active in PVA dehydrogenation, PVA oxidation, and β-diketone hydrolysis, the PVA-degrading enzyme from *A. faecalis* seems to have an unique catalytic activity for both the dehydrogenation reaction in the presence of PQQ co-factor, and the subsequent aldolase-like dehydrogenation reaction.

Another PQQ-dependent PVA-dehydrogenase was recently isolated from the cell-free extract of *Pseudomonas* sp. strain 113P3 capable of growing with PVA as its sole carbon source [59]. In that case, no PVADH activity was detected in the supernatant of the bacterial culture as suggested by the absence of PVA low molecular weight fractions in the culture broth. It was therefore assumed that high molecular weight PVA (DPn 1700) could be directly incorporated into the bacterial cells and then degraded by the specific dehydrogenase. This hypothesis represents a new feature of the physiological pathway for the microbial assimilation of water-soluble PVA. Indeed, it is generally assumed that molecular size limits the cellular permeability and hence the metabolization of polymer chains, as reported in the case of poly(ethylene oxide) [28,60]. On the other hand, high molecular weight PEGs (DPn 450) are degraded by an intracellular (periplasmic) PEG-dehydrogenase.

Interestingly, this enzyme binds PQQ as a prostetic group analogously to the membrane-bound PVA-dehydrogenase [30,61]. However, PEG enzymatic degradation takes place by exocleavage of C2 terminal units (an unzipping mechanism), whereas PVA chain cleavage occurs primarily via an endo-type random mechanism.

PVA-dehydrogenase from *Pseudomonas* sp. 113P3 produced β-diketone groups along the polymer chains that were supposed to be further hydrolyzed to low molecular weight PVA by a specific hydrolase [62]. The enzyme activity was not affected by the presence of a limited amount (1.5 mol%) of β-hydroxyketones produced by chemical oxidation of PVA, thus suggesting that the isolated membrane-bound PVADH was able to oxidize both the chemically oxidized PVA as well as the non-oxidized original PVA. The presence of β-diketone groups was essential for microbial assimilation and growth when PVA was the sole carbon source. However, the chemically oxidized PVA, containing only random distributed carbonyl groups, did not support any significant cell growth when supplemented to PVA-degrading bacteria. Apparently, the monoketone structure of chemically oxidized PVA negatively affected the PVADH production by PVA-degrading microorganisms [62].

Recently, the ultimate biochemical fate of partially hydrolyzed PVA was investigated in the presence of *Pseudomonas vesicularis* PD strain, a specific PVA-assimilating bacterium [63]. This microorganism metabolizes PVA by SAO oxidation of the hydroxyl groups, followed by hydrolysis of the formed β-diketones by a specific hydrolase. Both enzymes are extracellular and the polymer chains are cleaved outside the cells into small fragments by repeated enzyme-mediated reactions, according to the previously described degradation mechanism [56]. It was therefore suggested that the degradation products of partially acetylated PVA samples, represented by acetoxy–hydroxy fatty acids and hydroxy fatty acids, could be incorporated and assimilated inside the bacterial cells.

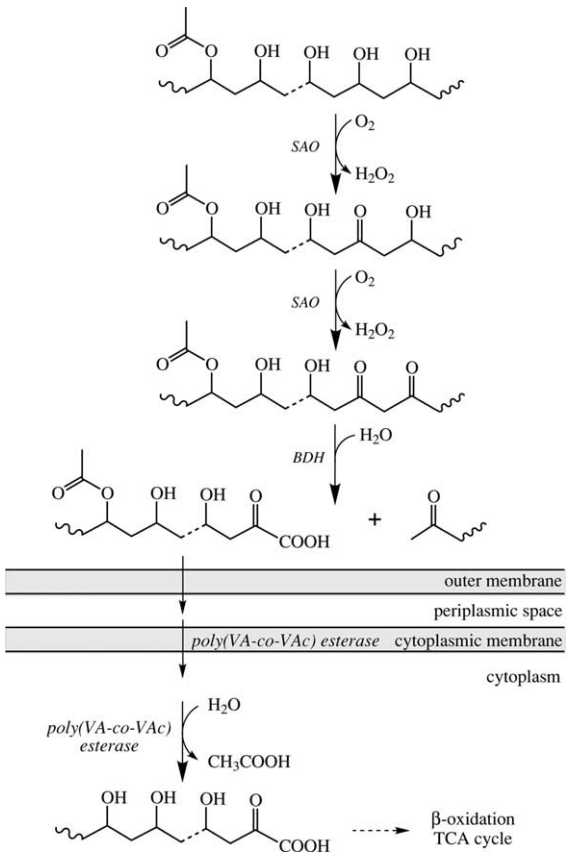
Based on this hypothesis, the effect of co-substrates in the synthesis of SAO and *β-diketone hydrolase* by *P. vesicularis* was investigated. The addition of 0.05% of acetic acid to the PVA-fed bacterial culture significantly increased the activity of both enzymes, although bacteria were unable to utilize acetic acid as a sole carbon source. Formic acid induced similar effects [64]. Higher enzymatic activity was induced by incubation in a rich medium containing beef extract (0.025%), peptone (0.05%), and thiamine (4.0 mg/l).

A specific esterase capable of hydrolyzing the acetyl groups of PVA molecules was purified from the cytoplasmatic fraction of *P. vesicularis* PD cells. The esterase was found to be associated to the cytoplasm membrane [63]. Both PVA molecular weight (MW) and HD significantly affected the enzyme activity. The highest hydrolysis rate was recorded in the presence of PVA samples having the lowest MW and HD. It was suggested that this esterase is particularly effective towards low molecular weight PVA fractions. Based on this assumption, a pathway for the ultimate metabolism of partially acetylated PVA, such as most commercially available samples, was then proposed (Scheme 5) [63].

The low molecular weight products, deriving from the enzymatic oxidation PVA and subsequent β-diketone and β-hydroxyketone hydrolytical cleavage, are a mixture of acetoxy-hydroxy and hydroxy fatty acids. These fragments migrate inside the cells, where deacetylation mediated by cytoplasmic esterase leads to the release of acetic acid and hydroxy fatty acids that are further metabolized in the cytoplasm through β-oxidation and the tricarboxylic acid cycle (TCA) [63].

2.2.2. Symbiotic bacterial biodegradation of poly(vinyl alcohol)

Shimao et al. [65], firstly established the symbiotic assimilation of PVA by bacteria. Several mixed PVA-utilizing microbial cultures were obtained from various environmental sources and in all cases two main bacterial components of the *Pseudomonas* genus were isolated from each culture. In one case, the two strains *Pseudomonas putida* VM15A and *Pseudomonas* sp. VM15C were identified as a pair of essential symbionts. Physiological studies showed that all symbiont cultures were comprised of one strain producing a PVA-degrading enzyme, and one supplying an essential co-factor. In fact, the first one was unable to degrade PVA in axenic culture, whereas bacteria could grow and assimilate PVA axenically in the presence of the culture supernatant of the second



Scheme 5. Biodegradation pathway of partially acetylated PVA [63].

type of bacteria [65]. Taxonomic studies performed on the different symbiotic cultures identified the PVA-degrading bacteria as belonging to the same species of *Pseudomonas* sp., whereas the essential co-factor bacteria were assigned to either *Pseudomonas* or *Alcaligenes* genus [66].

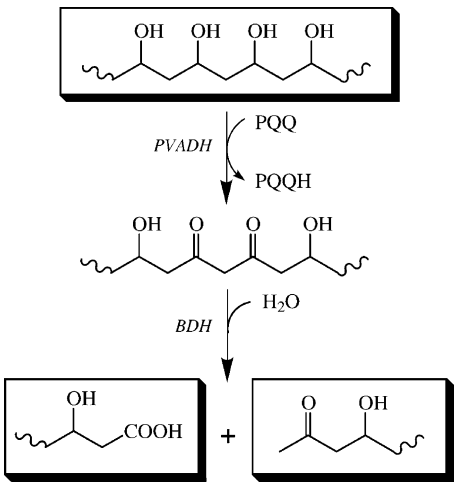
Further studies on two bacterial symbionts, *Pseudomonas putida* VM15A and *Pseudomonas* sp. strain VM15C, indicated that PVA symbiotic assimilation is based on the cross feeding of an essential vitamin-type co-factor, pyrroloquinoline quinone (PQQ), from VM15A to PVA-degrading VM15C [67,68]. PQQ was effective in not only determining VM15C growth, but also in enhancing growth rate and cell proliferation.

Two different PVA-degrading enzymes bound to the membrane fraction of *Pseudomonas* sp. VM15C were isolated and characterized [69]. Substantial *PVA-oxidase* activity was observed in the VM15C culture medium, even in the absence of PVA. The isolated strain showed almost the same productivity of the enzymatic protein both in a rich cultivation medium and in cultures supplemented with PVA as the sole carbon source. The low PVA oxidase production observed in the presence of glucose was attributed to a catabolic repression phenomenon [69]. It was suggested that the PVA oxidase activity of VM15C is constitutive, and susceptible to repression depending upon the incubation condition. The oxidase activity was located mainly in the cytoplasm and not in the periplasmatic space of bacterial cells. However, a large release of the enzymatic protein in the culture medium was observed when the bacterium was fed PVA [69].

The PVA oxidase activity was not affected by PQQ, and the membrane bound PVA-oxidase of *Pseudomonas* sp. VM15C did not contain PQQ as a coenzyme. Fractionation of the solubilized cell membrane of this bacteria also led to the isolation of a PQQ-dependent PVA-dehydrogenase (PVADH) [70]. This enzyme showed high activity toward some low-molecular weight secondary alcohols, but complete disregard for primary alcohols, similar to the membrane-bound oxidase isolated from the same microorganism. Furthermore, the cytochrome reduction coupled to PVA oxidation by the solubilized cell membrane fraction suggested that the polymer oxidation was coupled with the electron transport chain in the cell (Scheme 6) [70].

The PQQ-dependent PVA-dehydrogenase was assumed to be a quinoprotein (holoenzyme) capable of binding PQQ via non-covalent weak interactions that were prone to an easy coenzyme release. Sonicated extracts of VM15C cells grown in the absence of PVA still showed PQQ-dependent PVA dehydrogenase activity indicating that the synthesis of PVA-dehydrogenase is constitutive.

By considering the essential requirement of PQQ in the PVA utilization by *Pseudomonas* VM15C, a relationship between the membrane-bound PVA-oxidase and PVA-dehydrogenase was also proposed. Accordingly, the PQQ-dependent dehydrogenase might be an oligomeric enzyme with PVA oxidase as a subunit. In this case, PQQ should not play a direct role in the biochemical dehydrogenation of PVA since the oxidase is able to oxidize PVA on its own. PQQ



Scheme 6. Biodegradation pathway of PVA as mediated by a PVA-dehydrogenase PQQ-dependent in symbiotic bacterial culture [70].

might be involved in another enzyme subunit in the secondary electron transfer from the PVA-oxidase subunit to electron acceptors other than O_2, since the PQQ-dependent dehydrogenase did not use oxygen. This may also account for the significance of PVADH as the primary electron acceptor in the energy metabolism in absence of O_2 [70].

The structural gene encoding the PQQ-dependent PVA-dehydrogenase from *Pseudomonas* sp. VM15C was cloned and its entire nucleotide sequence determined [71]. The structural gene is associated to a plasmid, which, once introduced in an *Escherichia coli* strain, induced the synthesis and the expression of PVADH activity. PVA's ineffectiveness in inducing enzymatic activity in the *E. coli* clone confirmed that PVADH is constitutive in *Pseudomonas* sp. VM15C. The PVA dehydrogenase activity expressed by this microorganism was PQQ-dependent, and no activity was detected without the addition of exogenous PQQ.

Based on the amino acid sequence of this enzyme, a signal sequence, a heme *c*-binding site, and a PQQ-binding site were recognized as putative functional sites. In particular, a possible PQQ-binding site was identified based on the similarity within the aminoacid sequence of PQQ-binding sites in different bacterial dehydrogenases of monocarbynols, such as methanol and ethanol.

Ca^{2+} and Mg^{2+} are known to play an important role in the binding of PQQ to the apoenzymatic protein in many quino-protein dehydrogenases [72]. In the case of PVADH from *Pseudomonas* sp. VM15C, Ca^{2+} stimulated the enzyme activity. Mg^{2+} induced a similar effect, although to a lesser extent. Therefore, it was suggested that these cations are essential for PQQ-binding to the apoprotein and consequently in the dehydrogenase activity.

The similarity of the investigated PVADH with bacterial alcohol dehydrogenase and in particular with the quinoprotein *ethanol-dehydrogenase* was supported by the presence of an aminoacid sequence corresponding to a heme *c*-binding site. This prosthetic group appears to be involved together with PQQ in the dehydrogenation of PVA [71].

From a phylogenetic point of view, both PVA and synthetic polymers entered the natural environment only at a very late stage of life evolution. Because of this late arrival, most microorganisms do not yet recognize polymers as a carbon source. Nevertheless, it is possible that PVA-degrading enzymes, including PVADH from *Pseudomonas* sp. VM15C, were obtained in a recent evolutionary process, most likely starting from enzymes that degraded other substances in the original microorganisms. On the other hand, as indicated by Shimao et al. [71], PVA-dehydrogenase seems to be functionally unique. Indeed, its primary structure displays rather large differences as compared with that of other types of quinoprotein dehydrogenase. It was suggested that the isolated PVADH would have an unusual evolutionary history. It remains unclear how PVA, as well as other xenobiotic polymers such as PEG, could have induced a selective pressure and consequently an evolutionary process so efficient as to promote the synthesis of specific degrading enzymes in microorganisms.

Co-metabolic processes appear to play an important role in the microbial degradation and assimilation of PVA. Earlier studies by Shimao and co-workers [65,66], showed *Bacillus megaterium* strain BX1 able to utilize the synthetic polymer as its sole carbon source. This strain was isolated from the activated sludge of a textile factory by an enrichment procedure [73].

The mixed culture showed a two-step growth behavior. After an early stationary phase, the culture started to increase again, because of the assimilation of PVA degradation products most likely formed during the first growth stage [73]. However, *B. megaterium* BX1 showed poor growth and very

limited PVA degradation when cultivated in axenic culture. Significant PVA assimilation occurred only in the presence of a Gram-positive rod-shaped counterpart isolated from the original mixed culture. The latter bacterial isolate was not able to degrade PVA at all, indicating that PVA degraded as a consequence of the co-metabolism of the two bacterial strains. However, this co-metabolic process was not necessarily bound to cross feeding of an essential co-factor, as revealed by the ineffectiveness of PQQ to promote PVA assimilation by *B. megaterium* [73].

A constitutive PVA-degrading enzyme was produced by *Pseudomonas vesicularis* var. *povalolyticus*, PH, intracellulary accumulating the enzymatic protein when grown in a complex nutrient medium including tryptone and yeast extract without added PVA. This bacterium was isolated along with other two bacterial strains from PVA-enriched activated sludge. Characterization of *P. vesicularis* var. *povalolyticus* PH revealed that it requires thiamine as growth factor, and aminoacids like cystine, isoleucine, and tyrosine for the expression of PVA-degrading activity. Another strain identified as *Flavobacterium* sp. did not assimilate PVA, but was able to support the growth of the PVA-degrading *Pseudomonas* [74,75]. The intracellular enzyme activity increased with cell growth, reaching the maximum activity level at maximum growth. However, the activity in the culture supernatant continued to increase because of an active excretion process from the bacterial cells. Secretion of the specific PVA-degrading enzyme was effectively induced by PVA addition to the bacterial culture [76]. The crude enzyme was recovered from the cells by osmotic shock caused by treatment with sucrose or NaCl.

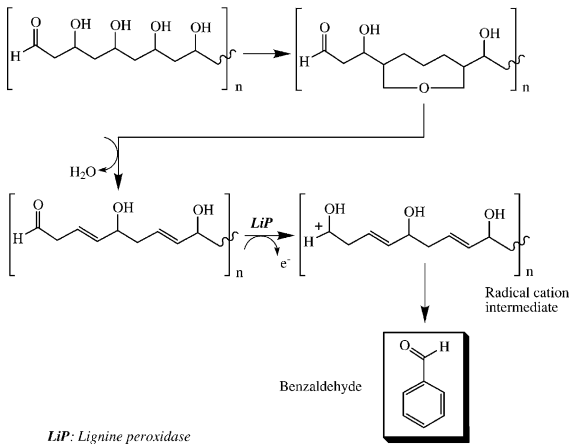
Kawagoshi and Fujita [77] purified a specific *2,4-pentanedione hydrolase* (2,4-PDH) from the same *Pseudomonas* PVA-degrading species by precipitation with ammonium sulfate followed by column chromatography. This enzyme exhibited hydrolytic activity over a broad pH range with a maximum at pH 8.0; 40 °C was the optimum temperature. The enzyme activity was inhibited by Hg^{2+}, Ag^{+}, NaF, 2,4-dinitrophenol, and *p*-chlorobenzoate, and it was markedly reduced by low concentrations of chloride and bromide anions. 2,4-PDH was able to catalyze the hydrolytic cleavage of PVA-oxidized sites. Therefore, the authors suggested that the peculiar hydrolytic enzyme produced by *P. vesicularis* var. *povalolyticus* PH was involved in the overall PVA biodegradation process [77].

2.2.3. Biodegradation of poly(vinyl alcohol) by fungi and yeasts

Limited information is available on PVA biodegradation mediated by microorganisms other than bacteria. However, the first report claiming PVA biodegradability concerned the activity of *Fusarium lini*, an occasionally phytopatogenic mycete [46]. In recent years, the fungal metabolization of PVA was further investigated [78,79]. The lignin-degrading basidiomycete *Phanerochaete chrysoporium* was used because of its versatility in the biodegradation of recalcitrant xenobiotic compounds, such as polycyclic aromatic hydrocarbons [80,81] and synthetic macromolecular systems [82,83]. Indeed, its enzymatic pool comprising *lignin peroxidase* (LiP), *manganese peroxidase*, and *laccase* is a powerful system able to perform monoelectronic oxidation (e.g. radical formation) of a large number of aromatic and non-aromatic synthetic compounds.

During the reported investigation, the ligninolitic enzyme LiP of *P. chrysosporium* promoted the degradation of PVA chains through the formation of carbonyl groups as well as double bonds, thus increasing the macromolecule unsaturation [78]. Accordingly, a substantial (approximately 80%) decrease in the average molecular weight was observed.

The first stage of the fungal degradation process was supposed to occur mainly in the amorphous phase of PVA, leaving behind crystalline structures that experienced degradation at a lower rate. The initial formation of a radical-cation by a complex sequence of redox reactions involving the ferric enzyme, hydrogen peroxide, and veratryl alcohol as an essential co-substrate was supposed to be a general feature of the PVA enzymatic degradation catalyzed by LiP. Mejía et al. [78] suggested a biochemical route in which the initial free-radicals were converted to epoxides, followed by water elimination and the formation of double bonds. This hypothesis was proposed based on carbonyl and double bond detection by FT-IR and UV analysis, along with the production of volatile benzaldehyde as a low



Scheme 7. Biodegradation pathway of PVA by *Phanerochaete chrysosporium* [78].

molecular weight end product. Indeed, the breakdown of radical cation intermediates could generate benzaldehyde through a rearrangement of seven carbon atom fragments (Scheme 7) [78].

PVA biodegradation by fungal species seems to be improved by polymer oxidative pretreatment [84]. The effect of pre-oxidation on the biodegradation of PVA was investigated in the presence of *Pycnoporus cinnabarinus*. This white-rot fungus is a producer of powerful ligninolytic oxidases able to degrade various industrial dyes. The oxidase activity of the selected fungal species was greatly enhanced by the addition of oxidized PVA in the culture medium. However, neither *lignin peroxidase* nor *Mn peroxidase* were detected, thus suggesting that *laccase* was the prevailing oxidase produced by *P. cinnabarinus* in the presence of PVA.

The extent of PVA degradation as monitored by titrimetric determination of PVA concentration in the cultivation broth [85] was significantly higher in the presence of chemically pre-oxidized polymer. The addition of glucose as a co-metabolite further enhanced the oxidase production and consequently the rate and extent of PVA depletion. It was also observed that *laccase* was the only active oxidase under co-metabolic conditions. However, the ultimate fate of PVA was not ascertained due to the lack of characterization of the degraded polymer.

An investigation aimed at identifying fungal microorganisms, particularly yeast-like ones, responsible for PVA biodegradation revealed that *Saccharomyces*, *Lipomyces*, and *Rhodotorula* spp. degraded and assimilated PVA (88% hydrolyzed, DPn = 1700). This process occurred both in the presence and absence of acetic acid, its salts, and esters in the culture medium. The polymer was also degraded by several other yeast species such as *Endomyces*, *Zigosaccharomyces*, *Pichia* and *Nadsonia*, but only in the presence of acetic acid derivatives as co-substrates [86,87]. Typically, approximately 70% abatement of COD was recorded in cultures of *Endomyces fibuliger* supplemented with 0.05% PVA and sodium acetate, while only an 8% COD drop was observed in the absence of co-substrate. An even more pronounced effect was observed in *Saccharomyces rouxii* cultures when sodium acetate was added as co-metabolite [86].

No further information exists regarding the biodegradation biochemistry of PVA by yeasts under axenic cultures, either as a sole carbon source or in combination with co-metabolites. Nevertheless, a specific PVA-degrading enzyme was isolated and purified from the vegetative structure of *Geotrichum fermentans* WF9101 grown on 2,4-pentanediol, a compound that mimics the constitutional unit of poly(vinyl alcohol) [88].

2,4-pentanediol

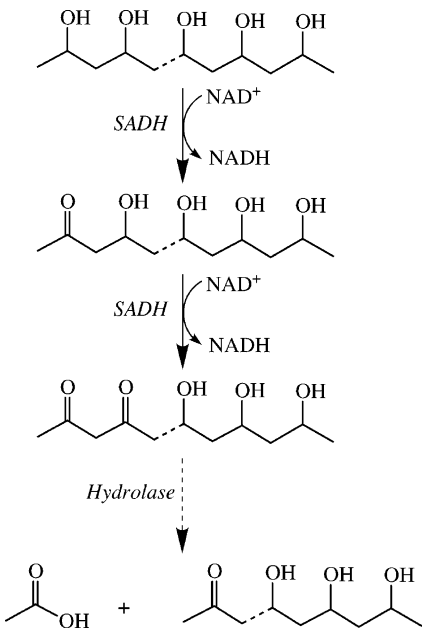
This fungal strain and one bacterium (*Bacillus megaterium*) were isolated from a PVA-degrading mixed culture obtained from the activated sludge of a textile factory. The *Bacillus megaterium* strain acted synergistically with another bacterium to degrade the PVA to oligomers [73,89]. The PVA-degrading enzyme from *G. fermentans* is not bound to the cell membrane. It was recognized as an NAD(+)-dependent *secondary alcohol dehydrogenase*, having relatively low stability and therefore fast deactivation. This enzyme was active towards several secondary alcohols and diols and was active specifically toward (S)-enantiomers.

Studies relevant to the purified dehydrogenase showed the kinetic parameters in the Michaelis–Menten equation, K_m and V_{max}, were significantly affected by the carbon chain length of the target

secondary alcohol. In particular, K_m increased and V_{max} decreased as the number of carbon atoms in the test compound changed with respect to the 2-hexanol, indicating an optimal activity towards secondary alcohols with medium chain lengths [88].

Analytical characterization of the degradation products of 2,4-pentanediol exposed to the fungal dehydrogenase led to the identification of both 4-hydroxyl-2-pentanone and 2,4-pentanedione. From these observations, Mori et al. [88], proposed a degradation pathway of vinyl alcohol oligomers resembling the attack of high molecular weight PVA by specific bacterial oxidase and dehydrogenase (Scheme 8). The PVA oligomer hydroxyl group was first oxidized to the corresponding 1,3-diketone by two sequential reactions catalyzed by the secondary alcohol dehydrogenase from *G. fermentans*. Then, a C–C breakdown mediated by a β-diketone hydrolase occurred. However, no definite identification of the enzymatic complex in the isolated fungal strain was carried out.

The fungal dehydrogenase was active towards PVA having an average degree of polymerization preferably lower than 20, with the upper threshold being 50. It was concluded therefore that the secondary alcohol dehydrogenase from *G. fermentans* might be involved in the biodegradation of PVA, although in a synergistic approach with other microorganisms it would be able to degrade high molecular weight fractions [88].



Scheme 8. Biodegradation pathway of oligomeric PVA by *Geothricum fermentans* [88].

2.3. Effect of structural features on biodegradation of poly(vinyl alcohol)

Relatively little information is available regarding the influence of microstructure characteristics on PVA biodegradability. Former investigations [51] reported that molecular weight, degree of saponification, and content of head-to-head units (1,2-glycol moieties) had very limited or no influence on the biodegradation process. Nevertheless, recent studies suggested that in some cases the structural characteristics of PVA might significantly affect the activity of several PVA-degrading enzymes, and hence polymer biodegradation rate and extent.

2.3.1. Degree of polymerization and degree of hydrolysis

The degree of polymerization and degree of saponification (hydrolysis) (DS) did not appear to have any significant influence on the biodegradation of PVA, at least for samples containing less than 20% of residual acetyl groups [50,51,53].

Matsumura and Toshima [90] reported that PVA samples having M_n between 90 and 0.53 kD were easily and equally degraded by PVA-degrading microorganisms. This investigation also showed that PVA-cleaving enzymes required a sequence of at least 3–5 vinyl alcohol monomeric units to exhibit catalytic activity. No significant difference in the degradation of different molecular weights of PVA was detected using a selected PVA-degrading mixed bacterial culture enriched from the sewage sludge of a paper mill treatment plant [91].

On the other hand, PVA molecular weight did affect the activity of a PVA-dehydrogenase (PVADH-S) purified from *Pseudomonas* sp. strain 113P3 [92]. The Michaelis constant (K_m) of PVADH-S decreased as the degree of polymerization decreased; the lowest K_m values were recorded in the presence of PVA samples having an average DPn of about 30. Further DPn reduction increased K_m, while the maximum

reaction rate (V_{max}) was not much affected [92]. These results strongly support the requirement of a minimal PVA chain length for exploiting enzymatic activity.

No major difference in the extent of biodegradation was observed among three PVA samples (PVA72, PVA88, PVA98) having different degrees of saponification (DS = 72, 88, and 98%, respectively) and molecular weights ranging between 6.6 and 88 kD [93]. However, the exponential phase of the culture fed with PVA72 started almost 10 days later than the other two samples (Fig. 2). The observed influence of the degree of saponification on the degradation rate is in contrast with previous observations by other authors [51], although they did not investigate samples having degrees of saponification lower than 88%. By contrast, a recent investigation showed an appreciable reduction of PVA-dehydrogenase activity as the degree of saponification lowered, whereas the degree of polymerization did not have any significant effect [59].

A sharp reduction in molecular weight was observed within a few hours of incubation in the supernatant of a selected PVA-degrading population [93]. This reduction was attributed to the endocleavage of polymer chains due to a random-type degradation mechanism. In this case, both molecular weight and degree of saponification did not affect the rate or extent of PVA cleavage by the extracellular enzyme.

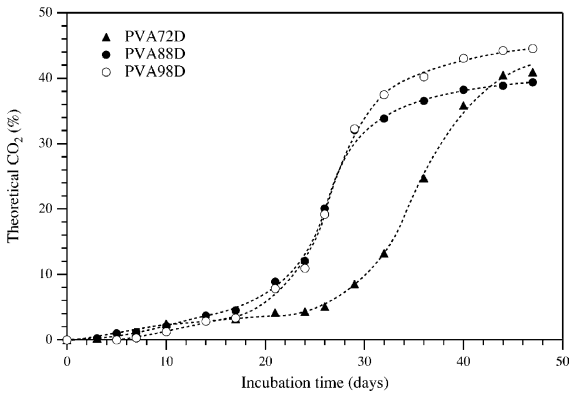


Fig. 2. Time profiles of mineralization of PVA samples having different degree of hydrolysis in aqueous medium in the presence of acclimated PVA-degrading microorganisms. Reprinted with permission from Polym Degrad Stab 2002;75:447. © 2002 Elsevier Science [94].

Molecular weight's effect was demonstrated in the case of a specific esterase that catalyzed the intracellular hydrolysis of PVA residual acetyl groups [63], the higher hydrolysis rates being recorded at lower DPn and DS values. NAD-dependent PVA-dehydrogenase from the fungus *Geothricum fermentans* [88] was also reported to act preferentially on low molecular weight PVA. A DPn equal to 50 represented the upper limit beyond which no degradation could be detected.

More recently, a F2 strain bacterium capable of assimilating PVA fractions with molecular weights up to 4.2 kD (Fig. 3) was isolated along with four other bacterial strains from an acclimated PVA-degrading mixed culture [94]. The degradation behavior shown by this strain requires the presence of enzymatic co-factors or symbiotic microorganisms; these requirements are indicative of a complex PVA assimilation pattern.

A clear effect of molecular weight on PVA mineralization was repeatedly ascertained in biodegradation tests carried out under incubation conditions aimed at reproducing natural environments. In particular, the preferential assimilation of low molecular weight fractions was observed under anaerobic conditions in the presence of anaerobic sludge and river sediment microorganisms [95], as well as in soil burial tests [96].

The reported results confirmed that the random cleavage of polymer chains, mainly mediated by

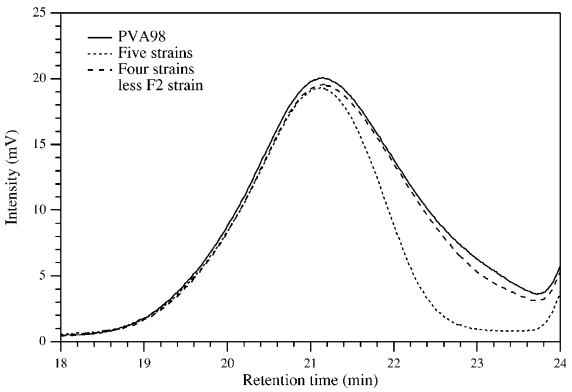


Fig. 3. SEC chromatograms of 98% hydrolysed PVA in the presence of different bacterial strains isolated from an acclimated PVA-degrading mixed culture. Reprinted with permission from Polym Degrad Stab 2002;75:447. © 2002 Elsevier Science [94].

extracellular enzymes and in a few cases by membrane bound ones, is indeed the most representative PVA degradation mechanism. This general scheme substantiates the low influence molecular weight plays on the biodegradation rate. Nevertheless, recent investigations appear to suggest that a different biodegradation mechanism is active in the presence of low molecular weight PVA samples. In particular, a biodegradation pathway resembling the β-oxidation of *n*-alkanes seems to occur in the presence of activated sludge microorganisms for PVA samples having a degree of polymerization lower than 40 [97].

2.3.2. Main chain stereochemistry

Recently, the influence of stereoregularity on PVA degradation was investigated in the presence of a PVA-degrading bacterial strain isolated from the wastewater treatment, plant of a textile factory [98]. This bacterium, belonging to the *Pseudomonas* genus, excreted a specific PVA-oxidase in the culture medium. However, it could not assimilate the synthetic polymer without the addition of yeast extract [98]. The cell growth rate and the supernatant viscosity were compared in cultures supplemented with either commercial PVA samples or highly isotactic PVA [99]. Significantly, a larger decrease in PVA content was recorded in cultures supplemented with the isotactic polymer than in those cultures containing the commercial atactic samples. The authors suggested that isotactic sequences were split preferentially by the enzymatic reactions. The observed differences should occur in the oxidation reaction of PVA 1,3-hydroxyl groups. In fact, stereochemical differences disappear when a β-diketone is formed from two adjacent secondary hydroxyl groups. The effects of tacticity on PVA biodegradation was attributed to the sensitivity of the PVA-oxidase enzyme to stereochemical constraints, as usually occurs in enzymatic reactions [98]. It was suggested by Fukae et al. [98], that the PVA-oxidase produced by *Pseudomonas* sp. strain A-41 must exhibit stereoselective behavior, since the substrate must have relatively long sequences for molecular recognition. This conclusion was further strengthened by previous reports indicating that PVA oxidase enzymes require a sequence joining more than three carbon atoms at both sides of the α-carbon bound to a hydroxyl group [52].

The influence of PVA microstructure on the dehydrogenation reaction was investigated by using a specific PVA-dehydrogenase (PVADH-S) produced by the PVA-degrading *Pseudomonas* sp. strain 113P3 [92]. This enzyme catalyzed the polymer oxidation in the presence of PQQ to give rise to the formation of β-diketone blocks along the macromolecular backbone. However, PVADH-S was not active on low molecular weight secondary alcohols, thus differing from the PVADH previously purified by Shimao et al. [70]. The PVADH-S V_{max}/K_m ratio increased in the presence of PVA samples containing 12–19% residual acetyl groups. This feature was further enhanced in the case of ethylene–vinyl alcohol block copolymers, due to the concurrent K_m decrease and V_{max} increase. Accordingly, it was suggested that PVADH-S substrate affinity is dependent upon the hydrophobic character of the vinyl alcohol copolymer, which is related to the relative content of either acetyl groups or ethylene blocks [92].

The reported kinetic parameters were also affected by the substrate stereochemistry. In particular, among the various 2,4-pentanediol isomers only the meso-form, corresponding to PVA isotactic sequences, was recognized as a substrate by the isolated PVADH-S. The much lower values of V_{max} measured for the syndiotactic isomer were attributed to its low solubility caused by intermolecular hydrogen bonding formation. Finally, 1,3-diols were dehydrogenated at a greater rate than 1,2-diols [92].

The content of structural links (head-to-head/tail-to-tail defects) did not demonstrate a significant effect on either the activity of specific PVA-degrading enzymes or the rate of mineralization [50,93].

2.4. Conclusive chapter remarks

The nature of PVA as a truly biodegradable synthetic vinyl polymer was repeatedly and intensively assessed. Nevertheless, the occurrence of specific PVA-degrading microorganisms in the environment appears to be uncommon and in most cases strictly associated with PVA-contaminated environments. Selective acclimation seems to be required, commonly observed for low molecular

weight xenobiotic compounds in natural microbial communities.

Most of PVA-degrading microorganisms were identified as aerobic bacteria belonging to *Pseudomonas*, *Alcaligenes*, and *Bacillus* genus. Some species degrade and assimilate PVA axenically, even though symbiotic association exhibiting complex cross-feeding processes is a rather common feature of PVA biodegradation.

On the other hand, few studies investigated the fungal degradation of PVA, mainly reporting the activity of ligninolytic species such as *Phanerochaete crysosporium*. More information would be really valuable in this area, especially taking into account the increasing attention on the production of disposable, single use PVA-based items for agricultural applications. The soil, the target environment, is widely colonized by fungi and actinomycetes, which play a fundamental role in the biochemical cycle of organic matter.

In solution, the major biodegradation mechanism of PVA is represented by the random endocleavage of the polymer chains. The initial step is the specific oxidation of 1,3-hydroxyl groups, mediated by oxidase and dehydrogenase type enzymes, to give β-hydroxylketone as well as 1,3-diketone moieties. The latter groups are susceptible to carbon-carbon bond cleavage promoted by specific β-diketone hydrolase, giving rise to the formation of carboxyl and methyl ketone end groups.

Enzymatic random endocleavage of PVA chains does not appear to be appreciably affected by polymer structural features, such as degree of polymerization and HD, at least while these features are in the 80–100% range. However, a positive influence on the hydrophobic character (e.g. residual acetyl group content) of the polymeric substrates on the activity of specific PVA-dehydrogenase was demonstrated. The activity of other PVA chains cleaving enzymes, such as PVA-oxidase, also showed dependence on the macromolecular microstructure.

Extracellular proteins constitute most of the isolated enzymes. A specific endocellular esterase capable of hydrolyzing residual acetyl groups occurs in the ultimate microbial metabolism of partially hydrolyzed PVA samples.

Many bacterial PVA-oxidases are constitutive, whereas some PVA-dehydrogenases are inducible enzymes that require essential co-factors, such as PQQ and bivalent cations, for an efficient exploitation of their activity. On the other hand, the activity of several PVA-oxidases is enhanced by acetic acid as well as by several aminoacids, whereas glucose acts as a repressive catabolite. This last finding is particularly interesting considering that several PVA-based blends contain natural polysaccharides, particularly starch.

Recent investigations support the hypothesis that other degradation mechanisms promoted by radical reactions induced by ligninolytic enzymes are involved in PVA microbial metabolism. Complete understanding of this metabolism in terms of both microbial ecology and environmental influence does not yet exist.

3. Biodegradation of poly(vinyl alcohol) under different environmental conditions

The environmental fate of PVA was primarily researched in relation to its extensive use in textile and paper manufacturing, which led to wastewater pollution caused by relatively large amounts of the vinyl polymer. Since the first studies carried out in the presence of domestic or non-acclimated activated sludge, long-term acclimation to PVA under conventional wastewater treatment processes was required for an efficient biological removal of the polymer [86,87]. On the contrary, negligible assimilation of the polymer was detected in the presence of non-acclimated domestic sludge microorganisms. Nevertheless, respirometric measurements demonstrate that PVA can be completely mineralized in the presence of selected microbial strains and under suitable incubation conditions [91].

Many studies were aimed at investigating the biodegradability of pure PVA, whereas PVA items dispersed in the environment are based on blown films. These films contain thermally induced modifications, and may contain process additives up to a maximum of 20% by weight. Recently, a study was undertaken under various environmental conditions in order to ascertain the degradative behavior of PVA-based blown films obtained by thermal processing as compared to unprocessed pure PVA samples. Different test conditions were selected, including simulated

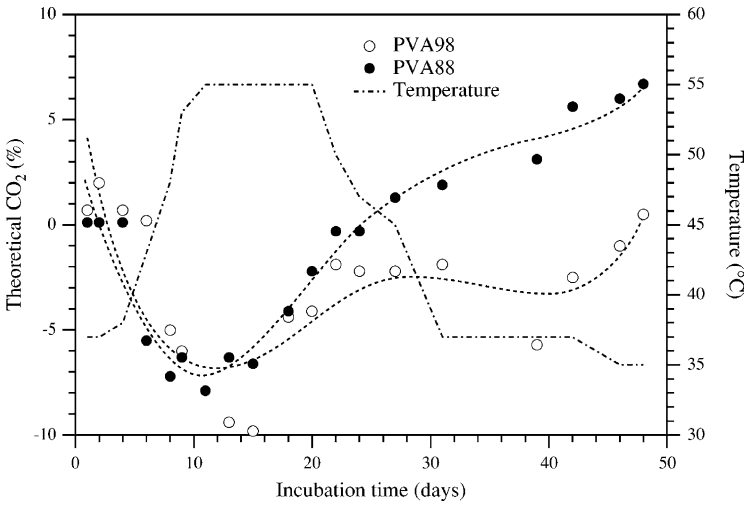


Fig. 4. Time profiles of mineralization of two different PVA based blown films in a simulated composing test. Reprinted with permission from Polym Degrad Stab 1999;64:305. © 1999 Elsevier Science [93].

aerobic composting, soil burial, and in aqueous media containing sewage sludge from different sources and PVA-acclimated microorganisms [93]. The rate and extent of mineralization of the film samples was monitored by respirometric measurements. The neat amount of evolved CO_2 was then converted to calculate the extent of degradation.

3.1. Biodegradation of poly(vinyl alcohol) under composting conditions

In a case study, the biodegradation propensity of PVA-based blown films in compost was evaluated [93] under incubation conditions partially derived from the ASTM D 5338-92 test [100] aimed at determining the degradation rate and extent for plastic materials under a controlled and reproducible test environment. Test materials were confined in a mature compost matrix and their relevant biodegradation measured as a percentage of the polymer carbon content converted to CO_2 during the test period. A temperature profile of consecutive mesophylic (e.g. 28–37 °C), thermophylic (e.g. 55–60 °C), and further mesophylic phases was applied to the test containers in order to mimic the thermal conditions occurring in open-field composting processes. Stabilized compost from urban solid waste was utilized as a microbial-active incubation media. Biodegradation of PVA-based plastic films did not exceed 7% in 48 days; this figure was only reached in the presence of film samples based on PVA with an HD of 88% [93]. It is worth highlighting that during the thermophylic phase of the simulated composting procedure, the CO_2 production of cultures supplemented with PVA-based plastic films was lower than that of the blanks. Most likely, this behavior can be attributed to a kind of noxious effect exerted by the polymer samples on the thermophylic microflora in the compost (Fig. 4).

Very moderate PVA biodegradation was also detected when using compost extract as a microbial source [101]. It is worth noting that the biodegradation extent (25%) of 88% hydrolyzed PVA, as recorded by BOD measurements after 300 days of incubation, was larger than the biodegradation extent (15%) of 98% hydrolyzed PVA. It was also observed that the biodegradation process for the latter sample was not appreciable until the 35th day of incubation [101].

These results confirmed previous investigations carried out by standardized laboratory procedures [102]. Under the adopted conditions, PVA underwent very limited biodegradation, reaching a plateau in the mineralization profile corresponding to approximately 12% of cumulative CO_2 conversion after 30 days of incubation. A starch sample used as reference was extensively mineralized (75%) by compost microorganisms under the same conditions [102].

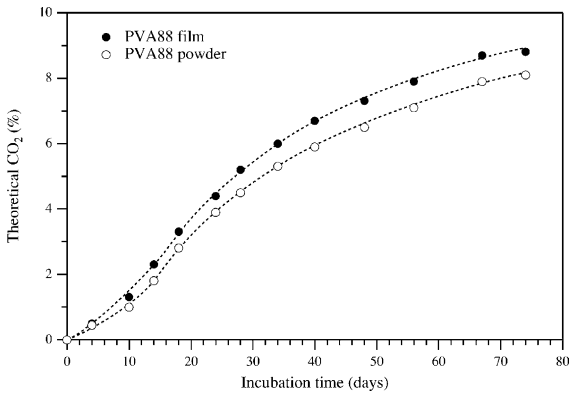


Fig. 5. Time profiles of mineralization of PVA based blown film and powder in simulated soil burial test. Reprinted with permission from Polym Degrad Stab 1999;64:305. © 1999 Elsevier Science [93].

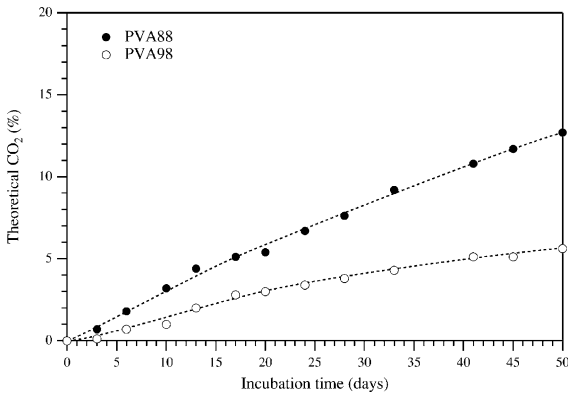


Fig. 6. Time profiles of mineralization of PVA samples having different degree of hydrolysis in simulated soil burial test [45].

3.2. Biodegradation of poly(vinyl alcohol) in soil environment

Simulated soil burial tests providing information regarding the soil biodegradation propensity of PVA-based blown films [93] were performed according to a procedure aimed at reducing the experimental error in the presence of polymeric materials that can be mineralized at moderate or slow rates [103].

PVA-based films underwent limited biodegradation (8–9%) in 74 days. (Fig. 5) in simulated soil burial respirometric tests. Apparently, polymer concentration and physical state did not affect the rate and extent of biodegradation, as indicated by the almost identical results obtained for different amounts of film or powder polymer samples [93]. Similar results were obtained in a prolonged experiment (up to 120 days of incubation) for pure PVA samples having 88% HD [103].

The mineralization rate's dependence on the hydrolysis degree was also evaluated in an additional soil burial experiment. This experiment used two different commercial PVA samples having 88 and 98% degrees of saponification, respectively, and relevant cast films. Very limited PVA biodegradation was observed. However, the two PVA samples exhibited small but significant differences; the sample with the lowest HD showed a slightly larger propensity to microbial assimilation (Fig. 6) [45].

SEC analysis was carried out before and at the end of the soil burial experiment on the PVA cast film having a 98% HD. A slight increase in average molecular weight accompanied by a significant reduction of the polydispersity index was observed. This behavior can be attributed to the leakage of low molecular weight fractions, as well as to the preferential microbial assimilation of short PVA chains (Fig. 7) [45].

The very limited propensity of fully hydrolyzed PVA to biodegradation in soil environments was repeatedly observed [104–107]. In particular, a detailed investigation was carried out on PVA sheets buried in 18 different natural soil sites, representing different compositions and climate conditions. Only very limited weight losses (less than 10%) were recorded after two years of incubation under natural weathering conditions [106]. In all cases, PVA specimens retrieved from the field tests did not shown traces of colonization by microorganisms, although they

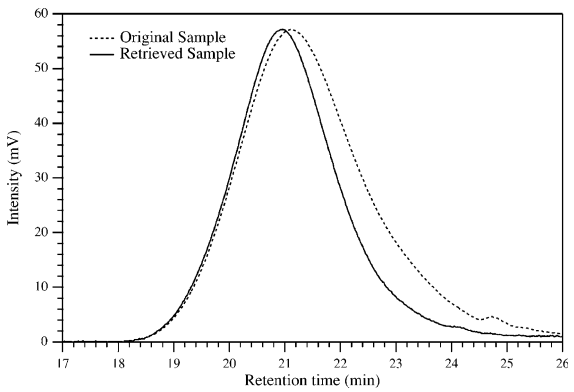


Fig. 7. SEC chromatograms of 98% hydrolyzed PVA film before and after simulated soil burial test [45].

were incubated in soils characterized by high microbial activity. Other polymers, such as PHB/HV and PCL, were attacked extensively by soil microorganisms under the same soil burial conditions [107].

This behavior might be attributed to the very scarce distribution of specific PVA-degrading microorganisms in soil matrices [108]. Indeed, no significant degradation in PVA concentration was observed after 28 days of incubation when the solid environmental samples (soil and compost) used in the respirometric tests were transferred to liquid cultures. This result confirmed that PVA-degrading microbial strains were absent in the utilized environmental matrices.

However, other possibilities must be taken into account when attempting to explain PVA's low propensity toward biodegradation by soil-associated microflora. In particular, the almost irreversible adsorption of PVA on soil's mineral and organic components can play an essential role [109]. Indeed, the development of strong interactions with solid substrates was one reason presented for the limited biodegradability of microbial polysaccharides and proteins observed in soil [110,111].

To clarify this point, an accurate investigation of the adsorption of PVA by soil was performed [112]. The resulting information provided insight into the nature of the interactions between PVA and different soil components. The influence of PVA's structural parameters, such as degree of saponification and molecular weight, on the adsorption process was also assessed. Natural soil is indeed a very complex, heterogeneous matrix, variable from site to site, consisting mainly of clays, silica, organic matter, and water. In order to simplify the task, the adsorption process was first investigated on montmorillonite (MM), quartz sand (QS), and humic acids (HA) that represented model compounds of the soil's main components.

Commercial poly(vinyl alcohol) samples having degrees of saponification ranging between 72.5 and 98% and different molecular weights (15–88 kD) were used in adsorption–desorption studies. Before use, all samples were characterized by SEC, FT-IR, and NMR analyses. Each material was suspended in a solution of PVA and water to investigate the kinetics of PVA adsorption on soil components.

The specific PVA adsorption on montmorillonite at equilibrium increased from 31 to 37 mg/g, increasing the degree of saponification from 72.5 to 98%. For the two samples having 88% saponification and different molecular weights, the specific PVA adsorption at equilibrium decreased from 34 to 28 mg/g, increasing the number average molecular weight from 36 to 88 kD. Moreover, the molecular weight of PVA remaining in solution increased with contact time, because of the preferential adsorption of low molecular weight fractions [112].

PVA adsorption on whole soil was investigated by suspending 5 g of farm soil in 200 ml of solution containing 1.0 mg/ml of 98% hydrolyzed PVA for 11 weeks. The adsorption isotherm that resulted showed PVA adsorption occurred primarily during the first few hours, with the process being complete within the first week. As in the case of montmorillonite, preferential adsorption of low molecular weight PVA fractions occurred. The equilibrium specific adsorption (8 mg/g) was much smaller than that (36 mg/g) recorded under the same experimental conditions in the presence of montmorillonite. This result is, however, in good agreement with the clay content (22.5%) in soil. Indeed, among the different soil components, only montmorillonite showed a significant PVA adsorption.

No detectable amount of PVA was released from montmorillonite samples when suspended in water, suggesting that the adsorption process is almost irreversible [112].

The influence of the adsorption by montmorillonite on the aerobic biodegradation of PVA was

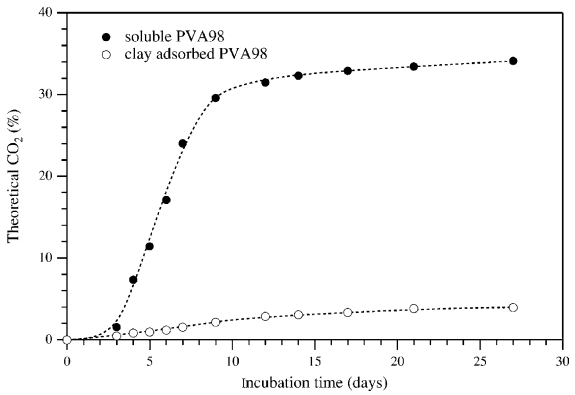


Fig. 8. Time profiles of mineralization of soluble and clay-adsorbed PVA in an aqueous medium in the presence of acclimated PVA-degrading microorganisms. Reprinted with permission from J Polym Environ 2000;8:67. © 2000 Kluwer Academic [112].

investigated in liquid culture containing an acclimated microbial inoculum [93]. Parallel experiments were performed in cultures containing either PVA alone or PVA adsorbed on montmorillonite. Kinetic plots (Fig. 8) clearly showed that PVA in solution and when adsorbed on montmorillonite reached 34 and 4% mineralization, respectively, within one month of incubation. The reported results seem to indicate that PVA adsorption on inorganic substrates effectively inhibits biodegradation processes, at least under the adopted incubation conditions [112].

The above reported data suggest that the comparable, very low extent of PVA biodegradation recorded under composting and soil burial test conditions may be ascribed to the adsorption of the synthetic polymer by inorganic and organic components present in soil. Accordingly, the small increase of PVA adsorption with the degree of saponification, indicating the active role held by polymer hydroxyl groups in the adsorption process, may also account for the slightly larger mineralization observed for the PVA sample having 88% degree of saponification with respect to the 99% DS sample.

3.3. Aerobic biodegradation of poly(vinyl alcohol) in aqueous environments

Many investigations were carried out on the biodegradation of PVA in aqueous media since it is a water-soluble polymer. In most cases, however, significant levels of biodegradation were reached only in the presence of acclimated PVA-degrading microorganisms [47,48]. A fairly limited biodegradation (13% after 21 days of incubation) of PVA-based blown films was also observed in aerobic biodegradation tests carried out in liquid cultures inoculated with municipal sewage sludge [93]. However, this result cannot be considered a conclusive one, due to the short incubation time. Indeed, a more active and specialized microbial population could be established in a longer period of 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 the paper mill wastewater treatment plant, the biodegradation extent of PVA and PVA-based blown films reached values comparable to that of cellulose.

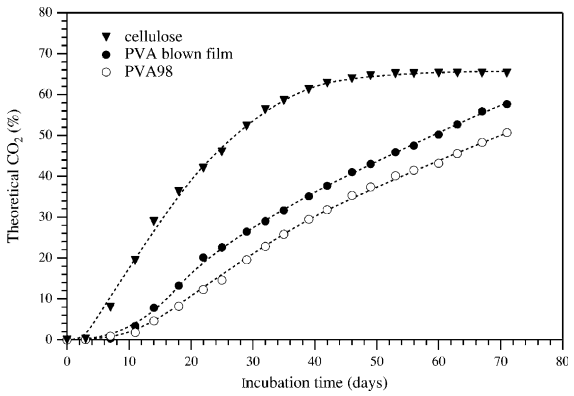


Fig. 9. Time profiles of mineralization of PVA-based blown films, 98% hydrolyzed PVA (PVA98), and cellulose in an aqueous medium in the presence of paper mill sewage sludge. Reprinted with permission from Polym Degrad Stab 1999;64:305. © 1999 Elsevier Science [93].

However, this occurred only after an appreciably longer incubation time (Fig. 9) [93].

The observed behavior can be explained by considering that microbial strains present in the paper mill sewage sludge are particularly active in the biodegradation of PVA, most likely because of the selective pressure exerted by the large amount of PVA in the waste-water from paper factories. As expected, a close correspondence between the CO_2 evolution profiles and the decrease of PVA concentrations was observed.

By taking into account these results, paper mill sewage sludge was enriched in liquid cultures in the presence of 250 ppm PVA as the sole carbon and energy source. The microbial culture resulting from repeated sequential transfers was used as acclimated inoculum in respirometric tests [93]. In the presence of this inoculum, the rate and extent of biodegradation of PVA samples reached values much larger than those recorded in the presence of the previously tested inocula. Moreover, acclimation of the microbial population to PVA led to a substantial decrease in cellulose assimilation by the same microorganisms, revealed by the very low biodegradation (1.5%) of the cellulose sample after 28 days (Fig. 10) [93]. In this case, cellulosolytic and PVA-degrading activity were conflicting. Particular attention should therefore be devoted to the degradation biochemistry and to the occurrence of co-metabolic processes, particularly in the presence of polymer blends and composites.

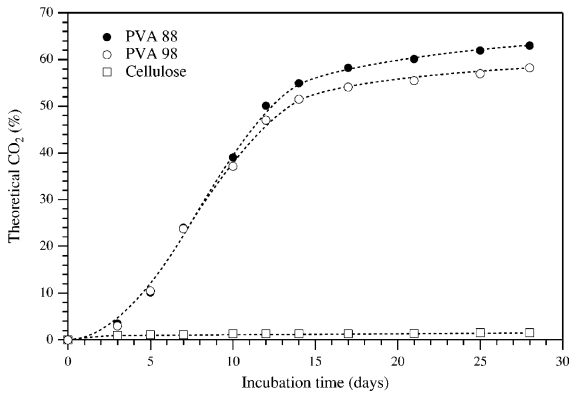


Fig. 10. Time profiles of mineralization of PVA-based blown films, 98% hydrolyzed PVA (PVA98), and cellulose in an aqueous medium in the presence of selected PVA-degrading microorganisms. Reprinted with permission from Polym Degrad Stab 1999;64:305. © 1999 Elsevier Science [93].

The reported data indicates that he biodegradability of a specific compound material and relevant items should not be thought of as universal. On the contrary, biodegradability is subject to the precise definition of infrastructure, time scale, and other conditions in which the biodegradation experiments are carried out.

3.4. Biodegradation of poly(vinyl alcohol) under anaerobic conditions

A detailed investigation of the anaerobic biodegradability of PVA was carried out by Matsumura et al. [95], using anaerobically preincubated microorganisms deriving from both river sediments and activated sludge from municipal sewage plants. In that study medium-high molecular weight (14 kD) PVA samples as well as low molecular weight (2.2 kD) samples were submitted to the indicated microbial inoculum under strictly anaerobic conditions.

The rate and extent of PVA biodegradation were monitored during the experiment by measuring biogas (carbon dioxide) evolution, TOC determination of the anaerobic cultures, and SEC evaluation of the polymer molecular weight and molecular weight distribution.

In the presence of anaerobic microorganisms from river water sediments, the overall CO_2 production promoted by cultures fed with low molecular weight PVA was similar to that recorded in the presence of D-glucose, used as a reference compound. Both rate and extent of CO_2 evolution were affected by PVA molecular weight, with lower values being observed for the higher molecular weight sample [95]. The percent biodegradation was evaluated by the actual to initial TOC ratio of the anaerobic cultures. The resulting values were consistent with the gas production. The overall biodegradation of the low molecular weight sample correspond to about 75%, whereas the high molecular weight samples reached lower levels (50–60%) during comparable incubation times. Lower but still significant biodegradability was recorded for both PVA samples in cultures inoculated with anaerobic sludge microorganisms.

SEC analysis showed that both low and high molecular weight fractions were effectively biodegraded under the adopted anaerobic conditions. However, it was also clearly evidence that low molecular weight fractions were degraded more quickly and preferentially by the anaerobic microorganisms. Moreover, the high molecular weight fractions remained as a residue in the anaerobic cultivation medium even after prolonged incubation time (125 days). This feature was more pronounced in the presence of anaerobic microorganisms deriving from activated sludge. In this case, an initial shift of the SEC peaks toward high molecular weights was observed without significant mineralization, as revealed by TOC measurements. Afterwards, the progressive shift at higher molecular weights was accompanied by a substantial decrease of the polymer concentration, although complete degradation was not reached after 6 months incubation [95].

Based on the reported results, the authors suggested a mechanism of PVA biodegradation under anaerobic conditions similar to the oxidation of hydroxyl groups to β-diketones followed by hydrolytic chain cleavage that is known to occur under aerobic conditions. However, under aerobic conditions the random scission of PVA chains generally caused a fast decrease in the molecular weight. On the contrary, the progressive shift toward higher molecular weight observed during the anaerobic biodegradation of PVA might suggest that a different biodegradation pathway exists under anaerobic conditions. This hypothesis should be demonstrated by further investigations. However, it was clearly established that under anaerobic conditions the potential biodegradation of PVA is influenced by the molecular weight.

The anaerobic biodegradation of PVA was investigated by monitoring the biogas evolution and TOC decrease in anaerobic respirometric tests performed in the presence of a microbial inoculum consisting of river sediment [113]. The potential biodegradability of disodium methylenemalonate/vinyl alcohol block copolymer was also investigated under these conditions.

A 60% PVA biodegradation was estimated by TOC decrease after 4 months of incubation, a value slightly larger than that of the malonate-type copolymer. In agreement with previous results, the copolymer propensity to biodegradation increased with the fraction of vinyl alcohol repeating units.

In contrast with the above investigations, PVA was found to show only minor degradation, ranging between 0 and 12% in 77 days, in anaerobic tests using digestion sludge according to ISO and ASTM standard procedures [114].

3.5. Conclusive chapter remarks

The ultimate biological fate of PVA appears to be largely dependent upon the kind of environment it reaches. Accordingly, high levels of biodegradation were observed in aqueous environments, even though these environments contained acclimated bacterial species often associated with PVA-contaminated waste water and sewage sludge.

On the other hand, moderate or negligible microbial attacks were repeatedly ascertained in soil and compost environments. Different hypotheses were tentatively suggested to account for these observations, such as the absence or scarce occurrence of PVA-degrading microorganisms in soil and compost matrices, the physical state of PVA-samples, and PVA's strong interactions with the organic and inorganic components of environmental solid matrices. All of these limit the ability of the PVA chain to cleave enzymes, and can be claimed as responsible for the small degradation extent recorded under the reported environmental conditions. Finally, observations on the preferential attack of low molecular weight fractions in soil and compost may suggest the occurrence of biodegradation mechanisms other than the most common random endocleavage, thus adding further substantiation to the cited hypothesis.

4. Blends and composites based on poly(vinyl alcohol)

4.1. Generalities

In recent years, environmentally degradable plastics (EDPs) have attracted growing attention because of their potential use in the replacement of traditional non-degradable plastic items deriving from fossil fuel feed stocks. In this connection, PVA has been widely utilized in specific merceological segments for the preparation of blends and composites with several natural, renewable polymers.

PVA's rheological properties, particularly its ability to produce highly resistant films and its hydrophilic character, account for the improvements in the mechanical properties and performances of natural polymers when mixed with PVA.

Accordingly, several investigations were carried out on the rheological and structural characterization of materials obtained by mixing PVA, either in solution or in the melt, with several natural polymers of vegetal, animal, and marine origin, such as cellulose, lignin, starch, silk, gelatin, chitin, and chitosan.

4.1.1. Poly(vinyl alcohol)/lignocellulosic materials composites

Nowadays, particular attention is devoted to the utilization of materials from renewable resources, such as agricultural over-productions and by-products as well as waste materials. In particular, these materials are investigated for the preparation of EDP items with the aim of reducing the negative environmental impact caused by the utilization of petrol-chemical compounds.

The search for EDP materials that can replace traditional synthetic non-degradable polymers in many applications, particularly in agricultural, dates back to the early 1970's.

A great deal of attention was focused on the production of biodegradable films for agricultural mulching, which would eliminate the cost required for their removal at the end of the life cycle. Deleterious environmental effects caused by burning or burial of non-degradable plastics would be reduced as well.

Films based on blends of cellulosic components were prepared over a wide range of compositions

by a solution-coagulation method using lithium chloride in, *N*,*N*-dimethylacetamide as the solvent [115]. Visual inspection and microscopic observations did not reveal any phase separation in the blends. Thermal and dynamic-mechanical testing confirmed the high level of miscibility between the two components in many amorphous regions, particularly in the presence of cellulose exceeding 60% by weight [116]. DSC studies demonstrated that the cellulose content drastically affected PVA crystallinity; this was completely suppressed in the presence of more than 70% cellulose. The high level of miscibility recorded in the amorphous regions was attributed to hydrogen bonding interactions among the hydroxyl groups of the two polymers [116]. Despite careful characterization, to the best of our knowledge no significant information on the biodegradability of PVA/cellulose blends is yet available in the literature.

Polymer composites containing lignocellulosic materials from agri-food industrial waste were prepared using sugar cane bagasse (SCB), orange and apple peels, and PVA as a continuous matrix [117,118]. Films were cast from PVA/water suspensions containing different types and amounts of lignocellulosic fibers, with or without the addition of plasticizers and crosslinking agents. In particular, glycerol and urea were utilized as plasticizers, the latter being a well-known nitrogen fertilizer chosen because of the PVA/lignocellulosic composites in agricultural applications.

Both the PVA/fiber ratio and the fiber type greatly affected the mechanical properties of the resulting films. In the case of PVA/SCB composites, the presence of a large amount of the heterogeneous, water-insoluble lignocellulosic raw material led to the formation of voids and dishomogeneities that strongly affected the integrity of the film [117]. It was also noted that composites containing 50% by weight of SCB showed a substantial increase in T_g with respect to that of PVA (from 50–55 to 65–70 °C). The T_g increase and the heterogeneity of SCB fiber distribution made PVA/SCB composites brittle and provided moderate strength, as indicated by mechanical testing. The tensile strength of the 50/50 PVA/SCB composite was 3.6 MPa, whereas a pure PVA cast film exhibited a value one order of magnitude larger (36 MPa) [117]. On the other hand, the use of glycerol and urea in 50/50 PVA/fiber blends yielded flexible films.

In all cases, PVA constituted the continuous phase in the blend, and the composite films were very sensitive to water and moisture. However, the addition of hexamethoxymethylmelamine or formaldehyde as crosslinking agents effectively enhanced the film's resistance to disintegration in water and reduced moisture uptake at high relative humidity (95%), with an improvement in overall mechanical properties [118].

Among the various PVA/lignocellulosic fiber composites, the biodegradation of a 50/50 PVA/SCB cast film, with and without other additives, was investigated both in aqueous and solid media by respirometric tests (Fig. 11) [117].

The mineralization of PVA/SCB cast films containing urea, glycerol, and 88% SD PVA was investigated in aquatic aerobic cultures inoculated with a selected PVA-degrading mixed bacterial population. Biodegradation levels approaching 35% were recorded after 40 days of incubation (Fig. 11) [117]. However, during the same period, PVA films that completely dissolved in the aqueous incubation medium showed the greatest extent of mineralization (45%), most likely because of the specificity of the microorganisms used as inoculum. These results seem to suggest that the PVA-degrading activity of selected microorganisms is depressed by the presence of a large

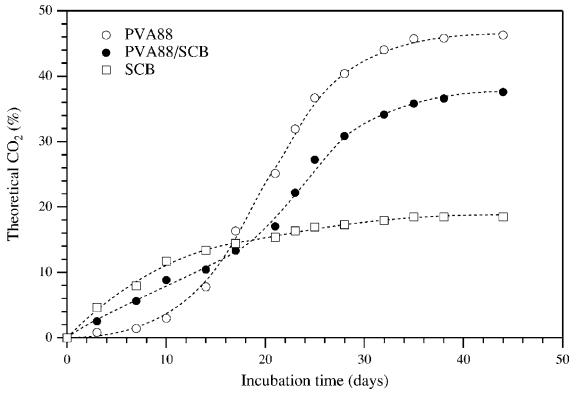


Fig. 11. Time profiles of mineralization of poly(vinyl alcohol)/sugar cane bagasse blend (PVA88/SCB) and relevant homopolymers (PVA88, SCB) in the presence of PVA-degrading microorganisms in an aqueous medium. Reprinted with permission from Macromol Symp 2000;152:83. © 2000 Wiley-VCH [117].

amount of lignocellulosic materials that also contain residual glucose, whose capability in reducing the activity of PVA-degrading bacteria was already reported [69].

The investigated PVA/SCB composites were prepared mainly for their utilization as agricultural mulching films. Therefore, their biodegradation propensity was investigated under conditions aimed at simulating soil burial. Tests were performed using a respirometric procedure aimed at evaluating the mineralization rate and extent of low biodegradability polymeric compounds in the presence of soil microorganisms. This procedure avoids large CO_2 production in the blanks, thus maximizing the signal-to-noise ratio [103].

Under the adopted conditions, SCB underwent a relatively intense mineralization process, reaching 35% of the maximum theoretical value after 150 days of incubation (Fig. 12) [117]. Within the same period, a 50/50 by weight PVA/SCB film exhibited a lower but still significant amount of mineralization (24%). In agreement with other reports [104–106], degradation of pure PVA did not exceed 5% under the adopted soil burial conditions.

In this case, the synthetic polymer seems to depress the biological attack of the lignocellulosic material, as opposed to what was observed in tests carried out in aqueous medium by using selected PVA-degrading microorganisms.

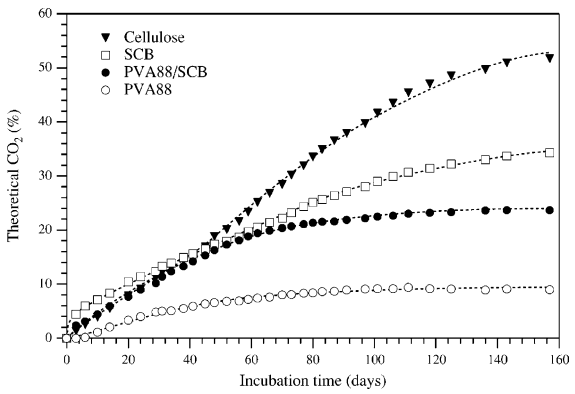


Fig. 12. Time profiles of mineralization of poly(vinyl alcohol)/sugar cane bagasse blend (PVA88/SCB) and relevant homopolymers (PVA88, SCB) in a simulated soil burial test. Reprinted with permission from Mecromol Symp 2000;152:83. © 2000 Wiley-VCH [117].

These results once more substantiate that environmental conditions, as well as the kind of microbial species present, strongly affect the biodegradation behavior of PVA and PVA-based materials.

4.1.2. Poly(vinyl alcohol)/starch blends and composites

Starch-based materials originally attracted a great deal of interest because of their low cost, real biodegradability, and renewable origins.

Starch is a mixture of amylose, a substantially linear polysaccharide of α-1,4 anhydroglucose units, and amylopectin, a highly branched polymer consisting of short α-1,4 chains linked by α-1,6 bonds. The relative proportion of the two components depends upon the starch source. In general, amylose is present as a minor component, its average content being around 25%. Nowadays, starch is widely available at a price much lower than that paid even for commodity polymers, thus offering an additional reason for its utilization as feedstock for biodegradable items. However, pure starch articles, even those derived from thermoplastic (e.g. gelatinized) starch, are usually brittle and moisture sensitive, thus strongly limiting their potential fields of application.

The relatively poor mechanical properties of starch-based materials have been tentatively ameliorated by adding large amounts of plasticizers, such as glycerol or ethylene glycol, or by modifying the chemical properties of starch itself. Since the first studies, improvement of its mechanical properties appeared to be bound to blending with PVA. Several studies showed that co-processing starch and PVA significantly improved the strength and flexibility of gelatinized starch. Cast films from water solutions of amylose or high-amylose starch are characterized by higher strength and elongation at break than cast films of starch alone [119]. The addition of formaldehyde as a crosslinking agent also enhanced the mechanical properties of cornstarch/PVA blends [120].

Starch-based films designed for potential agricultural mulching applications were obtained by heating and casting a mixture of starch, PVA, glycerol, and a surfactant, eventually crosslinked by the addition of formaldehyde [121]. The resulting films were then coated with a water-insoluble synthetic polymer such as PVC in order to improve their tensile strength.

However, the biodegradation propensity of these films was not investigated.

Degradable mulch films containing slow-release fertilizers were developed using PVA as the continuous phase [122]. Blend films were prepared by adding a urea–formaldehyde solution and other conventional plant nutrients to a mixture of starch, partially hydrolyzed PVA, and plasticizers. Films were also coated with poly(vinyl acetate) layers. Strong and flexible clear films were thus obtained. Mechanical characterization showed that the addition of ethylene glycol, starch, and urea increased the elongation at break of the composite films. Their degradation properties were investigated in accelerated weathering studies and by determining the dissolution rate of the prepared mulch film placed on top of farm soil in open field trials. The presence of starch and urea improved the tensile strength, elongation at break, and the dissolution rate of the films. However, these films were not submitted to true biodegradation experiments. Instead, their environmental degradability was claimed on the basis of weathering studies, thus assuming that the degradation process occurred as a consequence of the leaching of film components under the action of rain [122].

The possibility of the heat extrusion of mixtures consisting of starch, PVA, and water into a homogenous melt followed by mold processing was described by Bastioli et al. [123]. Extruded expanded foams of modified starch containing up to 10% PVA were also produced [124].

Shaped foam starch articles were obtained by baking a starch and water paste in heated molds; the items were formed as the starch gelatinized, expanded, and dried [125–127]. The resulting items were completely degraded in a few weeks in composting trials without any appreciable accumulation of residues.

An analogous procedure was utilized for the production of baked starch/PVA foamed articles in which the vinyl polymer was introduced in order to improve strength, flexibility, and water resistance [128].

Relatively little information is available on the compatibility and morphology of composites and blends based on starch-PVA mixtures. Characterization of solution mixtures indicated that the polysaccharide and the synthetic polymer are largely incompatible [129,130]. However, the improvement of mechanical properties of both cast and blown-extruded blend films suggested that there is at least a mechanical compatibility between the two components [121]. On the other hand, the process utilized in the preparation of PVA/starch mixtures could affect the compatibility. Accordingly, extruded starch/PVA films were expected to result in more homogenous dispersions because of the intense mixing and mechanical stress in the extruder, which helps to finalize the destructurization of starch granules. At least in the case of starch/PVA foamed articles, scanning electron microscopy (SEM) analysis indicated that the two polymeric components are mostly phase-separated, with starch granules immersed in a continuous PVA phase [128].

The potential biodegradation of these articles was investigated by recording both CO_2 production and O_2 consumption. Samples were placed in a closed respirometer using natural farm soil as the incubation substrate. After 82 days of incubation, soil samples supplemented with pure potato-starch trays showed a CO_2 evolution equivalent to 82% of the carbon content in the test sample. The mineralization extent recorded in the presence of the PVA/starch trays containing 10 and 20% PVA by weight was 73 and 65%, respectively, whereas a pure PVA sample underwent only 20% biodegradation. Moreover, test specimens retrieved from the soil were composed mainly of PVA. These results suggested that the vinyl polymer did not affect significantly the microbial assimilation of the polysaccharide in soil, at least under the investigated range of composition. However, no direct evidence of the possible enhancement of PVA biodegradability promoted by starch was attained. In fact, based on the adopted experimental set-up, it was impossible to attribute the observed CO_2 emission solely to the starch, the PVA, or to both components. In addition, gravimetric analysis could be strongly affected by the possible leaching of PVA from the foamed articles into the soil matrix.

The potential biodegradability of starch–PVA cast films crosslinked with hexamethoxymethylmelamine was investigated in respirometric tests by measuring the total CO_2 production resulting from the microbial mineralization of the test compounds confined in soil environment [104]. Crosslinked and uncrosslinked starch/PVA films containing 10–40% PVA were

examined, as well as control samples represented by pure corn-starch and PVA. Both the extent and rate of CO_2 evolution from corn-starch were very high, whereas soil alone and soil supplemented with pure PVA produced CO_2 at a lower rate and to a minor extent. Starch/PVA films degraded more slowly than pure starch, but still at a higher rate and to larger extent than pure PVA. Accordingly, the biodegradation was negatively correlated to the amount of synthetic polymer in the blends.

Interestingly, addition of the crosslinking agent to blends containing different amounts of PVA yielded cumulative CO_2 emissions similar to that recorded for uncrosslinked samples having the lowest PVA content. Apparently, crosslinking enhanced the mineralization of the PVA matrix contained in the blend films. However, the individual degradation rate and extent of each crosslinked polymer component was not investigated, thus limiting the validity of this assumption. Moreover, PVA-based films were not retrieved and their chemical and physical changes were not investigated.

Finally, it has to be mentioned that the addition of glucosidic materials to soil matrices could strongly enhance the mineralization of organic matter contained in soil, because of the so-called 'priming effect' [131]. Therefore, the recorded cumulative CO_2 emissions, and consequently the reported results, might be heavily affected by the addition of starch to the soil sample.

A detailed investigation aimed at understanding the degradation mechanism of PVA/starch based materials was recently carried out using an industrial grade Mater-Bi AF10H (trade mark) sample containing approximately 60% starch and natural additives, and 40% modified PVA and plasticizers [132]. In order to define the role played by each component in the biodegradation of the composite, degradation experiments were created using specific degrading microorganisms and enzymes for each blend main component (starch and PVA). Heterogeneous microbial populations such as those associated to sewage sludge were used as well.

The weight loss of Mater-Bi AF10H films was ascertained in liquid cultures inoculated with either activated sludge, a specific starch-degrading bacterium (*Bacillus subtilis*), or a PVA-degrading bacterium (*Pseudomonas vesicularis* var. *povalolyticus* strain PH) previously isolated from PVA-acclimated sewage sludge [133]. Enzymatic degradation tests were carried out using either commercially available α-amylase or the PVA-degrading crude enzyme obtained from the culture broth of *P. vesicularis* PH strain. An overall weight loss of about 40% was observed for specimens incubated in the activated sludge. The specimen weight decreased faster in cultures inoculated with the amylolytic bacterium; a degradation level of 45% was reached in a few days. In both cases the films maintained their shapes. A slightly larger weight variation (50%) was recorded when PVA-degrading *P. vesicularis* was utilized as the inoculum that caused the breakdown of films. A faster degradation was detected for specimens incubated in the presence of α-amylase, nevertheless film integrity was still maintained. On the contrary, in the presence of the PVA-degrading enzyme, film breakdown occurred early and 70% weight loss was reached.

The degradation extent of test materials as determined by gravimetric analysis cannot be related to their ultimate degradation or mineralization. Indeed, in degradation experiments performed in liquid culture, weight loss can be partially attributed to its dissolution into the aqueous phase. However, Ishigaki et al. [132] suggested that the weight loss might be due to the biological erosion of water insoluble PVA/starch-based plastics, releasing starch and/or PVA metabolites, such as low molecular weight organic acids and hydroxylated fatty acids or carbonyl compounds. These components would be easily assimilated by microorganisms in natural environments.

The degradation of Mater-Bi samples by activated sludge was tested by simulating the degradation processes occurring in natural environments. The reported results (ultimate weight loss did not exceed 40%) indicated that this type of PVA/starch material is not easily degraded under ambient conditions. Previous studies showed that the mineralization profile of analogous PVA/starch blend films leveled off at about 40% after 100 days incubation in the presence of activated sludge [134]. An erratic CO_2 evolution that did not exceed 75% of biodegradation followed during the next 200 days.

On the other hand, a faster rate and greater extent of degradation was reached using selected bacterial

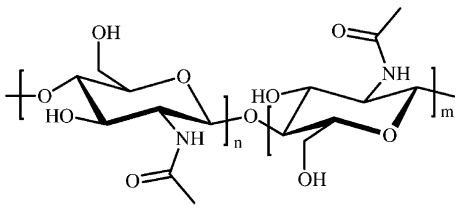
culture and/or specific enzymes able to catalyze the biodegradation of starch and PVA, which are the main components of the tested materials. Application of PVA-degrading enzyme was especially effective; the leakage not only of PVA but also of a significant amount of the starch fraction was supposed [132]. However, neither the PVA-degrading bacterium nor the selected enzyme show amylolytic activity. Therefore, the loss of the starch fraction appeared to be simply the result of its aqueous dispersion in water, as promoted by the degradation of the continuous PVA phase.

The higher degradation effectiveness promoted by pure enzyme solutions was attributed to four main factors: one, the high degrading enzyme activity at the start of the test, versus the lag phase that may occur in bacterial production; two, more favorable conditions for enzyme activity; three, the delay represented by the time required by bacteria to adhere to the plastic film, as opposed to enzymes, which act immediately upon contact; and four, the easier permeation of the enzyme into the plastic film bulk.

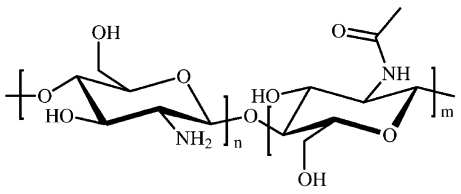
The slow and limited biodegradation of PVA/starch-based plastics in natural environments such as activated sludge may arise from the scarce distribution of naturally occurring PVA-degrading microorganisms and relevant enzymes. In fact, it was suggested that PVA-degrading bacteria seldom exist in natural environments, as they were detected only in four out of 100 screened soil samples [108]. On the other hand, it was also reported that microorganisms able to assimilate PVA are commonly distributed in various environments, even though long timeframes and selective pressure are required to reach a population density able to exhibit appreciable PVA-degrading activity [135]. This is most likely associated with the inducible nature of many PVA-degrading enzymes, thus substantiating the observed slow degradation under natural environmental conditions.

4.1.3. Poly(vinyl alcohol)/chitin blends and composites

Chitin and chitin derivatives were utilized in the preparation of miscible blends; these blends are among the various blends and composites made of



Chitin



Chitosan

PVA with natural polymers from renewable resources,

Chitin is a biodegradable natural polysaccharide and one of the most abundant biopolymers, mainly consisting of *N*-acetylglucosamine units connected via β-1,4 bonds. It is present in the exoskeleton of macroinvertebrates (insects, crustacea) and fungi. Chitin is quickly hydrolyzed by enzymatic routes, although it is highly resistant to chemical and physical agents.

Chemical derivatization of chitin may improve its processability. Indeed, the original polysaccharide is characterized by very low compatibility and solubility due to its strong crystalline structure.

Aoi et al. [136,137], described the syntheses of chitin-*graft*-poly(2-methyl-2-oxazoline) and chitin-*graft*-poly(2-ethyl-2-oxazoline) by reaction of partially deacetylated chitin with poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline), respectively. Blends of PVA and chitin-*graft*-poly(2-alkyl-2-oxazoline) were prepared in order to improve the environmental degradability of the vinyl synthetic polymer [138–140].

Miscibility of the chitin derivatives with PVA in blend films obtained by casting from aqueous solutions was demonstrated by DSC and FT-IR analysis [138–140]. The interactions of PVA with the graft chitin polymers were found to be roughly controlled by the number, chain length, and alkyl group of the poly(2-alkyl-2-oxazoline) branches. Thermal characterization of PVA/chitin derivatives blends showed that partially deacetylated chitin

increased the glass transition (T_g) of PVA while lowering its melting point (T_m) On the other hand, both T_g and T_m were depressed in blends containing the grafted chitin derivatives, thus indicating an increased mobility for the PVA chains. The tensile strength of the blends was comparable with that of pure PVA cast films. Moreover, the hydrophilicity increased by blending PVA with either partially deacetylated chitin or chitin-grafted derivatives.

The biodegradation behavior of chitin-based polymer hybrids, (PVA/partially deacetylated chitin and PVA/graft chitin copolymers) was determined under soil burial conditions. The relationships between their physical properties and degradation propensity were also investigated [96].

Degradation tests were carried out at time intervals in natural soil by monitoring the weight loss of both recovered chitin-based polymer blends containing 70–90% PVA and of control PVA films. Under the adopted conditions, PVA films scarcely degraded (89% recovered polymer), whereas the weight loss of the PVA/chitin derivatives blends ranged between 53 and 89% after 150 days incubation, thus suggesting that the PVA matrix was degraded. FT-IR analysis of recovered samples from soil burial tests showed the appearance of absorption bands that were assigned to ketone and β-diketone (enol-type) moieties. A correspondence between the recorded weight losses and β-diketone amounts was also evidenced.

The formation of β-diketone groups was attributed to a biologically selective oxidation process, in accordance with the biochemistry of enzymatic PVA degradation. However, the stereoregularity of the retrieved samples was unchanged, whereas biochemical investigations evidenced the preferential enzymatic attack of isotactic PVA sequences.

A slight decrease in the molecular weight of the polymer and a narrowing of its polydispersity were also observed, suggesting the preferential degradation of low molecular weight PVA fractions as well as occurrence of microbial attack restricted to the film surfaces.

Experimental data indicated that the thermal properties and miscibility of the blend might influence the degradation rate of the PVA component, thus suggesting that molecular level mixing can enhance the biodegradation of PVA.

In spite of the recognition of biologically originated β-diketones along the PVA chains, the reported results cannot conclusively account for the ultimate biodegradation of the vinyl polymer contained in the chitin-derivative blends, since the rate and extent of mineralization and the leaching of the synthetic polymer into the soil substrate were not analyzed.

One idea involving the leaching of the synthetic polymer indicates that the improved hydrophilicity as well as the larger water solubility of PVA/chitin-derivative blends could significantly affect the film persistence. The PVA leached out from the films could be then strongly adsorbed by soil components as previously reported [141,142].

4.1.4. Poly(vinyl alcohol)/gelatin blends

Among the various biopolymers, proteins are versatile materials that combine many suitable characteristics for technical applications, such as good processability both in the melt and in solution and good film-forming properties [143].

Blends based on proteic materials obtained by aqueous solution mixing display thermodynamic incompatibility over a wide range of compositions, both in the presence and in the absence of crosslinking agents. In spite of the distinct phase separation of the main components, the mechanical properties of cast films and molded items improved [144–147].

One of the most common proteic materials is the gelatin obtained from collagen, the main constituent of connective tissues in humans and animals. Gelatin is obtained by heating collagen above the helix-coil transition temperature. Gelatin consists of aminoacid residues in variable proportion and distribution along the macromolecular backbone.

The disposal of gelatin scraps generated by different manufacturing processes in the pharmaceutical, cosmetics, tannery, and food industries constitutes an environmental concern. This material strongly swells in water and has a large carbon and hydrogen content, which may lead to high oxygen demand once it reaches drainage systems, waste water treatment facilities, and eventually fresh water streams. Labor-intensive, expensive treatment is often required for correct management of gelatin waste disposal, driving the need for value-added solutions aimed at defraying the disposal costs. Accordingly, the applicability of gelatin

scraps from the pharmaceutical industry (waste gelatin, WG) as a component in the formulation of PVA blends and composites with another renewable agroindustrial by-product, SCB, as a natural filler was investigated [148].

In material processing, several variables may influence the ultimate properties of the products. Among them, the thermal properties and thermal history of the material often hold a crucial position in terms of the actual processability in the melt. In the case of gelatin, different transition temperatures is found depending on its source, because of water content, the drying process, and the composition and sequence distribution of amino and imino acid residues along the peptide backbone (primary structure) [149].

Potentially biodegradable PVA–waste gelatin (PVA/WG) films obtained by room temperature casting of water solutions were characterized by thermal and mechanical analysis [150,151]. The effectiveness of glutaraldehyde as a crosslinking agent in enhancing the mechanical performance of the resulting materials was also investigated. Virgin gelatin was used as the reference standard to gain a better understanding of the characteristics of WG and relevant PVA blends in the presence or absence of the crosslinking agent.

Thermal-gravimetric analysis evidenced that the thermal stability of WG was lowered by the presence of PVA. In fact, the thermal decomposition temperature of WG decreased from 201 to about 160 °C when blended with PVA [150]. A similar behavior was observed in the presence of virgin gelatin/PVA blends. The latter blends were incompatible, as indicated by the substantial independence of gelatin T_g from the blend composition. Therefore, it was assumed that in this case PVA acted as organic filler material for the virgin gelatin. The behavior recorded for WG/PVA blends was more complex, most likely because of the presence of glycerol as a waste material plasticizer. The glass transition temperatures of both PVA and WG components was slightly decreased by increasing the WG content, thus suggesting at least a partial compatibility between the two components, very likely mediated by glycerol. However, the formation of substantial, unmiscible blends of PVA and WG within the investigated composition range was confirmed by SEM, clearly showing the formation of a distinct two-phase system (Fig. 13) [151].

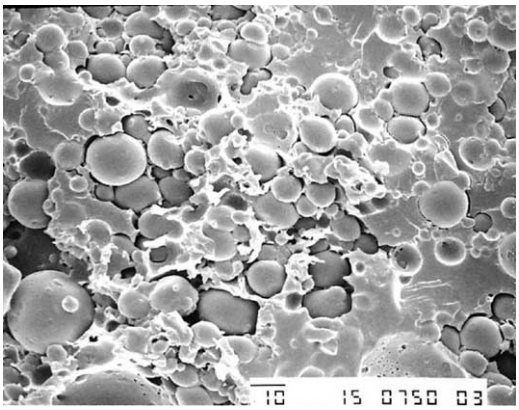


Fig. 13. SEM micrograph (750 ×) of the liquid nitrogen fracture of a 50/50 by weight PVA/gelatin blend film. Reprinted with permission from Biomacromolecules 2001;2:806. © 2001 American Chemical Society [151].

However, melting temperature determinations and tensile tests indicated a partial compatibility between the two components in PVA rich (80% by weight) systems. It was suggested that the amorphous regions of PVA and gelatin were compatible, or at least capable of interacting with each other in high PVA content systems. From a microstructural point of view, it appeared that PVA could induce protective action by forming an adsorption layer on the surface of globular particles of gelatin-rich phases [146].

Interestingly, analogous behavior was reported for blends of PVA with silk fibroin, another proteic material of natural origin [147].

Dynamic mechanical thermal analysis (DMTA) of WG/PVA films showed that the storage modulus of the blends decreased as the WG content increased. The same behavior was observed for the maximum of tan δ [151]. Tensile tests also showed a significant increase of the elongation at break after the addition of up to 20% WG to PVA. Correspondingly, both tensile strength and Young's modulus decreased. This behavior was attributed to the plasticization of the synthetic polymer promoted by the glycerol contained in WG. A drop of the investigated mechanical properties as measured against the values exhibited by WG itself was observed in blends containing more than 40% WG. The use of a crosslinking agent and the blending in aqueous solution with SCB, a fiber-rich lignocellulosic byproduct, were both evaluated in

order to improve the water resistance, thermal stability, and mechanical properties of WG/PVA films.

The thermal transition of the plasticized WG rigid blocks, recorded at 71 °C in the DSC analysis, shifted to higher temperatures and became less prominent as the glutaraldehyde content increased. Correspondingly, DMTA analysis showed a reduction of the storage modulus and an increase of the softening point. This behavior is in accordance with that reported by Fraga [149] for melt cast films crosslinked with formaldehyde. In fact, their DSC traces showed a broader temperature range of gelatin T_g, attributed to the crosslinking of gelatin chains involving the α-aminoacids present in the soft blocks with a consequent overall increase of structure stiffness.

Previous studies on WG cast films reported that both crosslinking with glutaraldehyde and blending with SCB improved the film cohesiveness and time of permanance when films were applied on soil [148].

The ultimate goal of the investigation was represented by the formulation of composite films based on hybrid polymeric blends and fillers from renewable resources for the production of self-fertilizing films. Therefore, a study of the biodegradation tendency of gelatin cast films containing PVA and/or lignocellulosic materials was carried out [148]. The influence of the PVA and natural lignocellulosic raw material, as well as of crosslinking agents on biodegradation rate and extent, on the films was ascertained in respirometric tests simulating soil burial conditions [103]. The biodegradation propensity was then compared with the thermal and mechanical characteristics of the blend films.

Films containing 17–20% of both PVA and SCB showed fairly limited biodegradation, not exceeding 40% mineralization after 30 days of incubation. On the contrary, pure WG films underwent about 60% biodegradation in the same time interval (Fig. 14) [152]. The detrimental effect on biodegradation was also observed for films containing the same percentage of either one of the fillers, although the synthetic polymer yielded a more evident negative influence.

The influence of the degree of crosslinking on WG biodegradation propensity was investigated in a previous study [148]. The reported results clearly demonstrated the negative influence of crosslinking on the biodegradation rate and extent. However, controversial results were obtained using increasing

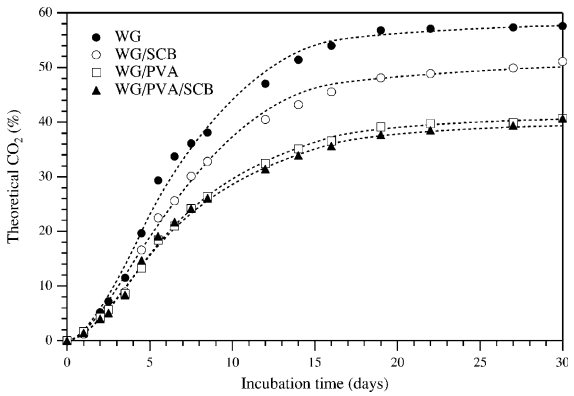


Fig. 14. Time profiles of mineralization of ternary blends of poly(vinyl alcohol)/sugar cane bagasse/waste gelatin (WG/PVA/SCB) and relevant binary blends (WG/PVA, WG/SCB) and pure waste gelatin (WG) in a simulated soil burial test. Reprinted with permission from Polym Degrad Stab 2001;73:549. © 2001 Elsevier Science [152].

amounts of glutaraldehyde. The addition of 0.25% glutaraldehyde to WG-based cast films containing 17–20% by weight of PVA and/or SCB did not induce any further reduction of the mineralization rate and extent (about 45% after 30 days of incubation) (Fig. 15) [152]. It seems that the negative effect induced on the bioassimilation by the presence of both PVA and SCB was strong enough to mask the effect of the dialdehyde at this concentration.

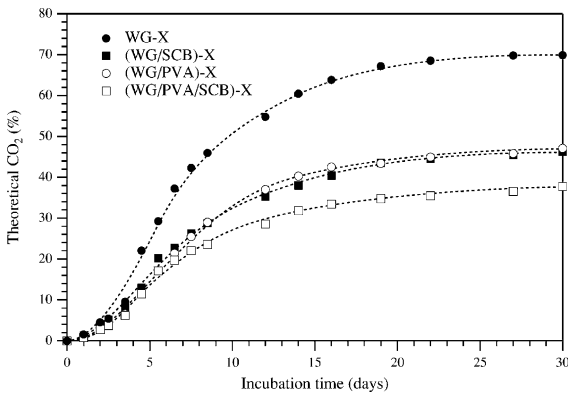


Fig. 15. Time profiles of mineralization of ternary blends of poly(vinyl alcohol)/sugar cane bagasse/waste gelatin (WG/PVA/SCB-X) and relevant binary blends (WG/PVA-X, WG/SCB-X) and pure waste gelatin (WG-X) based films crosslinked with 0.25% glutaraldehyde in a simulated soil burial test. Reprinted with permission from Polym Degrad Stab 2001;73:549. © 2001 Elsevier Science [152].

Experiments were then carried out to confirm the reduction of biodegradability caused by the addition of PVA to WG films, and to prove any stimulating effect of WG on the biodegradation of the synthetic polymer. Two series of WG/PVA cast films, one containing a constant amount of WG and variable amount of PVA, the other containing the same amount of PVA and variable amounts of WG were tested under simulated soil burial conditions. A pure WG film was also used as a reference material for the blends containing a fixed amount of WG. After 30 days of incubation, the mineralization profile of the WG sample reached a plateau at about 85% biodegradation (Fig. 16) [152].

Blend films containing at least 20% by weight WG reached extents of mineralization corresponding to 20%. All the other samples containing more than 80% PVA by weight experienced lower mineralization rate and extent (about 13% after 60 days), closely matching that of the pure PVA film (Fig. 17) [152].

The very large reduction in the biodegradation of WG-based films, caused by the addition of PVA, was tentatively attributed to a kind of physical coating of the proteic component by the vinyl polymer. This also agrees with the recorded immiscibility of the two macromolecular components. On the other hand, WG did not seen to favor the biological degradation of PVA by soil microflora, as indicated by the results recorded for different cast films containing increasing amounts of, WG, whose mineralization extent (12–14%) was strictly comparable to that of pure PVA [152].

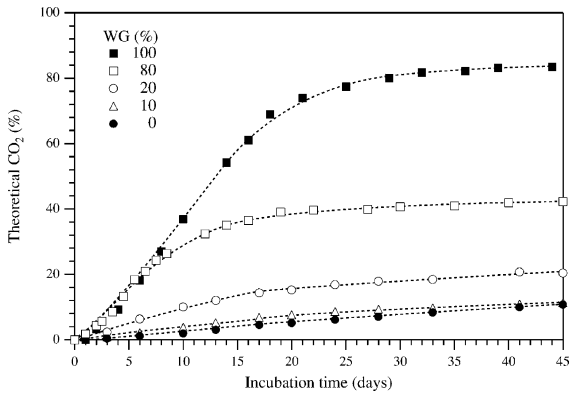


Fig. 16. Time profiles of mineralization of WG/PVA binary blends obtained by mixing 100 parts of waste gelatin (WG) and different amounts of poly(vinyl alcohol). Reprinted with permission from Polym Degrad Stab 2001;73:549. © 2001 Elsevier Science [152].

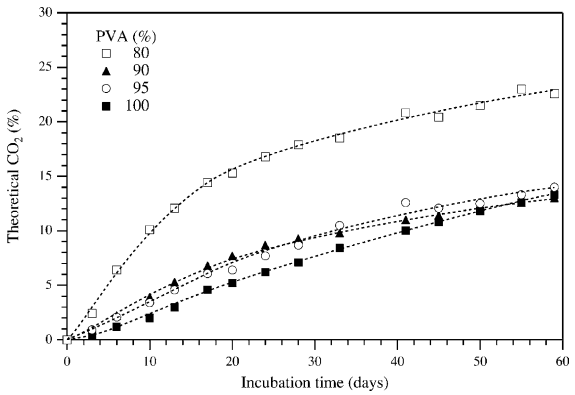


Fig. 17. Time profiles of mineralization of WG/PVA binary blends having different composition. Reprinted with permission from Polym Degrad Stab 2001;73:549. © 2001 Elsevier Science [152].

The fairly limited and slow mineralization of PVA under incubation conditions aimed at simulating soil burial was also reported [93,106]. This behavior was attributed to the strong and practically irreversible adsorption of the synthetic polymer by the different components of soil matrices, particularly clay [112].

4.1.5. Poly(vinyl alcohol)/poly(β-hydroxybutyrate) blends

Poly(ester)s of biosynthetic origin, such as poly(3-hydroxybutyrate) (PHB) and other poly(hydroxyalkanoate)s (PHA) (homopolymers and copolymers), are far from having a substantial impact on the widespread market of polymeric materials, even when restricted to considering environmentally degradable polymeric materials for packaging and biomedical applications. The major limiting factor is their relatively high price as compared with synthetic, biostable polyhydrocarbon polymers displaying comparable practical performances. Further drawbacks are connected to their production technology (work-up) and processing procedures (thermal sensitivity). Therefore, the potential blending of PHB with other less expensive synthetic polymers, eventually capable of improving the processing technology and their mechanical performances, represents an elective strategy for the production of PHB-based EDPs [153–156].

Recently, attention has been devoted to the preparation of both PHB/PVA hybrid blends [157] and graft copolymers [158,159] to obtain new

biodegradable polymeric materials with significantly improved toughness and ductility.

However, the potential biodegradability of polymer blends is not only determined by the biological assimilation of the individual components, but strongly affected by blend composition and phase structure (e.g. miscibility and crystallinity of the components), as well as by the surface to volume ratio of the investigated items.

Characterization of cast blends based on PHB and fully hydrolyzed PVA from hexafluoroisopropanol solution showed a partial miscibility of the two components in the amorphous region, depending upon blend composition, despite both polymers having a prevailing crystalline character [160]. This was expected to occur since the crystallization of the minor component was suppressed in the blends [161,162]. Investigation of the influence of PVA stereoregularity on the compatibility and crystallinity of cast films of PHB/PVA blends showed that syndiotactic PVA (sPVA) better provides compatible blends in a wide range of compositions [160,161]. This behavior was attributed to the ability of syndiotactic PVA to establish more extensive intermolecular hydrogen bonding interactions with the microbial polyester.

A PVA-grafted PHB copolymer was synthesized by an oligotransesterification reaction of racemic β-butyrolactone using porcine pancreatic lipase as a catalyst [158,159]. This synthetic material should have properties suited for acting as a versatile macromolecular compatibilizer in PVA/PHB blends.

The biodegradation behavior of a series of cast films obtained from PHB/PVA solutions having different compositions was monitored by BOD assay in respirometric tests carried out in the presence of a river water inoculum [162]. The effects of bulk and surface composition on the cast films, as well as of their crystallinity on the biodegradation potential, were also investigated.

BOD determinations revealed that the microorganisms from the river water inoculum could assimilate not only PHB and relevant blend films, but also PVA. It seemed likely that the inoculum would contain specific PVA-degrading species. However, it seems very likely that each polymer component in the blends was degraded through independent pathways, since the PVA-degrading microorganisms should be different from those utilizing PHB.

PVA also enhanced the mineralization rate of the PHB fraction in the blends. The accelerating effect of the PVA seemed to be related to the lowering of PHB crystallinity in polyester-rich blends, in accordance with previous findings showing that the PHB degree of crystallinity negatively affects the biodegradation rate [163].

Contact angle measurements demonstrated that the surface of PVA/PHB blends was more hydrophilic than that of PHB only films, most likely because of the preferential PVA dislocation at the surface regions as revealed by ATR FT-IR spectroscopy. The non-homogeneity of the film surface allowed the blends to swell more efficiently in water, thus helping to improve their propensity to biodegradation. In the early stage of the tests, the degradation rate recorded for PVA-rich films was larger than that of pure PVA, due to the plasticizing effect played by PHB, which reduced the crystallization propensity of PVA [162]. It was therefore concluded that blend compositions could effectively control the rate and extent of their biodegradation.

To confirm this hypothesis, the biodegradation behavior of PVA/PHB blend systems having a ‘compositional gradient’ was investigated in respirometric tests carried out in the presence of an inoculum from river water [164]. Ikejima and Inoue [164] define a ‘compositional gradient’ as a material whose chemical and physical properties change gradually along a specific direction throughout the bulk of the material.

It can be assumed that in the presence of solid substrates, such as those represented by PVA/PHB blend films, enzymatic reactions are restricted to the substrate surface in the early degradation stages and proceed into the inner layers once the surface has been eroded. Therefore, the generation of a compositional gradient in a blend system represents an attractive strategy to induce functional modifications capable of modulating the potential rate and extent of the material biodegradation.

In the reported study, solution-diffused PVA/PHB films were prepared by first casting a PVA solution onto teflon dishes, then adding an hexafluoroisopropanol solution containing equal amounts PHB and PVA on top of the former films. To obtain the best

compositional gradient, several experimental parameters, such as film drying rate, amount of solvent, radius of the teflon dish, and polymer concentrations, were varied and their relevant effects were thoroughly investigated [164]. In particular, a fast drying rate was shown to induce a large compositional gradient, revealed by the significant depression of the melting temperature and by the broadening of the melting PHB endotherm in DSC traces. This appears to be a consequence of the disordered crystalline structure of the blends. These results were confirmed by wide angle X-ray diffraction measurements of solution-diffused blends, which indicated a highly disordered structure of PHB in the blends [164].

The potential biodegradability of 50/50 by weight PHB/atactic PVA (PHB/aPVA) and syndiotactic PVA (PHB/sPVA) films prepared by solution-diffusion technique was investigated in respirometric tests. For comparison, the biodegradation of conventional PHB/sPVA films as well as that of pure PHB, aPVA, and sPVA films were determined. Solution-diffused PHB/aPVA blends were found to be slightly less biodegradable than the corresponding conventional cast films. This feature was more pronounced when the biodegradation extent (10% after 30 days) of solution-diffused blends was compared with that of PHB/sPVA cast films (20%). The observed behavior was attributed to the lower amount of exposed PHB on the gradient film surface as compared to the conventional solution-cast film.

The lower biodegradability of the PHB/sPVA film was attributed to the limited solubility of sPVA in water. The aPVA film reached a maximum biodegradation extent of about 10% after 15 days of incubation under the adopted conditions, whereas the sPVA cast film underwent only negligible degradation. A high level of mineralization (about 60%) was recorded for the pure PHB film [164].

The reported results indicated that microbial attack was restricted to the microbial polyester, whereas PVA microstructural characteristics, as well as the technique used in the blend preparation, strongly affected the overall biodegradation process of PHB/PVA systems.

Recently, a preliminary investigation into the biodegradation of graft copolymers and cast blends [165,166] from hexafluoroisopropanol solutions of PVA (88% HD) and synthetic atactic PHB was carried out. Respirometric tests were performed in aqueous medium using a PVA-degrading selected bacterial culture as the inoculum [167]. The biodegradation of pure PVA and pure atactic PHB samples with number average molecular weight of 0.6 and 10 kD for the graft copolymer and blends, respectively, was also ascertained under the adopted conditions. An appreciable mineralization of atactic PHB oligomers, graft copolymer, and the blends was observed early, reaching a plateau corresponding to a similar biodegradation extent (37–42%) after 6 weeks of incubation. Microbial assimilation of pure PVA and of high molecular weight (10 kD) synthetic PHB was delayed, displaying a lag phase of about 7 days. However, PVA reached about 50% mineralization, whereas the biodegradation profile of high molecular weight atactic PHB slowed down to an almost constant value of about 15% mineralization. SEC analysis demonstrated that the assimilation was restricted to the low molecular weight fraction (Fig. 18) [167].

These results are in partial agreement with previous reports on the unusual biodegradation behavior of amorphous high molecular weight atactic PHB, whose attack by specific PHB-depolymerase seems to take place only in the presence of a crystalline phase [165,168,169]. Consequently, PVA appears to enhance the biodegradation of amorphous high molecular weight PHB in PVA/PHB blends. This behavior is in agreement with a previous report on

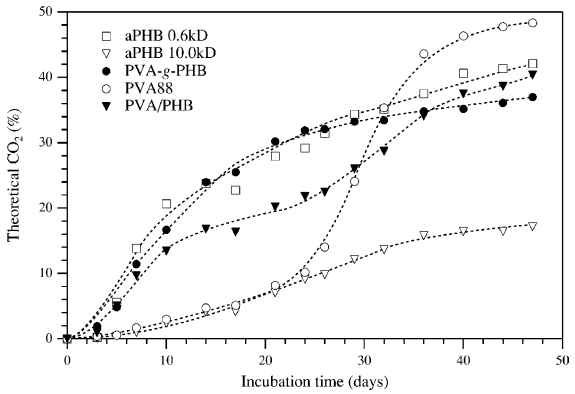


Fig. 18. Time profiles of mineralization of PVA/atactic PHB blend (PVA/PHB) and graft copolymer (PVA-*g*-PHB), and relevant homopolymers in the presence of PVA-degrading microorganisms. Reprinted with permission from Biorelated polymers. 2001.p.329. © 2001 Kluwer Academic [167].

the degradation of PVA blends with biosynthetic polyester samples [162]. It is worth mentioning that PVA-degrading microorganisms assimilated the atactic PHB oligomers, thus indicating that two specific biochemical routes may coexist in this microbial inoculum, without mutual catabolic repression.

4.1.6. Poly(vinyl alcohol)/poly(ε-caprolactone) blends

The production of PVA-based blends with other biodegradable synthetic polymers represents another field of interest. The good physical-mechanical properties of PVA can indeed promote a sort of upgrading of the synthetic aliphatic polyester, whose processability and ultimate performances are cumbersome and unsatisfactory to service requirements. On the other hand, their potential biodegradation varies from good to excellent under various environmental conditions.

A preliminary investigation was carried out through the preparation of blend films of PVA and poly(ε-caprolactone) (PCL) [170]. PCL is a semicrystalline thermoplastic polymeric material that can be effectively assimilated by many bacteria and fungi. These microorganisms are distributed over many kinds of environmental habitats, including landfill leakage, compost, sewage sludge, forest soil, farm soil, paddy soil, weed field soil, roadside sand, and pond sediments [171].

PVA/PCL films were obtained by casting hexafluoroisopropanol solutions of both polymers on teflon dishes. PVA was shown to have almost the same crystallinity in the pure state and in the blends, whereas crystallinity of the PCL component decreased as the PVA content of the blend increased [170].

Attention was mainly focused on the influence of PVA on the biodegradation of PCL. The potential biodegradation of the films was investigated by BOD measurements in axenic cultures of a specific PCL-assimilating actinomycete that was isolated from the compost deriving from the organic fraction of household waste. That microorganism did not grow in the presence of PVA as a sole carbon source, whereas a significant mineralization of pure PCL occurred. However, the PVA/PCL blends were not assimilated at all, even at a substantial PCL content (80% by weight). Apparently, PCL degrading activity was physiologically inhibited by the presence of PVA, which appears to exert a kind of physical block against the accessibility of PCL by the active esterase.

The biodegradation of pure PCL film was completely suppressed when the cultures were supplemented with PVA, even when PCL mineralization was already started. It appeared that addition of PVA to the aqueous culture medium caused a change in the surface properties of PCL films that strongly refrained inhibited their propensity toward biodegradation. Indeed, the contact angle values of PCL films suspended in distilled water dramatically decreased on addition of a PVA solution. This result proved that PVA tends to strongly adsorb on the PCL surface, substantially depressing the hydrophobicity required for guaranteeing the accessibility of the polymer bulk to esterase enzymes [170]. Although these results are impressive, the lack of direct information about the influence of PVA on the production and activity regulation of PCL-hydrolytic enzymes leaves us unable to rule out the occurrence of negative biochemical effects as an additional factor.

Detailed investigation of PVA/PCL film biodegradation in the presence of specific PVA-degrading microorganisms and mixed cultures including free and mixed enzymes should afford more information about the role played by each polymeric component in the environmental biodegradability of these materials. This information should help establish if polyester enzymatic degradation leading to small organic acid fractions can influence the biochemical route of PVA degradation, and if PVA biodegradation can affect PCL assimilation.

4.2. Conclusive chapter remarks

The potential replacement of traditional nondegradable plastic items from fossil fuel feed-stocks with biodegradable natural and synthetic polymers has attracted a great deal of attention. The usually poor mechanical properties of natural polymers can be efficiently improved when mixed with PVA to give blends and composite matrices. However, with the noticeable exception of cellulose, natural polymers such as starch, gelatin and lignocellulosic materials produced incompatible PVA blends.

Incompatible blends and composites were often found to undergo biodegradation to an extent well

below that expected as based on their chemical composition. In particular, no significant evidence exists regarding the potential stimulating effect played by natural polymers on the rate and extent of PVA mineralization. On the contrary, the poor biodegradability of PVA in the absence of suitable microorganisms appears to have a negative influence on the mineralization of materials utilized in the preparation of blends and composites. This effect is particularly evident in the case of PVA blends with gelatin and PCL, where the apparent depletion of the hydrophobic character typical of gelatin and PCL appears to be primarily responsible.

On the other hand, chemically modified chitin appears to improve the environmental degradability of the vinyl synthetic polymer in PVA-based miscible blends.

It must be mentioned that the activity of specific PVA-degrading enzymes could be strongly affected by the nature of the natural polymeric component in PVA-based blends. This is the case in polysaccharide matrices, whose repeating glucosidic unit was found to induce catabolic repression of PVA oxidase.

5. Vinyl alcohol block and graft copolymers

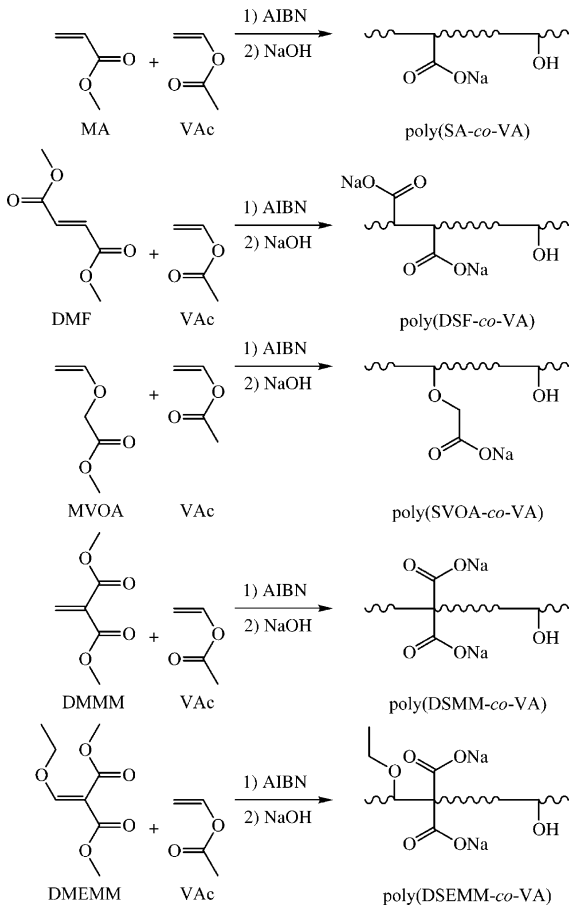
5.1. Poly(carboxylate-co-vinyl alcohol)

In the field of water-soluble polymers, high molecular weight poly(carboxylate)s such as poly(sodium acrylate) have been used as dispersing agents and detergent builders, replacing polyphosphates, the well-known eutrophycization inducers.

However, poly(carboxylate)s are known to be generally resistant to biodegradation, which presents environmental risks, particularly in large-scale applications and in the industrial field. Consequently, many efforts were spent in designing blocky copolymers comprised of PVA segments in order to obtain biodegradable water-soluble poly(carboxylate)s. Several types of water-soluble copolymers containing PVA sequences were synthesized by free-radical copolymerization procedures [113,172–174].

Functional vinyl monomers such as methyl acrylate (MA) and methyl vinyloxyacetate (MVOA), as well as 1,2-dicarboxylic acid (dimethyl fumarate, DMF) and 1,1 dicarboxylic acids such as diethyl methylenemalonate (DEMM) and diethyl ethylenoxymethylenemalonate (DEEMM) were copolymerized with vinyl acetate (VAc) by using 2,2′-azobisobutyronitrile (AIBN) as a free-radical initiator. Vinyl acetate blocks of different lengths were obtained by varying the comonomer molar ratio. Subsequent alkaline saponification of the resulting copolymers afforded the target vinyl alcohol copolymers (Scheme 9).

Thorough investigations were carried out in order to evaluate both the requirements of vinyl alcohol block length, as well as the influence of the type of functional vinyl monomers on the copolymer degradation by specific PVA-degrading microorganisms and enzymes. Indeed, degradation of vinyl alcohol blocks, in principle mediated by specific



Scheme 9. Synthesis of vinyl alcohol copolymers containing carboxylate groups [113,172–174].

PVA-degrading enzymes, could be influenced by both steric and electrostatic factors bound to the microstructure of the copolymer chains.

The minimum length of PVA blocks in the poly(carboxylate) copolymer needed for recognition by the PVA-degrading enzyme was then investigated. Tests were carried out in the presence of a selected PVA-degrading mixed culture using compositionally homogenous acrylate copolymers containing different amounts of vinyl alcohol units [172].

In the case of poly(sodium acrylate-*co*-vinyl alcohol) poly(SA-*co*-VA), BOD measurements performed on liquid cultures inoculated with PVA-degrading microbes containing mainly *Arthrobacter* sp. Evidenced the high biodegradability of copolymers containing more than about 80 mol% vinyl alcohol groups. A significant reduction of BOD values was recorded for poly(SA-*co*-VA) samples containing less than 75 mol% vinyl alcohol groups. SEC analysis indicated that poly(SA-*co*-VA) samples having a number average block length of more than about 5–6 vinyl alcohol units were cleaved by the selected microorganisms [172].

The same poly(SA-*co*-VA) copolymer that was degraded by the PVA-degrading mixed culture was mineralized by activated sludge microorganisms, suggesting that these polymers can be considered to be environmentally biodegradable. However, the biodegradation mechanism of poly(SA-*co*-VA) occurring in the presence of an activated sludge sample that did not contain specific PVA-degrading microorganisms was not clarified.

Enzymatic attack of biodegradable PVA segments may experience steric and electrostatic effects, caused the presence of neighboring functional groups. Therefore, the biodegradability of 1,1-dicarboxyl type copolymers was investigated in comparison with that of the corresponding 1,2-dicarboxyl type copolymers [113]. Poly(disodium methylenemalonate-*co*-vinyl alcohol) poly(DSMM-*co*-VA) [174], and poly(disodium ethoxymethylenemalonate-*co*-vinyl alcohol) poly(DSEMM-*co*-VA) were synthesized (Scheme 9). Their biodegradability was compared to that of poly(disodium fumarate-*co*-vinyl alcohol) poly(DSF-*co*-VA) in the presence of symbiotic PVA-degrading microorganisms tested on high molecular weight PVA. BOD measurements combined to SEC analysis were utilized to monitor the biodegradation process.

It was observed that 1,1-decarboxyl type copolymers underwent greater biodegradation than the corresponding 1,2-dicarboxyl copolymers having the same vinyl alcohol content [113]. Among the 1,1-dicarboxyl type copolymers, the biodegradability of poly(DSMM-*co*-VA) was larger than that recorded for poly(DSEMM-*co*-VA). It was suggested that a larger length of vinyl alcohol blocks was required in order to make the substrate suitable for PVA-degrading enzymes.

The steric hindrance exerted by ethoxyl groups apparently decreased the susceptibility of poly(DSEMM-*co*-VA) to enzymatic attack; this effect was lessened by a substantial increase of PVA block length. The minimum PVA block chain length required for biodegradation was about 2–3 and 3–4 monomeric units for poly(DSMM-*co*-VA) and poly(DSEMM-*co*-VA), respectively. A minimum block length of 5 and 7 vinyl alcohol units was necessary for the cleavage of the 1,2-dicarboxy type poly(DSF-*co*-VA) and P(DSMM-*co*-VA), respectively [113].

It was suggested that the recorded differences in biodegradation rate and extent might result from the different conformations of the polymers in aqueous solution. Apparently, 1,1-dicarboxyl type copolymers had a greater propensity to expand in solution than 1,2-dicarboxyl type ones because of the large steric hindrance created by the bulky 1,1-dicarboxyl groups of the methylenemalonate units in the polymer chains. This characteristic could favor the access of the biodegradable chain segments represented by vinyl alcohol blocks to PVA-degrading enzymes [113].

Further investigations were carried out by using the cell-free extracts of *A. faecalis* KK314, which was isolated from a river water inoculum [174], as a source of the PVA cleaving enzymes PVA dehydrogenase (PVADH) and β-diketone hydrolase [175]. Poly(disodium fumarate-*co*-vinyl alcohol) poly(DSF-*co*-VA), poly(disodium vinyloxyacetate-*co*-vinyl alcohol) poly(DSVOA-*co*-VA), and poly(disodium methylenemalonate-*co*-vinyl alcohol) poly(DSMM-*co*-VA) (Scheme 9) having different molecular weights and vinyl alcohol content were submitted to the action of the above PVA-cleaving enzymes.

In the case of poly(DSF-*co*-VA), a significant increase in the PVADH activity was observed in the presence of an average PVA block length larger than seven monomeric units. On the other hand, PVADH from *A. faecalis* KK314 was shown to require a minimum vinyl alcohol block length corresponding to about three units in isotactic sequence [174]. The preferential PVADH activity toward isotactic sequences was also confirmed by ^{1}H-NMR studies, showing the disappearance of isotactic triads in PVA samples treated with the PVA-cleaving crude enzymes. The observed differences were attributed to steric and electrostatic effects caused by the neighboring carboxylate groups.

Vinyl alcohol trimers and pentamers having bulky terminal groups were synthesized to gain a better understanding around the dependence of the biodegradability of PVA copolymers on PVA block length. These compounds were used as model substrates of PVADH [175], as well as of symbiotic PVA-assimilating microbes and of isolated PVA-degrading bacteria *A. faecalis* KK314 [58] and *Pseudomonas* sp. 113 P3 [176].

BOD measurements indicated that *A. faecalis* KK314 readily assimilated the isotactic pentamer as well as high molecular weight PVA samples. A slightly lower biodegradation rate was observed in the presence of the isotactic-type trimer. The atactic trimer underwent only moderate biodegradation, whereas 2,4-pentane diol, corresponding to the vinyl alcohol dimer, exhibited no degradation. In addition, no significant dependence of the biodegradability on the molecular weight of PVA was observed for samples longer than the octamer [58].

In the presence of *Pseudomonas* sp. 113 P3, high molecular weight PVA samples were better assimilated than low molecular weight model compounds. The vinyl alcohol dimer was not assimilated by the selected bacterial strain, and a significant influence of main chain stereochemistry was observed.

Similar results were obtained using symbiotic PVA-degrading microorganisms as the inoculum. Based on these results, it was established that the required minimum length of the vinyl alcohol block corresponded to a PVA trimer with a preferential isotactic-type configuration.

This hypothesis was substantiated by measuring the PVADH activity in the presence of various substrates, including 1,2-diols and monoalcohols. The isotactic pentamer induced higher PVADH activity among the tested polyols. Lower values were recorded using the atactic-type trimer, whereas no significant enzyme activity was observed in the presence of 2,4-pentanediol or using 1,2-diols and monoalcohols such as 1-butanol and 2-butanol [175].

Therefore, it can be hypothesized that the biodegradation of functional poly(carboxylate)s containing vinyl alcohol blocks occurs in two steps. The primary biodegradation is promoted by the cleavage of vinyl alcohol blocks mediated by specific PVA-degrading enzymes (PVADH and hydrolases) to give low molecular weight fractions. These can be further assimilated in a second step by non-specific PVA-degrading microorganisms.

5.2. Acrylic acid–vinyl alcohol graft copolymers

The hydrophilic character and gelling capability of PVA were exploited for the production of potentially biodegradable poly(acrylate)s. The vinyl polymer was used as a crosslinking agent in super-absorbent systems for applications in high capacity absorbing devices such as diapers, hospital products, and thickening agents.

PVA functionalization was achieved either by condensation-elimination reactions (e.g. tosylation-detosylation) to give randomly distributed double bonds, or by condensation with *p*-styrene sulfonyl chloride leading to functional groups containing double bonds pendant out from the PVA chains [177]. The functionalized polymers were then reacted with partially neutralized acrylic acid to form three-dimensional networks characterized by high-swelling rate and capacity in aqueous media. In principle, these materials should be biodegradable because of the biodegradability of PVA, leading to low molecular weight poly(acrylic acid), depending on the ratio of acrylic acid to functionalized PVA. The swelling behavior of different PAA-vinyl alcohol networks was investigated in buffered water solutions, and very large super-absorbent properties were detected. The dynamic swelling profiles in α-chymotrypsin and papain solutions were also used as an indication of the potential biodegradability of PVA links between PAA chains in the network. Swelling ratios were found to decrease within a few minutes upon the contact with

the enzyme solutions, thus indicating the start of enzymatic degradation leading to the loss of the three-dimensional structure of the investigated materials [177]. However, the ultimate environmental degradation of these super-absorbent polymeric systems may be definitely assessed only after further investigation in the presence of specific PVA-degrading microorganisms and enzymes.

5.3. Ethylene–vinyl alcohol copolymers

Copolymers of ethylene with vinyl alcohol are currently produced by the hydrolysis of poly(ethylene–vinyl acetate) synthesized by radical copolymerization of ethylene and vinyl acetate in methanol solution [178]. The ethylene content of commercial samples ranges between 30 and 45 mol%, which makes these systems practically insoluble in water. Poly(ethylene-*co*-vinyl alcohol) (EVOH) copolymers are currently utilized in food packaging because of their very effective gas barrier properties.

The increasing attention paid to the production of environmentally friendly materials stimulated several studies regarding the possible application of EVOH and their blends with natural polymers as biodegradable materials. Since the early 1990s, commercial grade EVOH/destructurized starch blends were produced and sold by Novamont under the trade name of Mater-Bi [179].

Little is known about the effective biodegradability of EVOH, and few investigations were carried out on both pure EVOH and EVOH/starch blends. In the latter case, it was found that the natural component of blend films can be effectively hydrolyzed by extracellular glucosidase when exposed to the metabolic activity of lake water and activated sludge microorganisms, even if highly shielded by the interpenetrating structure of the synthetic polymer. It was also proposed that the EVOH matrix was attacked by microorganisms adhering to the specimen surface [134].

Two EVOH/starch samples having different compositions in terms of both starch content and ethylene/vinyl alcohol ratio (trade name Mater-Bi AF05H, 60% starch, 40% EVOH containing 40 mol% ethylene; trade name Mater-Bi AF08H, 60% starch, 40% EVOH containing 20 mol% ethylene) were submitted to biodegradation tests along with a pure EVOH sample (40 mol% ethylene). Noticeable degradation levels after a very prolonged incubation time (about 80–90% after 300 days) were recorded for the AF05H and AF08H samples in aerobic respirometric tests carried out using activated sludge as the microbial inoculum. A relatively faster biodegradation rate was detected for the AF08H specimens, most likely due to its more hydrophilic vinyl alcohol copolymer, capable of enhancing the starch accessibility by enzymes. However, film residues retrieved from the degradation environment at the end of the tests were composed mainly of the synthetic component [180].

Three EVOH samples having the same chemical composition (60 mol% vinyl alcohol) but different molecular weights (12, 30, and 60 kD) and crystallinity (31, 4.7, and 58 J/g) were also tested under the same experimental conditions. The overall extent of biodegradation after 200 days of incubation reached 18, 26, and 9%, respectively. The highest value was reached in the presence of the amorphous sample in spite of its high molecular weight.

A lag phase as long as 10 weeks occurred before the start of significant EVOH mineralization of the crystalline samples by activated sludge microorganisms. Accordingly, it was suggested that the crystallinity of the synthetic component in EVOH/destructurized starch blends may play a fundamental role in determining their potential biodegradability [180].

A low rate of degradation was detected in the presence of pure EVOH samples using an amylolytic actinomycete, whereas a high biological activity was recorded in respirometric experiments when grown in the presence of EVOH/starch blends. In any case, the recorded biodegradation did not exceed the value expected from the content of the natural component [181].

The anaerobic biodegradation of Mater-Bi based films (AF05H and AF08H) was also investigated according to a standardized test procedure using sewage sludge from the primary digestion of a domestic biosolid treatment facility [182]. Degradation rate and extent were monitored during the experiment by measuring CH_4 and CO_2 evolutions in the headspace of serum vials [183]. Under anaerobic conditions, the first stage of biodegradation was represented by the breakdown of the polymer

chains into small fragments promoted by extracellular enzymes. Next, acetogenic bacteria took over to produce volatile fatty acids and esters along with CO_2 and H_2. The methanogenic step usually follows the acetogenic phase as a result of the ultimate biodegradation of fatty acid and esters under anaerobic conditions. Accordingly, CH_4 determination represents a useful parameter in the evaluation of the anerobic biodegradability of polymeric materials. Microbial conversion to methane reaching 30% of the theoretical value was recorded for the investigated Mater-Bi samples. Specimens recovered at the end of the biodegradation test were rather brittle because of the loss of physical strength. It is worth noting, however, that the recorded methane evolution was lower than the theoretical value (60%) expected for the complete digestion of the natural component present in both samples. Moreover, infrared spectroscopic analysis for the degraded samples did not evidence the formation of new functional groups. These results suggested that the anaerobic microbial assimilation was restricted to the starch component of the tested materials [183]. Nevertheless, ^{13}C NMR spectra clearly showed a significant increase in the intensity of resonances centered about 22, 26, 31, 35, and 40 ppm. This effect was more pronounced for the AF08H sample having the largest content of vinyl alcohol units. Therefore, it was suggested that the cleavage of polymer chains gives rise to the formation of functional end-groups [183].

Depending upon the exposure time, a 30–40% weight loss was recorded for Mater-Bi AF05H films in aerobic composting degradation experiments carried out with green waste under lab-scale conditions [184]. These experiments were compared with the enzymatic hydrolysis of the same sample as mediated by specific *α-glucoamylase* and *β-glucoamylase* produced by *Bacillus licheniformis* and *Aspergillus oryzae*, respectively.

The enzymatic degradation test revealed a rather limited dissolution (30% by weight) of carbohydrates from Mater-Bi films, whereas approximately 50% weight loss was observed. The 20% difference was attributed to the enzymatic attack of other components besides starch. However, no definite information regarding the biodegradability of the synthetic component of Mater-Bi samples was gained due to the lack of information about the enzymatic and composting degradation of pure EVOH samples.

The biodegradability of ethylene–vinyl alcohol copolymers has been recently investigated by means of radio-respirometric measurements of ^{14}C-labeled EVOH samples incubated for 500 days [185]. Radio-respirometric determinations represent a useful technique for monitoring small changes in a test material, especially when the biodegradation process is expected to occur at a very low rate. The determination of $^{14}CO_2$ deriving from the mineralization of labeled polymer samples can be performed very carefully by liquid scintillation counting, leading to reliable results collected over a long testing time [186].

Experiments were performed in a mineral culture medium inoculated with two fungal species, *Penicillium simplicissimum* and *Fusarium redolens* [185]. Films used in the degradation tests were obtained by extrusion of EVOH containing 44 mol% ethylene. Before testing, some of the films were exposed to UV irradiation in order to mimic abiotic environmental photodegradation. The sample weight loss was then compared with the amount of CO_2 produced by fungal degradation of labeled samples.

During the reported investigation, degradation levels significantly larger than that of the sterile controls were recorded for UV-irradiated EVOH samples maintained in the fungal-inoculated aqueous medium. On the contrary, the differences between inoculated and sterile EVOH specimens were very small in the case of non-irradiated samples. The maximum level of degradation recorded after 500 days of incubation was only 2.25%. Structural characterization of the recovered samples displayed a small increase in the carbonyl index as compared with the starting films.

The abiotic UV-degradation was shown to produce a decrease in the sample molecular weight, whereas the fungal activity did not induce any change. However, the molecular weight of irradiated specimens slightly increased after exposure to the biotic environment [185].

In conclusion, the reported study clearly showed that EVOH samples underwent only very limited changes of their molecular weight, crystallinity, and functional group content when submitted to biodegradation experiments. These results do not agree with those reported in previous studies on

the biodegradation of EVOH/starch blends, in which the degradation of at least about 80% of the synthetic component accompanied by a 13% reduction of the molecular weight was reported [180].

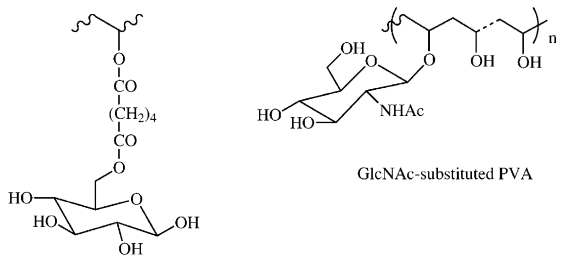
In a different investigation, several polymers, including a pure EVOH sample of unspecified ethylene–vinyl alcohol ratio, were exposed to an estuarine benthic community for one month. The metabolic responses due to the biological degradation of the tested materials were monitored by the flux variations of dissolved nutrients (ammonia, nitrate and nitrite, phosphate, and silica) and dissolved inorganic carbon (DIC) [187]. During the test period, EVOH did not show any significant degradation, demonstrated by the substantial identity of DIC fluxes from cores dosed with EVOH and controls.

Kunkel and Seo [188] investigated the enzymatic degradation of several polymers, including EVOH samples with ethylene contents ranging between 30 and 45 mol%. This study was aimed at mimicking polymer digestibility in the human digestive tract and/or by gut microflora. Accordingly, an enzyme cocktail containing α-amylase, amyloglucosidase, peptidase, protease, invertase, and lipase of both animal and microbial origin was utilized. On the basis of weight loss measurements it was noted that the used EVOH films were not degraded at all by the selected enzymes.

The biodegradation of EVOH (38 mol% ethylene) was investigated by weight loss and CO_2 evolution measurements in respirometric tests carried out in the presence of marine sediments and selected marine microorganisms [181]. No significant degradation was recorded, but a marine actinomycete was found to grow in the presence of EVOH as a sole carbon source, although at a rate too slow to be appreciated by respirometric measurements.

5.4. Glycosylated poly(vinyl alcohol)

Among the various PVA copolymers tailored to produce polymeric material with improved environmental compatibility and propensity to biodegradation, the enzymatic synthesis of sugar-branched polymers consisting of glucose, fatty acids, and poly(vinyl alcohol) was recently reported [189]. Intense research activities were devoted to the preparation of synthetic polymers containing sugar branches that would allow for the utilization of renewable resources in several industrial applications, such as surfactants and hydrogels [190,191].



Scheme 10. Glycosylated poly(vinyl alcohol) [192,194].

A PVA main chain esterified with adipic acid as a spacer arm and glucose as sugar moiety is an example of a sugar-branched PVA copolymer. The enzymatically synthesized 6-*O*-vinyladipoyl-D-glucose was polymerized in DMF/water mixture to give poly (6-*O*-vinyladipoyl-D-glucose) (Scheme 10) with number average molecular weights (M_n) ranging between 3.5 and 34.5 kD, depending on the composition of the DMF/water solvent [192]. The biodegradability of these materials in liquid cultures inoculated with forest soil was investigated by respirometric tests based on BOD measurements. The mineralization of different monomeric components of the copolymers was also ascertained. PVA samples having different molecular weights were tested as reference standards. Under the adopted conditions, fast degradation of glucose, adipic acid, and 6-*O*-vinyladipoyl-D-glucose occurred, whereas poly(6-*O*-vinyladipoyl-D-glucose) (M_n 7 kD) degraded at a lower rate. However, a large extent of mineralization (70%) was reached after 28 days, whereas a pure PVA sample having M_n of 75 kD underwent negligible biodegradation under the same test conditions. A negative correlation between the molecular weight of poly(6-*O*-vinyladipoyl-D-glucose) and its biodegradation was observed.

The driving force in controlling the rate and degradation extent of sugar-branched PVA polymers by natural soil microorganisms appeared to be the chain length (e.g. DPn). Accordingly, it was found that samples having estimated DPn ranging between 12 and 40 were substantially mineralized, as well as pure PVA samples having the same degree of

polymerization. On the contrary, high molecular weight PVA (DPn 1700) was not utilized at all by the soil microorganisms [192].

The reported results can be tentatively explained by considering that the utilized soil inoculum did not contain microorganisms able to degrade high molecular weight PVA, whose environmental occurrence has been reported to be rare [93,108, 132]. These results demonstrate that PVA samples having M_n lower than 13 kD can be degraded without the presence of selected PVA-assimilating microbes [192].

It is well established that selected microorganisms can accomplish the biodegradation of both high and low molecular weight PVA [90,91]. Still, little is known about the biodegradation of low molecular weight PVA samples, even though some authors reported the biodegradation of PVA having M_n in the 2–14 kD range by microorganisms present in river sediments and activated sludge [95,97].

These results are in keeping with the suggestion that at least two different degradation mechanisms are active in PVA biodegradation, depending upon the inoculum that may contain only ubiquitous microorganisms or be enriched in substrate-specific strains.

The acceleration of biodegradation of high molecular weight PVA containing sugar residues directly bound to the hydroxyl groups of PVA was recently reported [193]. Partially substituted PVA containing *N*-acetyl-D-glucosamine (GlcNAc) residues was synthesized by glycosidation reaction (Scheme 10) [192,194].

The resulting polymer showed different water solubility and thermal properties than those of PVA [194]. Respirometric biodegradation tests carried out in the presence of soil suspension indicated that GlcNAc-substituted PVA containing 31 mol% sugar residues reached 18% mineralization in 40 days, while a PVA sample underwent only limited biodegradation (2%) [193]. SEC analysis of retrieved samples indicated that main chain scission as well as deglycosidation of GlcNAc-substituted PVA occurred during the biodegradation test, whereas PVA did not show any significant change. The reported results suggested that the cleavage of PVA main chains may be significantly accelerated by the partial glycosidation of hydroxyl groups. Recognition of pendant sugar residues by PVA-degrading enzymes appeared to play an important role in the biodegradation mechanism, as well as in the acceleration of the biodegradation rate [193].

5.5. Conclusive chapter remarks

Many efforts have been made regarding the preparation of functional water-soluble polymers, such as poly(carboxylate)s, made susceptible towards sustaining biodegradation by insertion of biodegradable PVA segments. In particular, several types of water-soluble copolymers containing PVA sequences were synthesized by free-radical copolymerization procedures.

Biodegradation studies carried out in the presence of specific PVA-degrading microorganisms and enzymes evidenced that the enzymatic attack of PVA segments may be affected by the nature of neighboring functional groups, because of steric and electrostatic effects. It was also established that the minimum required length of vinyl alcohol blocks for efficient biodegradation corresponds to the PVA trimer with preferential isotactic-type configuration.

The available literature data, with the sole exception of the investigations carried out by Bastioli et al. [180], indicate that the environmental degradation of ethylene–vinyl alcohol copolymers (EVOH) is extremely low, independent of their composition. However, it is worth noting that in most cases the potential biodegradability of EVOH was not compared with that of PVA under the same conditions. The activity of PVA-degrading microorganisms and enzymes utilized in EVOH degradation tests was not ascertained as well. By taking into account the fundamental requirement of acclimated microorganisms for PVA degradation, it seems reasonable that the possible absence of specific microorganisms may have affected the information gained in EVOH biodegradation experiments under the adopted conditions.

The requirement of at least three isotactic vinyl alcohol units for the efficient biodegradation of poly(carboxylate-*co*-vinyl alcohol) copolymers [175] and the activity enhancement of PVA-degrading enzyme promoted by the hydrophobicity of regions neighboring hydroxyl groups in PVA model compounds, such as that occurring in ethylene–vinyl alcohol copolymers, suggest that degradation

studies in the presence of specific PVA-degrading microorganisms and enzymes are needed for a conclusive assessment of EVOH biodegradability.

It has been demonstrated that biodegradation processes of glycosylated PVA, obtained by polymerization of enzymatically synthesized 6-*O*-vinyladipoyl-D-glucose, are significantly hindered by high molecular weight, as opposed to the case of PVA chains. Finally, the effectiveness of direct chemical modification of PVA by a glycosylation reaction in accelerating the biodegradation rate of high molecular weight polymer samples has been very recently reported.

6. Conclusive remarks and perspectives

The repeatedly ascertained biodegradability of PVA in water media, along with its water-solubility and rheological properties, constitutes the driving force for its utilization in the preparation of EDP items based on PVA itself or its blends and composites with other biodegradable polymeric components and fillers of both natural and synthetic origin [195].

The improvement of the relatively poor mechanical properties of many natural polymers represents one of the main goals in the utilization of PVA for the preparation of blends and composites with polymeric materials from renewable resources.

These kinds of materials are often claimed as biodegradable simply by considering the nature of their components. However, it was clearly and repeatedly demonstrated that PVA can be efficiently degraded only in the presence of selected microorganisms whose occurrence in natural environments may be relatively uncommon. This information is in keeping with the limited biodegradation of many PVA-based items in natural solid matrices such as soil and compost, as well as in aqueous media not containing specific PVA-degrading microorganisms. This latter aspect can be considered of paramount importance, even though the potential influence of the structural characteristics of PVA-based blends and composites (e.g. compatibility and miscibility) should be further investigated, as should the studies on the relationship between composition, microstructure and biodegradability in the case of poly(carboxylates-*co*-vinyl alcohol) copolymers.

Finally, it must be stressed that the ultimate fate of PVA, as well as that of water-insoluble PVA-based items in the environment, especially in solid matrices such as soil, cannot be yet considered fully rationalized. In this connection, some evidence seems to suggest that structural parameters, such as the HD and the degree of polymerization, could play a crucial role in the biodegradation of PVA in soil matrices. On the other hand, these parameters do not appreciably affect the excellent biodegradability of PVA in aqueous media, as long as the tested samples are soluble under the selected conditions and in the presence of acclimated microbial strains.

These indications, along with investigations focused on the biochemical and genetic processes that are at the basis of the natural selection of PVA-degrading microorganisms and enzymes, may represent the guidelines for the development of research programs on the production and characterization of effectively biodegradable PVA-based materials. The widely documented eco-compatibility of poly(vinyl alcohol) makes it an extremely valuable candidate for applications in which the combined characteristics of water-solubility and biodegradability represents a compelling choice rather than a voluntary option.

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