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Study of properties of biodegradable polymeric materials based on polyester

Studium vlastností biodegradabilních materiálů na bázi polyesteru.

Doctoral Thesis

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Course:	2808V006 Technology of Macromolecular Compounds
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CONTENTS

ABSTRACT

The present state of art of biodegradable polymeric materials, especially with emphasis on the biodegradable polyesters, is widely described in the theoretical part. Considerable attention is devoted to the detailed analysis of the mechanisms of biodegradation process consisting of several phases, where depolymerization is the most frequently the rate limiting step in the whole process. During this process macromolecular chains are cleaved into smaller fragments, which can be subsequently mineralized by microorganisms. As it was shown, the depolymerization does not occur only due to the biological activity of microorganisms, but is also often initialized or accelerated by abiotic factors. Besides, environmental conditions and material properties having substantial influence on the rate of biodegradation and susceptibility to it were also described. The theoretical part further deals with the utilization of biodegradable polymers in controlled release system for bioactive substances. Attention is mainly focused on the possibility of preparation of micro and submicro particles with active substance incorporated into a polymer matrix and mechanisms of its release.

The practical work is divided into two thematic blocks. In the first one some aspects of biotic degradation of two representatives of biodegradable polyesters, polylactic acid, aliphatic and aromatic-aliphatic copolymer poly (butylene adipate-co-terephthalate) were studied and in the second one the possibility of their use as matrix for controlled release of bioactive substance was investigated. In the first part, the primary focus was the investigation of the effect of photo-oxidation on the intramolecular changes in the polyester and their subsequent impact on the biodegradation in the compost environment. It was found, that while in copolyesters the irradiation led to polymeric chain crosslinking, in polylactide chain scissions prevailed. However, these changes did not have a significant effect on the rate of biodegradation and final mineralization. Subsequently, the influence of the molecular weight and form of the PLA sample on rate and course of biodegradation was studied in more details. It was shown, rate of biodegradation decreased and initial retardation was discernible with increasing molecular weight. These phenomena are then more pronounced in samples with smaller active surface area.

The main theme of the second part was the preparation of systems for the controlled release of bioactive substances based on microparticles made from low molecular weight polylactic acid. Based on the obtained findings, three series of particle varying in their size and with various initial amounts of the active agent herbicide metazachlor were prepared. The release studies subsequently showed that it is possible to reach the desired rate of release by the selection of the particles with certain sizes and loading.

Keywords: biodegradable polymers, polyesters, biodegradation, factors affecting biodegradation, microparticles, control release

ABSTRAKT

Současný stav problematiky biodegradabilních polymerních materiálů se zvláštním zřetelem na biodegradabilní polyestery je široce popsán v rámci teoretické části. Značná pozornost je věnována detailnímu rozboru procesu biodegradace skládající se z několika fází, kdy ve většině případů bývá řídícím krokem proces depolymerizace. Během tohoto procesu dochází ke štěpení makromolekulárních řetězců na menší fragmenty, které jsou poté mineralizovány uvnitř mikrorganismů. Jak se ukázalo, samotná depolymerizace nemusí probíhat pouze vlivem biologické aktivity mikroorganismů, ale bývá také často iniciována nebo urychlena působením abiotických faktorů jako je například světelná energie. Velký vliv na rychlost biodegradace a náchylnost k ní mají, vedle environmentálních podmínek, samozřejmě také vlastnosti materiálu, které jsou v práci popsány.

Rešeršní část se dále zabývá využitím biodegradabilních polymerů pro kontrolované uvolňování bioaktivních látek. Pozornost je zaměřena na možnosti přípravy částic mikro a submikro rozměrů se začleněním aktivní látky do polymerní matrice a následnými způsoby jejího uvolňování.

Samotná práce je pak rozdělena do dvou tematických bloků, kdy v prvním z nich byly studovány některé aspekty biologické rozložitelnost dvou zástupců biodegradovatelných polyesterů, alifatické kyseliny polymléčné a aromaticko-alifatického kopolymeru poly(butylen adipát-co-tereftalát) a v druhém pak možnosti jejich využití jako matrice pro kontrolované uvolňování bioaktivních látek.

Prvořadá pozornost byla v první části zaměřena na sledování vlivu světelného záření na intramolekulární změny v daných polymerech a jejich následný vliv na biodegradaci v prostředí kompostu. Bylo zjištěno, že zatímco v případě kopolyesterů dochází k síťování polymerních řetězců, u PLA naopak k jejich štěpení. Nicméně tyto změny neměly významný vliv na samotnou rychlost biodegradace. Následně byl podrobněji studován vliv molekulární hmotnosti a formy vzorku PLA na rychlost a průběh biodegradace. Ukázalo se, že s nárůstem molekulové hmotnosti klesá nejen rychlost biodegradace, ale dochází i k určitému zdržení na jejím počátku. Tyto jevy se pak více projevují u vzorků s menším aktivním povrchem.

Stěžejním tématem druhé části byla příprava systému pro kontrolované uvolňování bioaktivní látky založeného na mikročásticích vyrobených z nízkomolekulární kyseliny polymléčné. Na základě získaných poznatků bylo připraveno několik sérií částic lišící se svou velikostí a obsahem enkapsulované látky, kterou byl zvolen herbicid metazachlór. Uvolňovací studie poté ukázaly, že je možné volbou částic s určitou velikostí a plněním dosáhnout požadované rychlosti uvolňování.

Klíčová slova: biodegradabilní polymery, polyestery, biodegradace, faktory ovlivňující biodegradaci, mikročástice, kontrolované uvolňování

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LIST OF SYMBOLS AND ACRONYMS

LDPE		low density polyethylene
MTZ		Metazachlor
MW	g/mol	Molecular weight
O/W		Oil-in-water
O/W/O		Oil-in-water-in-oil
PA		polyamide
PBAT		poly(butylene adipate-co-terephthalate)
PCL		poly(ε-caprolactone), poly(caprolactone)
PE		polyethylene
PEA		poly (esteramidy)
PET		polyethylene terephthalate
PGA		poly(glycolic acid), polyglycolide
PHA		polyhydroxyalkanoate
PHB		poly (β-hydroxybutyrate)
PHV		polyhydroxyvalerate
PLA		poly(lactic acid), polylactide
PLGA		poly(lactic-co-glycolic acid)
PLLA		poly(L-lactic acid), poly(L-lactide)
PDLA		poly(D-lactic acid), poly(D-lactide)
PDLLA		poly(DL-lactic acid), poly(DL-lactide)
POE		poly (oxyethylene)
PP		polypropylene
PS		polystyrene
PVA		poly (vinyl alcohol)
PVC		polyvinylchloride
MW		molecular weight
TPS		thermoplastic starch
T_m	°C	melting temperature
T_g	°C	glass transition temperature
W/O		Water-in-oil
W/O/W		Water-in-oil-water

LIST OF FIGURES AND TABLES

LIST OF PAPERS

PAPER I

STLOUKAL, P., JANDAK, J., HUSAROVA, L., KOUTNY, M., COMMEREUC, M., VERNEY, V. Identification of several factors affecting biodegradation of arometic-aliphatic copolyester. DEEE 2010 WSEA conference, 30.11.-2.12.2010. Puerto de la Cruz, Tenerife, ISBN: 978-960-474-253-0.

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STLOUKAL, P., VERNEY, V., COMMEREUC, S., RYCHLY, J., MATISOVA-RYCHLA, L., PIS, V., KOUTNY, M. Assessment of the interrelation between photooxidation and biodegradation of selected polyesters after artificial weathering. *Chemosphere* 2012, vol. 88, no. 10, p.1214-1219.

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PAPER IV

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PAPER V

STLOUKAL, P., SEDLARIK, V., HUSAROVA, L., KASPARKOVA, V., KOUTNY, M. Preparation of submicroparticles based on biodegradable co-polyester DEEE 2010 WSEA conference, 30.11.-2.12.2010. Puerto de la Cruz, Tenerife, ISBN: 978-960-474-253-0, ISSN: 1792-6653

PAPER VI

STLOUKAL, P., KUCHARCZYK, P., SEDLARIK, V., BAZANT, P., KOUTNY, M. Low molecular weight poly(lactic acid) microparticles for controlled release of the herbicide metazachlor: Preparation, morphology, and release kinetics. *Journal of Agricultural and Food Chemistry* 2012, vol. 60, no. 16, p. 4111-4119.

THEORETICAL BACKGROUND

During the last century, the utilization of synthetic polymers such as polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyamide (PA) gradually increased in many areas of human life. Their dominant position in many industrial fields and applications comparing to other materials has been achieved, mainly due to their relatively low cost and good mechanical, physical and chemical properties including lightweight, facile processing, strength, insulating properties, resistance to oxidation, and microbial degradation, etc. However, in parallel with the dramatic increase in production and the constantly improving resistance to all forms of degradation thus extending durability of these materials, problems concerning of the waste management has being emerged.

Nowadays, global production of a wide variety of petroleum-based synthetic polymers amounts approximately to 245 million of tons per year in 2008 [1]. A considerable portion of this production is processed as disposable products, thus alarming amount is rapidly introduced in the environment or disposed in the landfills as industrial and municipal waste. These potentially huge environmental accumulation and municipal waste disposal that could persist for centuries promoted research activities worldwide and led to the formation of several action and strategies how prevent and avoid these problems.

Two of the solutions involved were either the modification of current conventional polymers or the development of new alternatives in order to achieve in both cases their degradability by any or all of possible mechanisms: biodegradation, photodegradation, environmental erosion and thermal degradation [2]. Already in the 1980s, the conventional polymers usually polyolefins for instance PE were blended together with starch and other biodegradable materials to improved their degradability. However, after biodegradation of starch, the polymer was disintegrated leaving small fragment of PE, which unfortunately were not further degraded. In parallel, the new materials with similar properties as conventional polymers, which could be susceptible to microbial attack making them biodegradable, were being developed [3].

Although, worldwide production of non-degradable petroleum-based polymers remained dominant, biodegradable polymers seem to be increasingly attractive mainly due to environmental and economic aspects associated with waste disposal and increasing expenses of petroleum based production [4].

Concurrently, another area, which has generated an enormous amount of interest and also initialized the research concerning biodegradable polymers, has been medicine [5].

1 BIODEGRADABLE POLYMERS

The group of polymers that can be completely converted by microorganisms to carbon dioxide, water, mineral substances and biomass, with no negative environmental impact or ecotoxicity [6] is called biodegradable polymers. According to ISO and CEN definition, degradable plastics are those in which degradation results in lower molecular weight fragments produced by the action of naturally occurring microorganisms such as bacteria, fungi and algae [3].

Some of biodegradable polymers can also be referred as compostable. However, to be truly compostable, they must fulfil additional criteria such as compatibility with the composting process, degradation rate consistent with other known composting materials, no negative impact on quality of compost and leaving no visible, distinguishable or toxic residue. Typical compostable materials should be degraded in industrial composting process during 12 weeks at elevated temperature over 50°C [7].

The biodegradation occurs more easily in polymers having incorporated heteroatoms such as oxygen in their backbone chains [8]. Due to presence of heterogroups, which provide a site for a chemical attack, polymers like polyesters, polyethers, polyamides or polyurethanes are more susceptible for biodegradation than e.g. polyolefins [9]. Nowadays, number of commercially available materials that meet certain standards for biodegradable materials is produced.

1.1 Type of biodegradable polymers

Biodegradable polymers can be classified according to their origin or the method of preparation [4]. On the basis of origin they can be further divided into either biodegradable polymers, which are primarily based on renewable resources, or synthetic biodegradable polymers produced from fossil resources [10].

Biodegradable polymers made from renewable resources include:

- Polylactic acid (PLA)
- Polyhydroxyalkanoate (PHA): Poly (β-hydroxybutyrate) (PHB) and polyhydroxyvalerate (PHV)
- Thermoplastic starch (TPS)

Biodegradable polymers made from petroleum include:

- Poly-ε-caprolactone (PCL)
- Poly (vinyl alcohol) (PVA)
- Poly (esteramidy) (PEA)
- Poly (oxyethylene) (POE)

- Aliphatic polyesters based on diols and dicarboxylic acids
- Aromatic-aliphatic copolyesters

Biodegradable polymers can be produced in principle in three different ways. The most widespread method is a conventional synthesis using both monomers from non-renewable resources (PCL, copolyesters) and monomers derived from renewable sources (PLA). The other possibility is the biotechnological route, when polymers can be produced either through the microbial fermentation (PHB, xanthan, etc.) or genetically modified maize (PHA) followed by their extraction. Lastly, they can be also directly isolated from biomass (e.g. starch), which could be subsequently modified with help of a plastification agent e.g. with glycerol (TPS).

Biodegradable polymers are often blended together or with the conventional polymers and other composites to achieve the optimal material properties or enhance biodegradability [11]. They are also used as blends with natural materials especially with starch or cellulose to reduce their price or further accelerate the rate of biodegradation. On the other hand, these natural raw materials often cannot be processed as thermoplastic materials due to their poor physico-chemical properties and therefore have to be mixed with polymers or indispensable additives to improve their processability.

1.1.1 Biodegradable polyesters

Among biodegradable polymers, especially polyesters play a major role mainly due to their good chemical and physical properties [12]. In frame of this work, the attention is mainly paid to two representatives of polyesters: Polylactic acid (PLA) and Aromatic-aliphatic copolyesters (PBAT, Ecoflex)

1.1.2 Polylactic acid (PLA)

Structure and preparation

Polylactic acid (PLA) is linear aliphatic thermoplastic polyester with lactic acid as the elemental structural monomer unit. The scheme of PLA molecular structure is presented in Figure 1.

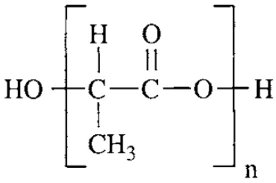

Figure 1: Chemical structure of polylactic acid

PLA can be produced either by polycondensation of lactic acid, which is known as poly(lactic acid) or by the ring-opening polymerization of the lactide [13].

The lactic acid itself can be prepared by either chemical synthesis mainly based on the hydrolysis of lactonitrile or by microbial fermentation of agricultural renewable resources rich on carbohydrate substances. The biotechnological preparation of lactic acid is increasingly interesting mainly due to its low environmental impact and the production of optically pure L- lactic acid in contrast to racemic mixtures produced by chemical synthesis [14].

Properties

PLA is highly transparent, colourless thermoplastic with good processability and water solubility resistance, in many aspects similar to polystyrene [4]. Properties of PLA can be affected by several factors including isomers composition, molecular weight, processing conditions.

Stereochemical composition has significant influence on melting temperature, rate of crystallization and degree of crystallinity [3]. PLA can be either amorphous or semicrystalline, when optically pure polylactides: poly (L-lactide) (PLLA) and poly (D-lactide) (PDLA) tend to be semicrystalline while those with lower optical purity (poly (D,L-lactide) (PDLLA)) are more amorphous [15].

Poly-L-lactide (PLLA) has a good tensile strength, low extension, high modulus approximately 4.8 GPa, degree of crystallization about 37%, glass transition in the range of 60-65 °C and melting point approximately 175 °C [16].

1.1.3 Aromatic-aliphatic copolyesters (PBAT, Ecoflex)

The attempt to combine the biodegradability of aliphatic polyesters with the good material performance of aromatic polyesters has given conception to the aromatic-aliphatic copolyesters. One of the most promising copolymers nowadays is poly(butylene adipate-co-terephthalate) (PBAT), which is commercially available under the trade mark Ecoflex (BASF, Germany), PBAT (EnPol Ire), etc.

Structure and preparation

PBAT is produced by a standard polycondesation technique from 1,4-butanediol, adipic acid and terephthalic acids. The schematic structure of PBAT is shown in Figure 2.

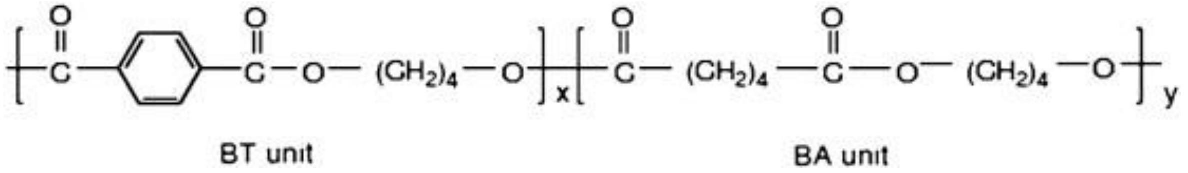

Figure 2: Structural scheme of PBAT [17]

Properties

PBAT shows good mechanical and thermal properties at a proportion of terephthalic acid higher than 35% mol and good biodegradability if the same is lower than 55% [18]. The mechanical properties of Ecoflex can be compared with those of low-density polyethylene (LDPE) in many aspects. The comparison of the several basic material properties and mechanical characteristics of 50μm blow films are presented in Table 1.

Table 1: The comparison of selected properties of PBAT and LDPE [19]

Properties	**PBAT**[a]	**LDPE**[b]
Mass density (g m^{-1})	1.25-1.27	0.922-0.925
Melting point T_m (°C)	110-115	111
Glass-transition temperature (°C)	-30	
Hardness (according to Shore)	32	48
Transparency (%)	82	89
Tensile strength (N mm^{-2})	32/36	26/20
Ultimate elongation (%)	580/820	300/360
Permeation rates of oxygen (Cm^3 (m^{-2} d bar))	1600	2900
Permeation rates of water vapor (g (m^{-2} d))	140	1.7

[a] Ecoflex (BASF, Germany); [b] Lupolen 2420 F(LyondellBasell, Netherlands)

Films are transparent, flexible and mechanically resistant due to relatively high tensile strength of the material exceeding LDPE. It mainly differs from LDPE in barrier properties because of its significantly higher water vapour permeability. However, an important feature of copolyester Ecoflex is its compostability.

2 BIODEGRADATION

Biodegradation or biotic degradation of organic compounds is a biochemical decomposition caused by the naturally occurring microorganisms such as bacteria and fungi. Organic compounds can be degraded either under aerobic conditions in the presence of oxygen to carbon dioxide, water, and microbial biomass, or under anaerobic conditions without oxygen to carbon dioxide, water, methane and biomass [20].

2.1 Mechanism of biodegradation

Biodegradation is a complex process (Figure 3) including several phases: biodeterioration, depolymerization, assimilation and mineralization.

Generally, since polymer molecules are too large and water insoluble to be able to penetrate through the membrane into the cells of microorganisms, where the most of biochemical processes take place, firstly they have to be depolymerized, in other words converted to low-molecular water-soluble fragments [21].

2.1.1 Aspects of biodegradation

Biodeterioration

During process called biodeterioration the resistance and durability of materials is weaken by the action of microorganisms, or/and environmental abiotic factors [22]. The growth of microorganisms adhering to the polymer surface can provoke formation of cracks, increase the size of pores and others defects, that may even lead to the disintegration of a material into small fragments [23, 24]. Such disruption can also be caused by physico-mechanical cyclic events including cooling/heating, freezing / thawing and wetting/ drying. As a consequences polymer chains are partially exposed and thus more accessible to depolymerization.

Depolymerization

Parallelly, catalytic agents excreted by microorganisms particularly extracellular enzymes and to some extend free radicals or abiotic factors including light, heat and chemicals could provoke the scissions of polymer chains. The principal classes of enzymes catalyzing the cleavage of chains are hydrolases for instance lipases and esterases [21]. In the course of depolymerization, molecular weight is gradually reduced and water soluble intermediates are generated: oligomers, dimmers and monomers.

Assimilation

During the following phase called assimilation, water soluble intermediates penetrate across the plasmatic membrane and inside the cytoplasm of microorganisms are integrated into the microbial metabolism pathways to produce energy, biomass, and primary and secondary metabolites.

Mineralization

Finally, as a result of metabolic processes, end-products such as CO_2, CH_4 (in the case of anaerobic biodegradation) H_2O, biomass are released. This step is known as mineralization [22].

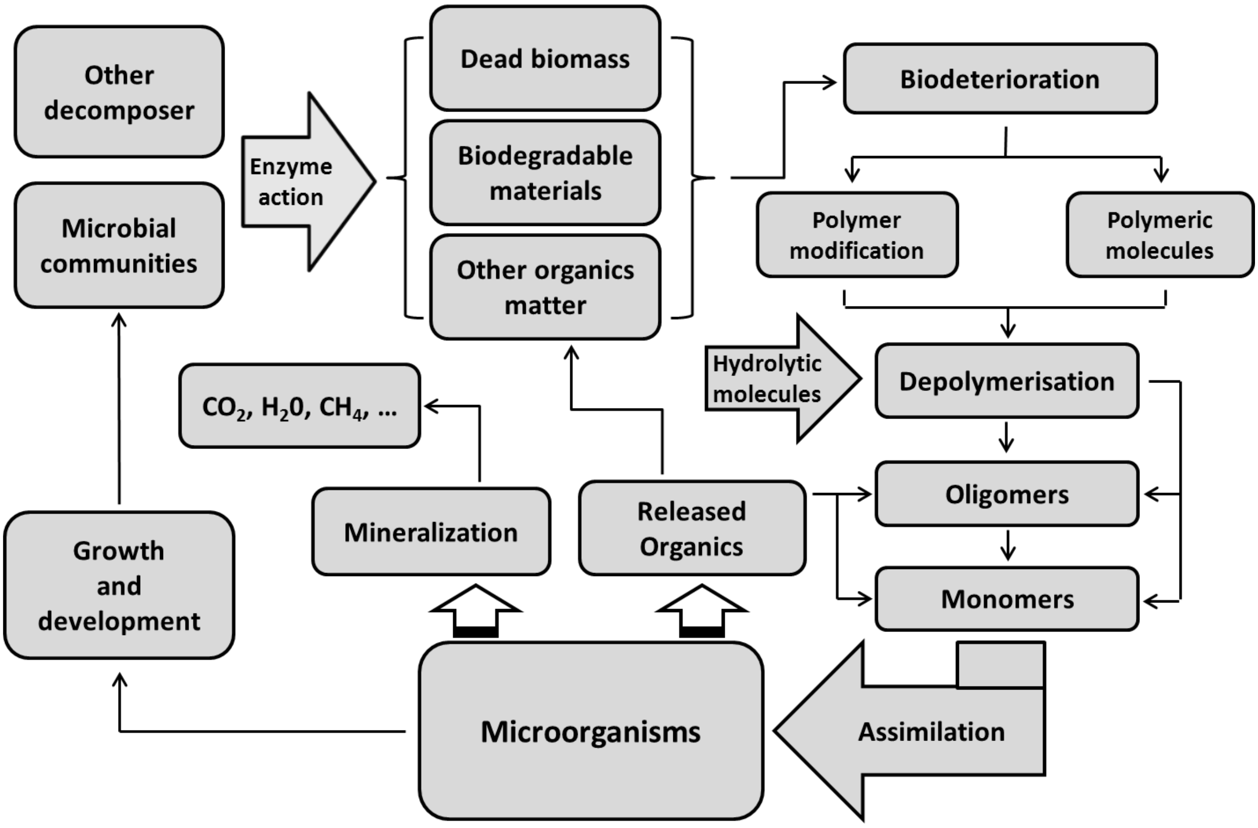

Figure 3: Polymer biodegradation scheme [22]

2.1.2 Abiotics factors

In the biodegradation process, the biological activity of microorganisms is predominant, nevertheless in nature abiotic degradations plays a certain part in the decomposition of organic matter along with biotic factors. Mainly in the first stage of biodegradation, either during biodeterioration or depolymerazation, abiotic factors should not be neglected. Abiotic processes contribute to biotic factors either as a synergistic aspect or direct initiation of chain scissions. Abiotic factors enhancing biodegradation include mechanical disturbance, photo, thermo and chemical degradation.

Mechanical degradation

Degradation of polymers by a mechanical action can be caused by several forces including compression, tension and shear stress as a consequence of many common environmental factors such as air and water turbulences, snow pressure and deterioration by animals. Mechanical factors do not have the dominant role rather they contribute synergistically with the other abiotic factors however the erosion of materials for instance cracking can activate the whole biodegradation process [22].

Photo degradation

Photo-oxidative decomposition induced upon exposure to UV and visible light is considered as one of the main sources of polymer degradation under ambient conditions [25]. The mechanism of photodegradation include the generation of unstable radicals by absorbtion of photon energy which can subsequently lead to the chain scission either via Norrish I or Norrish II mechanisms, oxidative processes or crosslinking reactions due to the recombination of created free radicals from Norrish I [26]. Unstable polymer hydroperoxides (POOH) are produced as primary products of oxidation of free radical.

Thermal degradation

Intensity of thermal degradation processes depends upon temperature i.e. the higher temperature the higher rate of degradation. Degradation provoked by heat usually takes place above melting temperature when solid phase of polymer is changed to liquid, nevertheless a large number of polymers undergo degradation under normal conditions as well. The mechanism and products of oxidation reaction occurring during thermo degradation are comparable to those resulting from photo-oxidation [27]. Principal difference consists in the initiation either by heat or light leading to degradation processes [28].

Chemical degradation

Primary chemical transformations in polymers are evoked by the atmospheric forms of oxygen (O_2 or O_3) usually catalyzed by heat and light as was already described above. Moreover, the environmental pollution and other chemicals such as agrochemicals may also cause some changes in polymer properties [29]. One of the most important chemical degradation reactions regarding the biodegradable polymers is the abiotic hydrolysis. Polymers undergo random scisson of hydrolytically unstable covalent bonds such as in ester anhydrides and amide esters groups. [30].

2.1.3 Types of biodegradation mechanisms

Depolymerization is the most frequently the rate limiting step in the whole process. It could be controlled by two different mechanisms either bulk or surface erosion (schematically present in Figure 4) In the case of surface erosion mechanism, the extracellular enzymes are not able to penetrate deeper into polymer matrix due to their considerable size therefore they act on the polymer surface only [21,31]. Loss of matter occurs from surface, but the molecular weight of entire polymer material does not change. As for the bulk erosion, the reduction of molecular weight occurs in the entire polymer matrix due to penetration of chemicals mainly H_20 inside the material, and provoking bond cleavage [32].

Both mechanisms usually contribute to the biodegradation to different extent nevertheless one of them is usually dominant. If the rate of scission of polymer chains is higher than rate of diffusion of chemical agents through the polymer matrix material surface erosion predominates otherwise the mechanism occurs via bulk erosion [33].

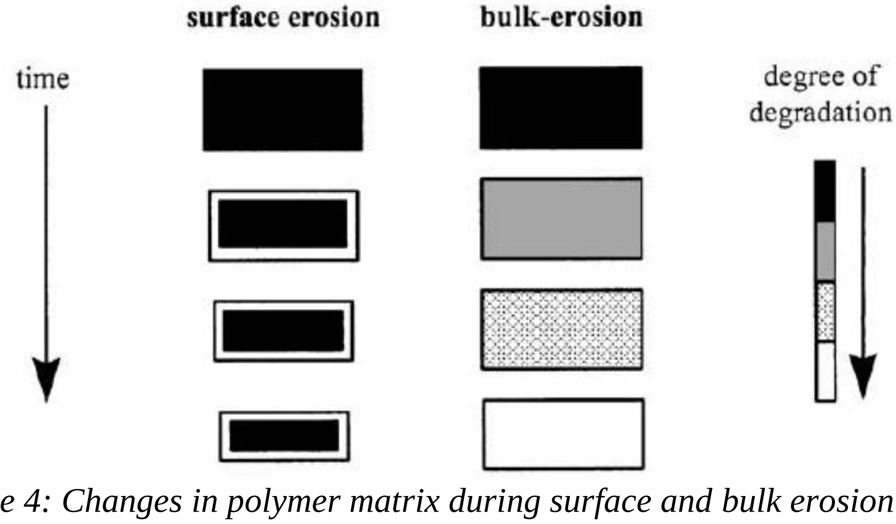

Figure 4: Changes in polymer matrix during surface and bulk erosion [33].

2.2 Factors affecting biodegradation

Biodegradation of polymers is affected by various factors including properties of polymeric material (mobility of polymer chains, crystallinity, tacticity, length and sequence distribution of aromatic and aliphatic chains, molecular weight and type of functional groups, specific surface, additives, etc.), types of organisms (mesophilic and thermophilic) and the environmental conditions, in which biodegradation takes place (pH, temperature, humidity, the presence of nutrients and others) [2].

2.2.1 Properties of polymeric materials affecting biodegradation

Mobility of polymer chains and polymer crystallinity

The term mobility of polymer chains means the ability of chains to temporarily escape from crystallites for a certain distance [34, 35]. The mobility plays an important role in the biodegradation of polyesters because it has been speculated that hydrolase enzymes secreted by microorganisms initiate the process [34]. The active site of the hydrolases (mainly lipases and esterases) is usually located in a certain cavity on the enzyme and therefore, a polymer chain must be mobile sufficiently to reach this catalytic centre [31].

The mobility is closely related with the crystallinity of polyester and the difference between its melting temperature and the temperature, in which biodegradation occurs. Whereas the polymer chains in the amorphous phase (above the glass transition temperature Tg) are highly mobile, thus easily degraded, in the crystalline domains of the polymer, chains are tightly bound and in consequence the mobility of chains is very low [31].

Since mobility relates to the melting point of the material, it seems unlikely that high temperature-melting polyesters such as PET can be degraded at an acceptable rate. However, according to [36] the aromatic polyesters having high melting point may be degraded by means of *T. fusca* hydrolases.

Moreover, the organization of molecular structure acts as a rate-limiting factor of chemical degradation which is considerably affected by the diffusion rate of O_2 and H_2O. In tightly fixed crystalline domains, the diffusion is restrained thus the extent of oxidative and hydrolytic degradation is reduced in comparison with the disorganised amorphous phase, where chemical degradation takes place more easily [22].

Length and sequence distribution of aromatic and aliphatic chains

According to several studies [37] the rate of biodegradation of the aliphatic-aromatic copolyester depends exactly on length and sequence distribution of monomer in the copolymer. Generally it is known, that while aliphatic polyesters are easily biodegradable, aromatic are practically biologically inert. In the case of their combination, the biodegradability is mainly affected by the tightness of polymer chains fixation (mobility) in crystalline domains of copolyester. Mobility characterized by the melting point of the material can be correlated with the length of aromatic sequences in an aliphatic aromatic copolymer [34]. As the content of aromatic sequences increase, crystallites are formed more easily and consequently melting point of aliphatic-aromatic copolymers rise, thus the biodegradation rate is efficiently controlled by the aromatic sequences [38].

However, negative correlation between suitable thermal properties improving physical characteristics and better biodegradability of polymer makes it difficult to design a material with both advantages and must result in a certain compromise of material properties [38].

Tacticity

Stereochemical composition could substantially effect properties of polymer such as crystallinity thus also its biodegradability. In the case of PLA, optically active enantiomers PLLA and PDLA consisting of isotactic sequences tend to be crystalline while optically inactive form PDLLA, which comprises both isotactic and atactic sequences, are rather amorphous, therefore more easily degradable. Furthermore the degree of stereoregularity has influence on hydrophilicity of polymers, which is an essential aspect in microbial depolymerization as well as hydrolysis [39]. For instance, lower tacticity of PDLLA chains cause higher tendency to hydrolysis due to weaker intramolecular interactions, which facilitate the attack of water molecules [40].

Specific surface

Specific surface of polymer can greatly accelerate the degradation rate, if it proceeds as surface erosion process [41, 42]. Increasing the absolute surface area of material, on which enzymes can be adsorbed, resulted in an increase of the enzymatic cleavage rate thus overall rate of biodegradation.

Molecular weight

Molecular weight (MW) and MW distribution are important factors in the mechanism of biodegradation. Generally, biodegradability of polymers decreases with increasing molecular weight. While, monomers dimers and oligomer as water soluble intermediates of polymers are much easily mineralized by microorganisms, long insoluble polymer chains have to be firstly cut to into smaller water soluble fragments [43]. It also produces an increase in the glass transition temperature leading to the deceleration of degradation, because glassy polymers are degraded more slowly than rubbery ones [44].

Additives

Various fillers, stabilizers, antioxidants, pigments, non-polymeric impurities such as catalysts residues from polymerization, transformation product of additives etc. can have considerable influence on resistance to biodegradation. In recent years, photosensitive additives called prooxidants (transition metal salts) accelerating degradation processes in polymers via oxidation have been developed. The prooxidants are able to catalyze the breakdown of high molecular weight polyolefins, which are originally non-biodegradable, to lower molecular weight product thus make them oxo-biodegradable [45].

2.3 Biodegradation of PLA

PLA undergo natural degradation in different environments such as compost, although the susceptibility to microbial degradation is lower in comparison with other aliphatic polyester such as PCL, PHB and PBS [4]. The mechanism of PLA degradation involves firstly the hydrolysis of esters linkage induced by both abiotic and enzymatic hydrolysis, which is followed by bacterial assimilation of the water soluble residues [42]. Generally, rate of biodegradation increase with temperature, amorphous PLA is more susceptible for degradation than crystalline one and PLA with low molecular weight is easily degraded than with high molecular weight.

PLA is completely degraded under compost conditions due to high humidity and temperature above 50°C. In compost thermophilic actinomycetes, mainly genus *Amycolatopsis*, were isolated as PLA degrading microorganisms [46]

In the soil under real conditions, the biodegradation rate is considerably slower comparing to the compost mainly due to low temperature and the consequently low rate of abiotic hydrolysis, and probably limited number of

PLA degraders [47]. However, some mesophilic organisms capable of PLA degradation notably some Gram-negative bacteria were isolated [48].

2.4 Biodegradation of PBAT

The biodegradability of aliphatic-aromatic polyesters decreases with the content of aromatic monomers having the threshold of biodegradability at about 50–60 % of terephthalic acid [12]. The crucial factor controlling biodegradation of aromatic-alifatic copolyesters is the mobility of polymer chains relating to their melting temperature.

Biodegradation process is initiated by extracellular enzymes, which catalyze hydrolysis of esters bonds in the polymer. Several studies [49, 10] have proven that thermophilic actinomycetes play dominant role in the initiation of copolymers degradation. Particularly, an actinomycete strain indentified as *Thermomonospora fusca* and its extracellular hydrolase (TfH) was isolated. TfH having responsibility for polyester cleavage combines the characteristics of lipase and esterase due to its ability to hydrolyze dissolved esters, which is typical for esterases [21]. The PBAT copolyesters can also be degraded in soil at ambient temperature but the process is significantly slower [50].

2.5 Methods for biodegradability testing

There exist various methods for testing biodegradation of biodegradable polymers. Some of these methods were established as standards for Testing Biodegradable Plastics by international organizations such as the International Standard Organization (ISO) and American Society for Testing and Materials (ASTM). ISO standards are virtually identical to the standards approved by the European Commission for Standardization (CEN) and the German Institute for Standardisation (DIN) [6].

These standards are usually based on the measurement of typical characteristics indicating, that the material undergoes a degradation process. These characteristics include loss of weight [51], changes in surface properties [48] tensile strength [52] and chemical and physical properties [51], carbon dioxide production [54], bacterial activity [55], reduction in the average molecular weight and distribution [56] and so on.

Measurement of “loss of weight” can easily be realized on one hand, but on the other hand, it is limited by polymer fragmentation and loss of integrity during biodegradation.

Visual changes on the surface, which can be observed by optical, electron microscope, etc. such as holes and cracks, bio-film and changes in colour do not prove the biodegradation in the meaning of mineralization but can serve as a first indicator of the action of microorganisms or abiotic factors during biodeterioration and depolymerization [57].

Some mechanical properties, for instance tensile strength, are very sensitive to small changes in molecular weight of the polymer, which is often an indicator of degradation. Measurements of molecular weight or intrinsic viscosity changes by gel permeation chromatography or rheology provide definite information about chain scissions but almost nothing about the ultimate mineralization of polymers. The extent of mineralization of the material can be determined by respirometric methods, which evaluate the amount of carbon dioxide produced or oxygen consumed by microorganisms.

2.5.1 Respirometric methods

Methods consisting in the measurement of the evolved carbon dioxide in the aerobic environment or the consumption of oxygen are the most often methods used in the laboratory testing of polymers biodegradility. Extent of mineralization corresponds to the proportion of CO_2cumulatively generated from tested material to the theoretical amount of CO_2 in the material, which can be either calculated from its chemical composition or determined by elemental analysis [58].

Evolved CO_2 can be measured by several techniques classified as cumulative measurement respirometrics (CMR) and [2] direct measurement respirometrics (DMR). Classical acid-base titration of evolved CO_2, which is entrapped in a sodium or barium hydroxide, is used in cumulative measurement. Solution of hydroxide with dissolved CO_2 is titrated manually with acid solution with a visual indicator [59]. In the direct measurement technique, amount of CO_2 is analyzed directly from the headspace of the closed bioreactors using gas chromatography or an infrared gas analyzer.

3 APLICATIONS OF BIODEGRADABLE POLYMERS

Nowadays, biodegradable polymers have found a wide range of applications as a replacement of conventional non-degradable polymers mainly in the area of food industry, agriculture and medicine.

3.1 Food industry

The increasing utilization of biodegradable polymers in food applications is one of the responses to serious ecological problems which arose with the use of synthetic polymers due to their non-biodegradability and problems related with their recycling [43]. Typically, fields of application lies in both disposable products including packaging films, cups, bags, bottles, disposable celery and long-term products, such as various boxes for food and liquid containers.

3.2 Agriculture

The volume of plastics films used in agriculture have achieved about 1 000 000 ton per year during the last century [6]. Nowadays, polymeric film are applied as various covers for greenhouses, tunnels over garden row and as mulching films to control soil temperature and humidity, increase the efficiency of fertilizers, herbicides etc, and mainly to improve product quality [60]. In the case of replacement by biodegradable films, the material can be either ploughed into the soil if sufficient degradability is guaranteed or disposed after collection in composting facilities [61]. Moreover, it can be used for composting bags, temporary planting pots and in some special applications such as delivery system for fertilizers and pesticides and seed coatings [62, 63].

3.3 Medicine

In the last three decades there has been obvious shift from conventional to biodegradable (hydrolytically and enzymatically degradable) polymers in many biomedical applications [16]. Typical current applications includes implants for the fixation of fractured bones and tissues together such as clips, bone pins and plates, sutures and scaffolds for tissue engineering [5]. Furthermore, biomaterials can be used for disposable products (syringes, blood bags, and catheters) or as controlled drug delivery vehicles [64].

3.4 Controlled release system for bioactive compounds

In the last three decades, the bioactive compounds delivery systems have attracted increasing interest in the pharmaceutical industry and agriculture, because they are able to reduce some undesirable side effects of these bioactive molecules considerably [65]. In the case that bioactive agent is incorporated into biodegradable micro and nanoparticles such delivery system offer several important advantages comparing with the conventional devices. Besides the possibility to control the release rate of incorporated drugs leading to an increase of drug efficiency thus reduction of drug dose needed they also provide easy administration and no need to remove a biodegradable polymer carrier [66].

In agriculture, particles loaded with various agrochemicals could prevent the unwanted phenomena associated with the their conventional applications such as leaching through the soil, volatility, degradation (photolytic, hydrolytic and microbial) etc. and simultaneously extend their activity in soil, improve their stability and reduce unwanted toxicity thus safety for the environment and for operators along with easier handling and application [67].

3.4.1 Structure

According to the internal structure of the particles they can be classified into two groups: nanospheres and nanocapsules [68].

Nanospheres have matrix type of structure, when the entire mass is solid with no differences between core and shell of particle. The shape of nanospheres is largely spherical, when bioactive agent can be either absorbed at surface or incorporated inside the particles [69].

On the contrary, nanocapsules consist of polymeric membrane surrounding cavity with inner liquid core, in which bioactive agent is usually dissolved. While the liquid core consisting of oil phase serves as carrier for nonpolar liphophilic compound, an aqueous core is suitable carrier for polar water soluble compound [70,71]. Active compound may be also adsorbed on capsule surface as in the case of spheres [72].

Encapsulation of active agents inside particles whether applied in spheres or capsules is better for the protection of fragile molecules against degradation or suppression of some unwanted phenomena such as burst release [73, 74]. During the burst effect, undesired initial fast release of drug from particles occurs among others due to the weak adsorption of the drug on the surface of nanoparticles [75].

The incorporation of a bioactive agent into polymeric carrier should be achieved during preparation already. However, in the case that a drug is not able to associate with the carrier during preparation or easily degrade due to process conditions, adsorption on the surface of previously prepared system can be applied [73].

3.4.2 Method of preparation

Over the years, many methods for preparation of polymer nanoparticles have been developed. They can be divided into two main categories according to whether the formulation is prepared through the polymerization of monomer or directly from macromolecules. The majority of these methods comprise of two steps including firstly the creation of emulsified system of monomers or polymers followed by second step when particles are obtained by specific mechanism such as precipitation or polymerization. However, there are a few methods, which lead to the formation of nanoparticles in the first process step

[76]. Methods most frequently used for the preparation of loaded particles are listed below.

Emulsification/solvent evaporation method

Solvent evaporation technique is one of the most preferred encapsulation method due to its relative simplicity, with no need for specialized equipment [77,78]. In the first step, solution of polymer and drug containing the volatile solvent is emulsified in aqueous phase using high-energy homogenization (homogenizer, ultrasonication). During the second step, polymer solvent is evaporated, which leads to precipitation of polymer with enclosed drug in the form of micro- or nanoparticles dispersion [79, 80]. A drawback of this method may be the presence of some residual organic solvent [81].

This procedure can be applied to incorporation both polar and nonpolar compound, depending on the modification of emulsion system [82] Oil-in-water emulsion (O/W) is suitable system especially for substances with low polarity [83]. Oil-in-oil emulsion system is used for substances which, although they are classified as non-polar, exhibit considerable solubility in water [84]. Less common modifications of method such as water-in-oil-in-water (W/O/W) and oil-in-water-oil (O/W/O) utilize several emulsification steps in principle [85]. In both cases the middle phase acts as a barrier against leakage of the encapsulated drug from the internal to the external phase in which it is readily soluble [81].

Nanoprecipitation (solvent displacment, Interfacial deposition)

Nanoprecipitation, also called solvent displacement or interfacial deposition, is the fast and one of the most feasible methods used widely for the preparation of both nanospheres and nanocapsules [86]. Polymer dissolved in water-miscible solvent is injected into a stirred aqueous solution in the presence or absence of surfactants depending on the type of polymer. Instantaneous diffusion of the solvent into aqueous solution leading to polymer deposition on the phase interface results in formation of polymer particles suspension [87]. Hereby particles with well defined size typically about 200 nm and narrow distribution can be prepared [69]. Disadvantage could be low polymer concentration in the organic phase leading to consumption of the large amount of solvent.

Emulsification−diffusion method

The method consists in formation of drug loaded particles by purely diffusion mechanism. Firstly, both polymer and drug intended for encapsulation are dissolved in a partially water soluble solvent, which is saturated with water to achieve phase equilibrium. Subsequently, solution of polymer is emulsified into aqueous solution with emulsion stabilizer and diluted with pure water, which causes diffusion of solvent from the droplets to the continuous phase thus formation of nanoparticles. Finally, depending on the boiling temperature solvent is eliminated either by evaporation or filtration [88].

The main advantages of method are simple control of the particle size and high efficiency of encapsulation [89]. Disadvantages may be leakage of water-soluble substances into the aqueous external phase [90].

Salting out method

In principle, this method is similar to the emulsification diffusion of solvent with main differences consisting in using of solvent totally miscible with water i.e., acetone and aqueous phase containing a salting-out agent (anorganic electrolytes such as magnesium chloride, calcium chloride, etc.). Particles are obtained by reverse salting-out effect, when dilution of the emulsion with large amount of water, lead to precipitation of polymer [90].

Interfacial polymerization

It is very well established technique of particle preparation consisting in polymerization of two reactive monomers or agents dissolved in two non miscible phases. Organic phase is composed of lipophilic monomer, drug and surfactant while water phase contains hydrophilic monomer and a water soluble surfactant. The reaction taking place on the interface of oil droplets dispersed in continuous aquatic phase emulsion leads to the formation of particle dispersion [91].

The advantage is the possibility to control particle wall thickness and porosity having considerable influence on drug loading and release by the monomer concentrations and their molecular weight [73].

3.4.3 Release Mechanisms

The release of bioactive agents from biodegradable particles can occur via two main mechanisms: diffusion or erosion mechanism (bulk vs. surface erosion) [92]. Both mechanisms (present in Figure 5) may contribute to the release of the drug nevertheless one of them is usually prevailing.

In systems controlled by diffusion, an active compound diffuse through the polymer on the basis of concentration gradient. The diffusion of the released compound is strongly affected by its solubility and diffusivity depending on properties of both the active agent and the polymer matrix. For instance, the rate of release of some poorly water-soluble substances can be found insufficient.

As for erosion controlled systems, the drugs are gradually released to the surrounding medium by erosion of particles due to presence of hydrolytically or enzymatically cleavable bonds [93]. This technique is suitable for compounds with low water solubility. Erosion can occur either as surface or bulk erosion. If the rate of surface degradation exceed the rate of water diffusion into the polymer, mechanisms is referred to as surface erosion. It is a desirable mechanism because, magnitude of erosion thus rate of release may be controlled by surface area of particles [94]. Bulk erosion occurs in entire polymer matrix when water penetration is faster than surface erosion.

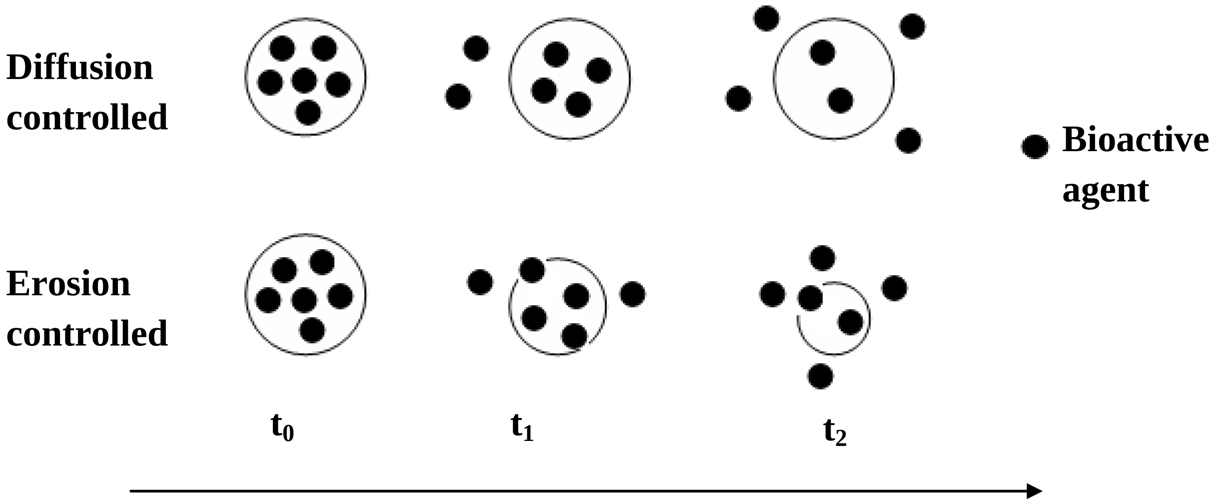

Figure 5: Mechanisms of release

AIMS OF DOCTORAL STUDY

The primary aim of doctoral study is the investigation of biological decomposition of biodegradable polyesters especially aliphatic polylactic acid (PLA) and aromatic-aliphatic copolyester poly(butylene adipate-co-terephthalate) (PBAT) and their utilization in selected special applications. The aims of work can be divided into several items:

- Study of the influence of selected environmental factors, particularly sun irradiation on the properties of biodegradable polymers and the rate of their biodegradation.

- Investigation of some material properties of polymeric polyester materials and their influence on the rate and course of biodegradation.

- Preparation of controlled release systems with a bioactive agent based on micro- and submicro- particles made from biodegradable polyesters.

SUMMARY OF THE PAPERS

In the following chapter, short summaries, major results and findings published in the attached Papers I to VI are presented. This chapter is divided into two thematic parts according to main aims of doctoral study: investigation of biological decomposition of biodegradable polyesters and their utilization in release system based on micro- and submicro- particles.

Investigation of biological decomposition of biodegradable polyesters

Paper I: Identification of several factors affecting biodegradation of aromatic-aliphatic copolyester

In the Paper I the effect of sample forms on the biodegradation of the aromatic-aliphatic copolyester was studied and compared in the soil and compost environments.

The material was introduced into the biodegradation tests in four forms differing above all in their specific surface areas, two films with different thickness and two other forms with higher specific surface areas, solvent precipitated powder of the polymer and deposition of the polymer on high specific surface area inert material. The course of biodegradation was monitored by gas chromatography equipped with TCD detector.

In principle, the copolyester proved its good biodegradability under compost conditions. It was shown that, the rate of biodegradation is greatly affected by specific surface of polymer specimens, when the biodegradation of sample forms with higher specific surface was significantly accelerated over sample specimens with low specific surfaces. However, for the most applications the polymer is applied in the form of films with a relatively low specific surface area and subsequently retarded biodegradation can be observed making him relatively poorly acceptable in an industrial composting plant.

Biodegradation was also tested in five various well-characterized agricultural soils however in non of the soils any significant biodegradation of any of the polymer forms was observed.

This contrast between biodegradation in soil and compost could be explained by the presence of large amount of highly active microorganisms in compost especially thermophilic actinomycetes producing extracelullar lipases and/or peptidases, which play critical role during the depolymerization process. According to another hypothesis, polyesters are hydrolyzed abiotically or enzymatically thus the increased temperature in compost (about 60°C) kinetically accelerates the reaction.

Paper II: Assessment of the interrelation between photooxidation and biodegradation of selected polyesters after artificial weathering

The Paper II deals mainly with the investigation of influence of photodegradation processes, taking place in the polymeric materials exposed to sunlight, on the biodegradation of selected polyesters. Free commercially available biodegradable polyester, two types of aromatic–aliphatic copolyesters (PBAT and Ecoflex) and polylactic acid intended for utilization as agricultural mulching films were chosen for the study.

Structural changes in polymeric films at the molecular level during experimental photooxidation simulating the exposure of materials to sun irradiation were evaluated in series of experiments. Initial information about the extent of eventual crosslinking in the samples receiving different doses during photooxidation was obtained from the determination of gel content. Changes in molecular weight (MW), MW distribution and crosslinking were elucidated in advanced rheological experiments. Moreover, structural changes were also monitored by non-isothermal thermogravimetry. Irradiated as well as non-irradiated samples in the form of films and sample forms with higher specific surface area were then subjected to biodegradation tests under composting conditions.

In the course of photooxidation processes all three of the biodegradable polyesters underwent deep changes on the molecular level resulting in rearrangement of the polymeric chains. In both copolymers with very similar chemical composition irradiation lead to crosslinking of polymer chain thus gradual increase of insoluble gel fractions. This process appeared to be significantly faster and also reached a higher extent in Ecoflex with higher content of the aromatic constituent, which should play apparently important role in the crosslinking mechanism, in comparison with PBAT. In PLA lacking any aromatic constituent gel fraction was not formed.

The gel fraction measurements corresponded with results from rheological experiments. The both viscosity and elasticity of copolyesters increased sharply and reached linearity in both components, which is characteristic for gel behaviour. Increase in slope steepness with irradiation time indicating the extent of crosslinking was more pronounced for Ecoflex again. In PLA, contrary to the former, photochemical reactions were not accompanied with crosslinking but instead provoked chain scissions. The increase in the content of char residue detecting by non-isothermal thermogravimetry may be taken as an evidence of some crosslinking in photooxidated copolyesters.

Biodegradation experiments showed that, despite pronounced structural changes, the extent of photooxidation was not the decisive factor significantly modifying the rate of biodegradation in all three investigated materials. The non-irradiated samples and those exposed to various degrees of photooxidation were comparable without any trend in biodegradability of differently exposed samples. According to expectation, the specific surface area of the sample specimens proved to be more important factor affecting biodegradation. It means, for example, that biodegradability of agricultural films might be enhanced to certain extent by their fragmentation during decomposition.

Based on the above findings it can be concluded that photooxidation of agriculture mulching films, made from the investigated materials and caused by sun irradiation during their field application, should not represent a problem for their subsequent biodegradation.

Paper III: Biodegradation of High Molecular Weight Polylactic Acid

Paper III was closely focused on biodegradation of high molecular PLA in compost and evaluation of its acceptability in industrial composting plants regarding the rate of decomposition. Likewise in paper I, various sample forms, film, fibre, powder and thin coating on inert surface differing in their specific surface areas were tested.

In accordance with previous findings from Paper II, the material confirmed its good biodegradability under composting conditions and all studied forms was shown to be acceptable for industrial composting. Contrary to the expectations, no significant differences in resulting mineralizations after 90 days period were observed for sample in the form of fibers, film and powder with various specific surfaces. However, the specific surface area of samples can contribute to different courses of biodegradation, in particularly different lengths of lag phases at the beginning of process and the rate of mineralization. The clearly faster biodegradation without any lag phase and with higher degree of mineralization was only detected for the thin coating on porous material with highest specific surface area.

Paper IV: Interrelations of molecular weight and sample form in the biodegradation of poly(L-lactic acid)

In Paper IV the study from previous paper III was extended to investigation of biodegradation of four PLA grades differing in their molecular weights (MW) in the interval from about 34 to about 160 kg mol^{-1}. The main aim of the work was evaluate the influence of MW on the rate and course of biodegradation. As in the preceding works, PLA samples were processed each into three different sample forms under identical experimental conditions. The material properties particularly tacticity and the related crystalinity, that could be critical for the interpretation of biodegradation results, were determined by polarimetry and differencial scanning calorimetry, respectively. Biodegradation of samples were monitored and evaluated in parallel processes of hydrolysis and microbial decomposition by comparing data from different experimental techniques employed.

Material characterization revealed that crystallinity of samples was roughly comparable, with somewhat higher values found in one case. The crystallinity of powder forms were always lower than film forms, reflecting the probable shorter time for crystallization during powder form preparation. Degree of crystalinity corresponded to the measured content of the D monomer.

The rate of biodegradation decreased with increasing MW of investigated samples with characteristic lag-phase or autocatalytic shape of the curves for higher MW samples. While for the two higher MW samples, the lag phase of about 20 days at the beginning of process was detected, it was apparently missing with the lowest MW PLA. For PLA with molecular weight 61000 g.mol^{-1}, curves exhibited some level of acceleration at about day 25, instead of the characteristic lag phase. As in the previous papers the specific surface area was also identified as an important factor promoting fast biodegradation especially for samples with higher MW. Whereas for the lowest MW PLA specific surface area of the film sample did not limit biodegradation rate comparing the other forms, for higher MW film samples biodegradation was markedly retarded. The initial retardation appeared gradually more pronounced with increasing MW for all sample forms.

The course of abiotic degradation was quasi identical with the course of biodegradation for all PLA grades investigated, suggesting that the abiotic hydrolysis appeared to be the major depolymeration mechanism and the rate controlling step of the biodegradation process.

Preparation of controlled release systems with a bioactive agent based on micro and submicro particles.

Paper V: Preparation of submicroparticles based on biodegradable co-polyester

The Paper V focuses upon the study of the preparation of submicro particles from biodegradable copolyester Ecoflex by the oil-in-water (O/W) solvent evaporation technique at reduced pressure. The main scope of study was investigation of the influence of various process parameters and conditions such as stirring speed, amplitude of sonication and concentration of polymer on the particle diameter. Size of particles was measured either by dynamic light scattering (Zetasizer) or with the help of optical microscope for particles with higher diameters.

During the study it was shown that it is possible to prepare micro and submicro polymer particles with this method. Among the investigated processing parameters the ultrasonication was shown to be crucial step of the method enabling to prepare particles with diameters under 1 μm, depending on the amplitude of sonication. Without sonication the typical mean diameter ranged from 1 to 10 μm decreasing with the stirring speed. The concentration of polymer also found to have a significant influence on final diameter, when smaller particles were formed with lower concentrations of polymer.

Paper VI: Low Molecular Weight Poly(lactic acid) Microparticles for Controlled Release of the Herbicide Metazachlor: Preparation, Morphology, and Release Kinetics

In Paper VI herbicide metazachlor (MTZ) frequently used in agricultural was encapsulated in biodegradable low molecular weight poly(lactic acid) micro- and submicroparticles, and its release to the water environment was investigated. With the help of knowledge from previous papers, three series of particles (S, M and L) varying in their size and with various initial amounts of MTZ were prepared by the O/W solvent evaporation method with gelatine as fully biodegradable and eco-friendly surfactant. The particle size and distribution were evaluated with the help of light scattering and optical microscope, respectively. These parameters as well as particle morphology were also observed by scanning electron microscopy. Efficiency of MTZ encapsulation and the amount released in subsequent experiments was measured by high performance liquid chromatography. To clarify the effect of particle properties, especially crystallinity, on MTZ release thermal analysis of particles was performed. PLA hydrolysis in aquatic environment was measured by dissolved organic carbon analysis to determined influence of abiotic erosion on the kinetics of MTZ.

Particles with maximum loadings corresponding to only 30% of the theoretical herbicide content were prepared due to polymer precipitation during solvent evaporation for higher loadings. Their diameters, which were controlled mainly by ultrasonication parameters and stirring speed, varied from 0.6 to 8 μm. S series of particles differed from M series by only about 200 nm, whereas L series particles were about 10 times larger. However the size of particles in individual series was not affected significantly by the MTZ loading. All formulations appeared to be spherical and also not affected by the amount of MTZ incorporated, with the exception of the highest loaded formulations in L series, which exhibited deviations from the spherical shape. Encapsulation efficiency reached about 60% and was lower for smaller particles (S and M series).

MTZ release was characterized by the typical nonlinear biphasic profile, where the initial rapid phase was followed by a considerably slower phase. Generally, it was found that the rate of release depends inversely on the z-average and directly on herbicide loading. Higher release rate of highly loaded formulation could have been enhanced by plasticizer effect of MTZ in PLA matrix decreasing Tg, which was close to the temperature employed in release experiments.

The kinetics of MTZ release from larger particles tended to be a diffusion-controlled, while the release from smaller ones was strongly influenced by an initial burst release. Low degree of PLA hydrolysis shown, that particle erosion has not considerable influence on MTZ release from PLA particles under given conditions.

CONCLUSIONS

The conclusion of the presented doctoral thesis is summarized in following items.

According to the expectation, the PBAT copolyester proved to be easily degraded in compost in contrast to soils, where is the rate of biodegradation significantly slower. The size of specific surface of polymer specimens showed to be significant factor accelerating the biodegradation.

Photooxidation simulating the exposure of materials to sun irradiation induced deep changes at molecular level in all three investigated polymers (PBAT, Ecoflex and PLA). The processes led to rearrangement of the polymeric chains, while in the case of both copolyesters (Ecoflex, PBAT) the events result in the polymeric chain crosslinking. On the contrary in PLA photochemical reactions provoked chain scissions. However, despite pronounced changes in their structure the rate of biodegradation in compost was not significantly modified by the extent of photooxidation. The specific surface area of the sample specimens was also shown to be more decisive factor. It can be concluded that in the case of application of investigated materials as mulching film on the fields the photooxidation caused by sun irradiation should not represent a problem for their subsequent biodegradation.

On closer investigation of PLA materials, it was found that the rate of biodegradation followed the pattern of increasing MW with characteristic lag-phase or autocatalytic shape of the curves for higher MW samples. In agreement with previous studies the specific surface area again proved to be an important factor promoting fast biodegradation especially for with higher MW samples. Abiotic hydrolysis appeared to be the major depolymerization mechanism controlling the rate of biodegradation process.

Regarding the preparation of micro and submicro particles by the oil-in-water (O/W) solvent evaporation technique, ultrasonication was found to be the crucial steps among the other investigated processing parameters affecting the size of particles. Application of sonication enables to prepare particles with diameters under 1 μm depending on the amplitude.

On the basis of obtained findings three series of polymer micro- and submicroparticles made from biodegradable PLA varying in size and loaded with various initial amounts of the herbicide metazachlor were prepared. Metazachlor release was characterized by the typical nonlinear biphasic profile, where the initial rapid phase was followed by a considerably slower phase.

Generally, it was found that the rate of herbicide release decreased with increasing size of particles and is lower for lower herbicide loadings. Whereas release of metezachlor from larger particles tend to be control by diffusion the kinetics for smaller particles was considerably influenced by an initial burst release. The study proved that it is possible to prepare release system of the herbicide metazachlor with relatively high water solubility based on PLA microparticles, when desired release profile could be tuned by combination of different formulations.

CONTRIBUTIONS TO THE SCIENCE AND PRACTISE

The present study provides the following contributions to science and technology.

- Assessment of the intramolecular changes in the aromatic-aliphatic copolyesters and poly(lactic acid) after photooxidation and the effect on the biodegradability in compost environment.

- The comprehensive study of the influence of poly(lactic acid) molecular weight on the rate and course of biodegradation in compost and hydrolysis as a driving factor.

- Progress in optimization of the oil-in-water (O/W) solvent evaporation technique for micro and submicro particles from aliphatic-aromatic copolyester.

- Creation of the fully biodegradable system for controled release of herbicides from low molecular weight PLA and gelatin as surfactant with potential applications in agriculture.

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LIST OF PUBLICATIONS

Articles published or accepted for publication in impact-factor journals (Jimp)

1. STLOUKAL, P., VERNEY, V., COMMEREUC, S., RYCHLY, J., MATISOVA-RYCHLA, L., PIS, V., KOUTNY, M. Assessment of the interrelation between photooxidation and biodegradation of selected polyesters after artificial weathering. *Chemosphere* 2012, vol. 88, no. 10, p.1214-1219.

2. STLOUKAL, P., KUCHARCZYK, P., SEDLARIK, V., BAZANT, P., KOUTNY, M. Low molecular weight poly(lactic acid) microparticles for controlled release of the herbicide metazachlor: Preparation, morphology, and release kinetics. *Journal of Agricultural and Food Chemistry* 2012, vol. 60, no. 16, p. 4111-4119.

3. KUCHARCZYK, P., URBANKOVA, M., STLOUKAL, P., KOUTNY, M., SEDLARIK, V. Influence of natural inorganic impurity on the synthesis of poly(L-lactic acid), properties and nanofabrication. *Journal od Applied Polymer Science* 2013, vol. 130, no. 1, p. 63-69.

Conference proceedings (D)

1. STLOUKAL, P., KOUTNY, M., SEDLARIK, V., KUCHARCZYK, P. Biodegradation of high molecular weight polylactic acid. 6th International conference on time of polymers (TOP) and composites, Ischia, Italy, 10–14 June 2012, ISBN: 978-0-7354-1062-6.

2. KUCHARCZYK, P., SEDLARIK, V., STLOUKAL, P., BAZANT, P., KOUTNY, M., GREGOROVA, A., KREUH, D., KURITKA, I. Poly (L-lactic acid) coated microwave synthesized hybrid antibacterial particles. Mezinárodní konference NANOCON 2011, 21-23. September 2011, Brno, Czech Republic, ISBN: 978-80-87294-23-9.

Other results (O)

3. KUCHARCZYK, P., STLOUKAL, P., KOUTNY, M., SEDLARIK, V. Biodegradable polymers from renewable resources: Effect of calcium inorganic impurity on polycondensation products of 2-L-hydroxy propanoic acid. The 1st WSEAS International conference on Biomedicine and Health Engineering, Zlín – The Czech Republic, 2012

4. KUCHARCZYK, P., STLOUKAL, P., KOUTNY, M., PAVELKOVA, A., SEDLARIK, V. Synthesis of PLA-PEG copolymers and their chain extending with di-isocyanate compounds. Structure, properties, degradation and potential utilization. 7th MoDeSt Conference, Prague, Czech Republic, September 2-6, 2012.

5. STLOUKAL, P., KUCHARCZYK, P., SEDLARIK, V., KOUTNY, M., Preparation of submicroparticles from poly(lactide)-poly(ethylene glycol) block copolymer Polymer in medicine 2012, Prague, Czech republic, 5 July, 2012

6. HUSAROVA, L., STLOUKAL, P., COMMEREUC, S., VERNEY, V., KOUTNY, M. Thermophilic microbial communities degrading selected synthetic polymers, Environmental Microbiology & Biotechnology conference 2012, Bologna, Italy, April 10-12, 2012.

7. DVORACKOVA, M., STLOUKAL, P., KOUTNY, M., GREGOVSKA, M. Biodegradability of Aliphatic-Aromatic Copolyester in Aqueous Anaerobic and Aerobic Environment. Proceedings of the 4 International Conference on Evironmental and Geological Science and Engineering, Barcelona, Spain, September 15-17, 2011, p. 141-1146, ISBN: 978-1-61804-032-9.

8. HUSAROVA, L., KOUTNY, M., STLOUKAL, P. Influence of long term storage on thermooxidation behavior of prooxidant containing low density polyethylene. International Conference on Development, Energy, Environment, Economics 2011 (DEEE 11), 10.-12. December 2011, ISBN 978-1-61804-055-8.

9. KOUTNY, M., STLOUKAL, P., COMMEREUC, S., VERNEY, V. Composting of some biodegradable synthetic polyesters can be influenced by sample shape and surface quality. European Polymer Congress, 26.6.-

1.7.2011, Granada, Spain, ISBN 978-84-694-3124-5.

10. STLOUKAL, P., KOUTNY, M., SEDLARIK, V., KUCHARCZYK, P. Optimization of biodegradable polymeric submicroparticles preparation. 5th International Conference on Energy and Development - Environment - Biomedicine 2011 (EDEB 11) Corfu Island, Greece, July 14-16, 2011, ISBN 978-1-61804-022-0.

11. KUCHARCZYK, P., SEDLARIK, V., KITANO, T., MACHOVKY, M., BARAK, M., KOUTNY, M., STLOUKAL, P., SAHA, P. Characterization of Partially Biodegradable Poly(L-lactic acid)/Poly(methyl methacrylate) Blends as Potential Biomaterials. 5th International Conference on Energy and Development - Environment - Biomedicine 2011 (EDEB 11) Corfu Island, Greece, July 14-16, 2011, ISBN 978-1-61804-022-0.

12. STLOUKAL, P., KOUTNY, M., SEDLARIK, V., KUCHARCZYK, P. Factors influencing encapsulation efficiency of biologically active compound into PLA submicroparticles. 2nd International Conference on Development, Energy, Environment, Economics 2011 (DEEE 11), 10-12. December 2011, Tenerife, Spain, ISBN978-1-61804-055-8.

13. VERNEY, V., COMMEREUC, S., ASKANIAN, H., COLLIN, S., KOUTNY, M., STLOUKAL, P., PERCHET, G., SANVOISIN, J., TROQUET, J. Interrelation between photodegradation and biodegradation of environmentally degradable polymers (PLA and PBAT). Frontiers in Polymer Science, 29-31.5.2011. Lyon,France.

14. STLOUKAL, P., JANDAK, J., HUSAROVA, L., KOUTNY, M., COMMEREUC, M., VERNEY, V. Identification of several factors affecting biodegradation of arometic-aliphatic copolyester. DEEE 2010 WSEA conference, 30.11.-2.12.20010. Puerto de la Cruz, Tenerife, ISBN: 978-960-474-253-0, ISSN: 1792-6653.

15. STLOUKAL, P., SEDLARIK, V., HUSAROVA, L., KASPARKOVA, V., KOUTNY, M. Preparation of submicroparticles based on biodegradable co-polyester DEEE 2010 WSEA conference, 30.11.-2.12.2010. Puerto de la Cruz, Tenerife, ISBN: 978-960-474-253-0, ISSN: 1792-6653.

16. STLOUKAL P., ROY N., SAARAI A., SEDLARIK V., KOUNY M. Preparation of submicroparticles based on biodegradable co-polyester. PLASTKO 2010, 13-14. April 2010, Zlín, Czech Republic, ISBN 978-80-7318-909-9

17. STLOUKAL, P., KOUTNY, M. Biodegradabilní plasty: současnost a perspektivy Průmyslová ekologie 24.03.2010 - 26.03.2010, Zruč nad Sázavou, Czech Republic, ISBN: 978-80-86832-50-0

Utility models (F)

1. SEDLAŘÍK, V. – KUCHARCZYK, P. - SÁHA, P. - KOUTNÝ, M. - STLOUKAL, P. DRBOHLAV, J. – NĚMEČKOVÁ, I. – NEHYBA, A. - BINDER, M. - ŠALAKOVÁ A. Biologicky odbouratelný materiál, zejména pro použití v humánních nebo veterinárních aplikacích a v zemědělství. Užitný vzor (n. 22603), published 2011 – co-author

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Education

2009 - to date **Doctoral studies**	Tomas Bata University in Zlin Faculty of Technology Technology of Macromolecular Compounds Doctoral Topic: Study of properties of biodegradable polymeric materials based on polyester
2007 - 2009 **Master's degree**	Institute of Chemical Technology in Prague Faculty of Chemical Engineering Process Engineering and Informatics Master thesis – Modelling and simulation of the processing of solids using
2004 - 2007 **Bachelor's degree**	Institute of Chemical Technology in Prague Faculty of Chemical Engineering Process Engineering, Informatics and Management Bachelor thesis – Contribution to risk assessment of chemical processes

Training abroad

4/2012, 10/2012	Montanuniversität Leoben, Austria AKTION project Development of effective technology for production of biodegradable polymer nanocomposite films with advanced properties.
4/2010 – 6/2010	Centre National pour Evaluation de Photoprotection, CNRS - Blaise Pascal University, AUBIERE cedex, France Erasmus work placement An investigation of the changes in the biodegradable polymers after irradiation with UV; preparation of samples for biodegradation.

Work on projects

2012 – to date	Project EUPRO-LE12002 Centre for promotion of international cooperation in research and development in technical fields Member of research team
2012 – to date	Centre of Polymer Systems project reg. number: CZ.1.05/2.1.00/03.0111 Member of research team
2012 – to date	Aktion Česko- Rakousko, 63p24 Development of effective technology for production of biodegradable polymer nanocomposite films with advanced properties. Member of research team
2012	Grant IGA/FT/2012/005 Development and application of microspheres on the base of newly synthesized biodegradable block copolymers. Member of research team
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PAPER I

Identification of several factors affecting biodegradation of aromatic-aliphatic copolyester

Petr Stloukal, Jiri Jandak, Lucie Husarova, Marek Koutny, Sophie Commereuc and Vincent Verney

Abstract — Aromatic-aliphatic polyesters aspire to be an eco-friendly replacement of conventional non-biodegradable polymers. Biodegradation of studied aromatic-aliphatic copolyester was estimated in soil and compost. The material was introduced into the tests in four forms differing above all by their specific surface. The copolyester in principle proved to be biodegradable under compost conditions. Biodegradation was also tested in five well selected and characterized agricultural soils. Here its biodegradability was not found to be significant. The specific surface of sample specimen was shown to be an important factor affecting the rate of biodegradation in compost, where biodegradation sample forms with higher specific surface was significantly accelerated over sample specimens with low specific surfaces.

Keywords — biodegradation, composting, copolyester, mulching film, soil.

I. INTRODUCTION

PLASTIC litter represents not only a serious environmental but also a social problem. Public is greatly alarmed at the omnipresence of plastic litter in the environment. The problem is particularly striking in less developed countries where functioning of waste management systems is insufficient. It should be pointed out that still the most recommended way of plastic waste treatment remains recycling but for many applications this approach tend to be economically not feasible due to the high cost of collection, separation and cleaning of the raw material. Plastic films are also still more extensively used in agriculture as mulching films. Here also the collection and especially cleaning is problematic.

In the past years growing interest is paid to the development of biodegradable polymers that could be able to replace conventional polyolefin films in many applications including mulching films and packaging applications [1,2]. The particularly perspective branch of such materials is represented by synthetic copolyesters with content of aromatic and aliphatic components [3,4]. Changing the component ratio one is able to balance processing properties of the copolyester and its biodegradability [5,6,7]. In principle, aliphatic parts of the copolyester favor biodegradability of the polymer [8]. Several these materials were already commercialized and they are available on the market [9].

However biodegradation of such materials was not extensively enough studied especially regarding different conditions where and how the biodegradation should occur.

The present study deals with one particular example of the described materials. We wanted to show that its biodegradation can be greatly affected by the form of the sample specimen and can differ in various environments.

II. MATERIAL AND METHODS

A. Material

The polymer used throughout the study was aromatic aliphatic copolyester containing units of terephtalic acid, adipic acid and 1,4-butane-diol (molar ratio 22:28:50, respectively).

B. Polymer processing

Raw polymer in the form of pellets was processed to obtain different forms of the polymer with different specific surfaces.

Films. Films were prepared by compression molding at 140°C. According to the inserted steel frame films of 100 μm and 300 μm were prepared.

Powder. Chloroform solution of polymer (50 mg/ml) was poured into four volumes of ethanol and the mixture was vigorously stirred. Obtained precipitate was filtered out and dried on air. Resulting powder was characterized by microscopy (Fig. 1A, 1B). There were of irregular shape with diameters up to 500 μm. Electron microscopy with higher magnification revealed the important specific surface of the particles (Fig. 1B).

Thin coating on inert surface. Calculated volume (4 ml) of the chloroform solution of polymer (50 mg/ml) was applied on the surface of pre-weighed porous inert material (perlite, 7 g) and stirred. Then the solvent was stripped out with air leaving the polymer coating on the surface of perlite.

C. Biodegradation experiment

Three components were weighed into 500 ml biometric flasks: polymer samples (200 mg), mature compost or any of tested soils (5 g of dry weight) and perlite 7 g. Biometric flasks were equipped with septa on stoppers. Sample flasks

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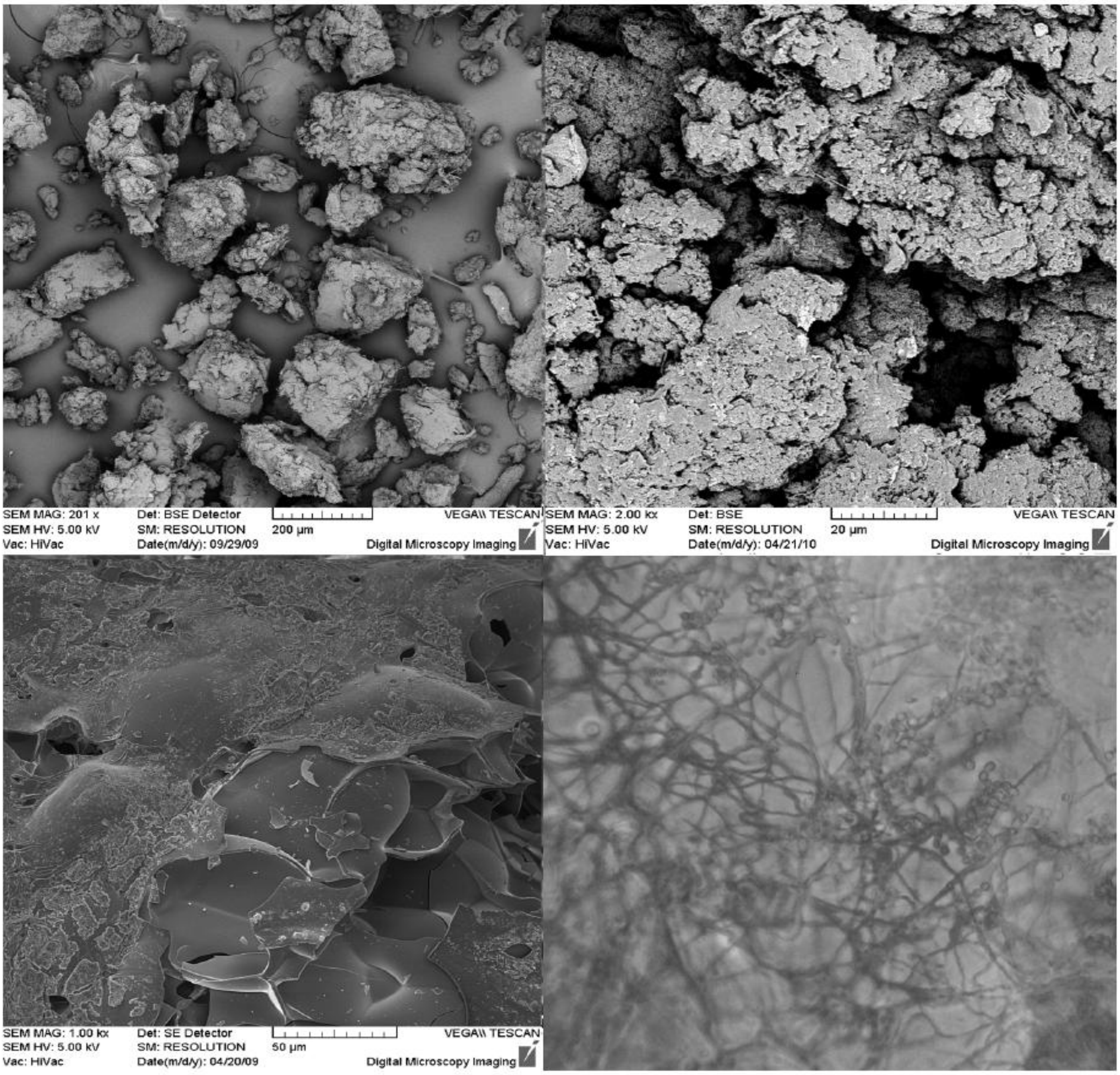

Fig. 1. A, electron microscopy of precipitated polymer powder (200×); B, electron microphotography of polymer powder at higher magnification (2000×) showing high specific surface of the powder particles; C, electron microphotography of polymer coating (upper left part of the microphotograhpy) on the surface of perlite (1000×); D, optical microscopy of bacterial filaments on the surface of 100 µm polymer film after incubation in compost (1000×), filaments were stained with carbolfuchsin and then observed under immersion oil, endospores typical for thermoactinomycetes are apparent as tiny nodules, the picture could not be made well focused everywhere because the film was not perfectly flat.

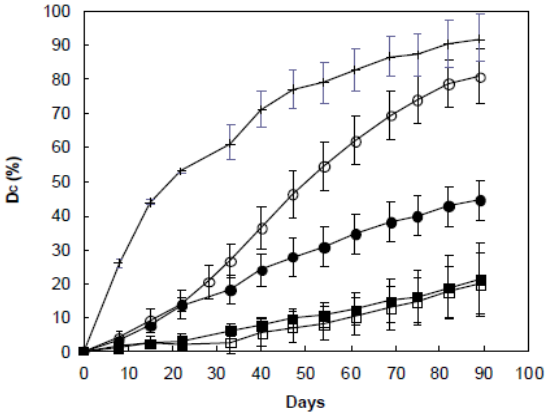

Fig. 2. Biodegradation of different forms of the polymer in compost experiment. □, thin film (100 µm); ■, thick film (300 µm); ○, powder; ●, thin coating; +, cellulose (reference compound). Error bars correspond to twice standard deviation (n=3).

D. Microscopy

For optical microscopy of the surface biofilm the polymer film fragment was fixed with 4% formaldehyde (overnight at 4°C) and then stained with carbolfuchsin. For scanning electron microscopy a polymer sample was coated with thin gold/palladium layer and observed in VEGA LMU (Tescan, CZ) instrument.

III. RESULTS

Investigated copolyester was designed to be a biodegradable material with potential applications in agriculture. Preliminary experiments showed (data not presented) that biodegradation of relatively thick compression (100 µm) molded polymer films appeared to be relatively slow. We decided to test whether this phenomenon was caused by low specific surface of the films. To answer the question we prepared two other forms of the polymer with higher specific surfaces, solvent precipitated powder of the polymer and deposition of the polymer on high specific surface inert material (perlite). Furthermore we intended to compare the polymer biodegradation under composting conditions and its biodegradation in a set of selected soils.

A. Biodegradation in compost

Results from compost experiment are well illustrated by Figure 2. Samples in forms of thin and thicker films behaved almost equally and end up with about 20% carbon mineralization after 90 days of incubation. Final values must be evaluated as relatively poor and hardly acceptable in an industrial composting plant.

However for the two forms of sample with higher specific surfaces the results are clearly more optimistic with about 45% carbon mineralization in the case of polymer coating on teh material with high specific surface (perlite). The fastest and most pronounced biodegradation was observed with the powder of the polymer, where it reached more than 80% at the end of the observation period and was comparable with the biodegradability of microcrystaline cellulose powder used as a reference material.

B. Biodegradation in soil

Biodegradation of the described forms of polymer was also tested in soil environment. Five agricultural soils were used in the test and their classification types and basic structural and physical-chemical characteristic were determined (Table I). Basic microbiological characteristic were also obtained (Table II) to prove that all five soils were fertile and capable to support biodegradation.

However, the results of biodegradation experiments with all five soils were identical; in no one of the soils any significant biodegradation of any of the polymers forms was observed, levels of mineralization observed was not significantly different from zero.

IV. DISCUSSION

The polymer proved its good biodegradability under composting conditions. However, our experiments clearly showed that specific surface of polymer specimens affects greatly the rate of biodegradation. For most applications the polymer will be applied in the form of films with a relatively low specific surface and subsequently retarded biodegradation. It must be admitted that the films used throughout our experiments were thicker than films that would be prepared for real applications. We was not capable to prepare films thinner than 100 µm by compression molding, in real industrial production the film would be prepared by blowing process and

TABLE I
PHYSICAL-CHEMICAL PROPERTIES OF SOILS

Soil	Horizon	Soil type	Soil texture	pH H_2O	pH KCl	SOM %
S1	Plough layer	Eutric Cambisol	Loam	7.10	5.18	2.56
S2	Plough layer	Haplic Luvisol	Silty clay loam	6.44	6.33	1.67
S3	Plough layer	Haplic Chernozem	Silt loam	7.28	6.28	3.05
S4	Topsoil	Haplic Fluvisol	Silt loam	7.04	5.49	4.30
S5	Plough layer	Calcic Chernozem	Loam	8.06	7.52	2.07

pH H_2O = Soil pH in distilled water, pH KCl = Soil pH in M KCl, SOM = Soil organic matter [10] .

TABLE II
BASIC MICROBIOLOGICAL CHARACTERISTIC OF SOILS

Soil	Filamentous fungi	Aerobic chemoorganotrophs	Actinomycetes
S1	6.89×10^4	4.59×10^6	3.44×10^6
S2	3.78×10^3	5.26×10^6	2.92×10^6
S3	5.37×10^4	5.65×10^6	3.30×10^6
S4	2.25×10^5	9.81×10^6	6.18×10^6
S5	6.65×10^4	4.83×10^6	4.83×10^6

Numbers represents colony-forming units (CFU) per gram of soil dry weight.

would be thinner (about 20 μm).

On the contrary, the polymer revealed to be non biodegradable in soil environment at least under conditions and time scale defined in our experiments. This finding is not completely surprising, because biodegradation of similar materials in soil was not clearly reported in the literature yet. Also longer time studied polyester poly lactid acid (PLA) was shown to be virtually non-biodegradable in soil environment, while readily biodegradable under composting conditions [11,12, 13].

Reasons for such behavior of polyesters are not completely understood. It is known that extracelullar lipases and/or peptidases play critical role during depolymerization process and make polymer fragments available for microorganisms. Thus higher ability of compost specific microbial communities to produce such enzymes could be an explanation of faster biodegradation of described polyester in compost environment. This explanation can be supported by the observation of biofilm of bacterial filaments bearing endospores (Fig. 1D) that could be tentatively indentified as thermo-actinomycetes, which is the typical taxonomic group participating on the decay of organic matter in compost.

Not fully rejected there is still the hypothesis that polyesters can be hydrolyzed abioticaly or enzymatically and the increased temperature in compost (about 60°C) kinetically accelerates the reaction.

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Errata

Missing text in II. Material and methods, C. Biodegradation experiment

Sample flasks were incubated at 25°C for biodegradation in the soil environment and at 58°C for biodegradation under compost conditions. Head space gas was sampled at appropriate intervals through the septum with a gastight syringe and then injected manually into a GC instrument (Agilent 7890). Sampling was operatively adapted to actual CO_2 production and O_2 consumption. From the CO_2 concentration found, the percentage of mineralization with respect to the initial sample carbon content was calculated. Endogenous production of CO_2 by soil or compost in blank incubations was always subtracted to obtain values representing net sample mineralization. In parallel, oxygen concentration was also monitored to provide a control mechanism so as to ensure samples did not suffer from hypoxia. Three parallel flasks were run for each sample, along with four blanks and positive control flasks with microcrystal cellulose for both compost and soils.

PAPER II

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Assessment of the interrelation between photooxidation and biodegradation of selected polyesters after artificial weathering

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ABSTRACT

Three commercially available biodegradable polymers, two different aromatic–aliphatic copolyesters and polylactic acid, intended for the fabrication of agricultural mulching films, in addition to other applications, were subjected to a series of tests with the aim of studying the relationship between their photooxidation and biodegradation. Photooxidation resulted in the rearrangement of polymeric chains, in the case of both copolyesters the events led to polymeric chain crosslinking and the formation of insoluble polymeric gel. The tendency was significantly more pronounced for the copolyester with the higher content of the aromatic constituent. As regards polylactic acid photochemical reactions were not accompanied by crosslinking but instead provoked chain scissions. A biodegradation experiment showed that, despite marked structural changes, the extent of photooxidation was not the decisive factor, which significantly modified the rate of biodegradation in all three materials investigated. The specific surface area of the sample specimens was shown to be more important.

1. Introduction

Polymer films are increasingly used in agriculture, both for traditional packaging applications, but recently there is also a rising demand for so-called mulching films as well (Espi et al., 2006). These are applied directly to the surface of soil, and their purpose is to retain moisture in the soil and moderate the surface temperature. The proper use of the mulching films can additionally lead to the reduction of pesticide application. In recent years these agricultural practices have witnessed a strong increase in popularity, and are applied on significant areas of farmland (Briassoulis, 2007). For all agricultural applications and, especially for the mulching, it is highly desirable that the films are made from a biodegradable polymer. In such case, the material can be either plowed into the soil, if its degradability in soil is sufficiently guaranteed (Suyama et al., 1998; Trinh Tan et al., 2008), or may be processed after collection along with other organic waste in composting facilities.

Useful materials for mulching films, that exhibit satisfactory mechanical and processing properties and are available for a reasonable price, include various polyesters and copolyesters either synthetic or fully or partially derived from renewable resources (Amass et al., 1998; Rudnik, 2008). Much attention is paid to materials based on polylactic acid (PLA), which can be obtained from renewable resources, and to possible improvements of its relatively poor physico-chemical and processing properties (Martin and Avérous, 2001). Another important group of applicable materials comprises aliphatic–aromatic copolyesters (Muller et al., 2001; Chen et al., 2007; Han et al., 2009). These materials exhibit favorable processing and mechanical properties that are positively influenced by the content of the aromatic component. On the contrary, an aliphatic component can support biodegradability of the material (Witt et al., 1996a,b; Marten et al., 2005). Examples of such commercially available polymers are Ecoflex (BASF) and PBAT (EnPol Ire) as used in the study presented here.

When applied to the soil surface, polymer materials are heavily influenced by environmental factors, among which, the influence of solar radiation is probably crucial. The materials can prematurely degrade and thus their integrity and function can be compromised (Kale et al., 2006), or vice versa, one could take advantage of solar

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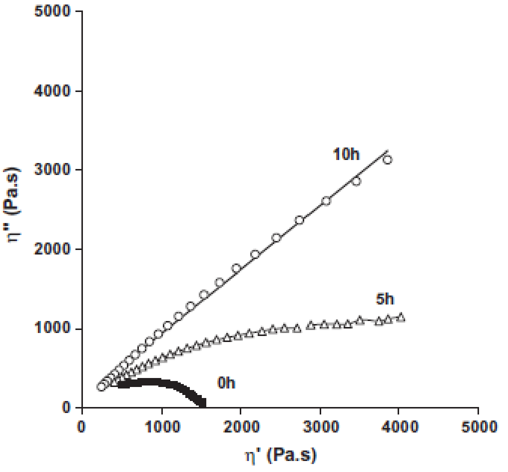

Fig. 1. Cole–Cole plots for Ecoflex (non-aged and aged 5 and 10 hs) at 140 °C.

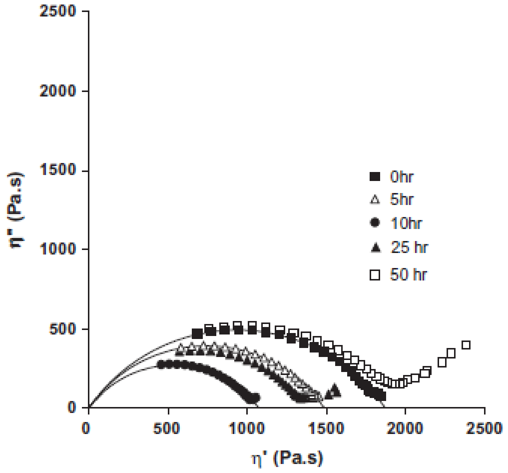

Fig. 2. Cole–Cole plots for PLA at 180 °C, non-aged and irradiated for 5, 10, 25 and 50 h.

radiation to start the rapid oxidation of the material leading to biodegradable products in so-called oxo-biodegradable materials (Koutny et al., 2006). Kijchavengkul et al. (2008a,b) conducted an extensive study of particular aromatic-aliphatic copolyester films in the laboratory and under field conditions. The authors observed relatively rapid changes in the investigated materials in the outdoor field experiment and also in the corresponding laboratory experiments where materials were exposed to long-wave ultraviolet radiation. At the molecular level, the deterioration of mechanical properties was correlated with the crosslinking of polymer chains and the formation of a gel fraction. It was also stated that samples with higher gel contents were biodegraded to lesser extend during laboratory composting tests (Kijchavengkul et al., 2008b). The authors concluded that photoinitiated changes can restrain subsequent biodegradation of these materials therefore deteriorating an important part of its functional properties. It should be noted that the behavior described could be markedly influenced by fillers incorporated in the studied materials, specifically by titanium dioxide, which is known to accelerate photochemical processes (Allen et al., 2004), and carbon black, which in turn could significantly protect the samples against the effects of radiation to which they were exposed. In the study presented here, the authors decided to investigate photodegradation in two materials, types of aromatic-aliphatic copolyesters, that varied in the content of the aromatic component. As previously indicated (Kijchavengkul et al., 2008b), photochemical reactions that lead ultimately to the crosslinking of the material take place on aromatic rings. PLA was studied in parallel as a reference, as well as other potentially useful materials for similar applications. Structural changes during photodegradation in fully controlled laboratory experiments were evaluated, and samples with different exposure levels were then subjected to biodegradation tests. The samples investigated contained no fillers that might affect the rate and/or the mechanism of photochemical and biodegradation processes.

2. Materials and methods

2.1. Materials

Ecoflex, BASF, Germany; PBAT G8060 (polybutyleneadipate-co-butyleneterephthalate), EnPol Ire chemicals, Korea; PLA (polylactide) 4042D, Nature Works, USA. All in the form of compression molded 100 μm thick films. The content of aromatic and aliphatic constituents in Ecoflex and PBAT were determined in ^{1}H-NMR experiment (Herrera et al., 2002) with a polymer solution in deuterated chloroform (50 mg mL^{-1}). For Ecoflex the method found 75.8 mol% of aliphatic and 24.2 mol% of aromatic components; for PBAT 78.0 mol% and 22.0 mol% of aliphatic and aromatic, respectively.

2.2. Photooxidation

Samples were exposed to UV irradiation at 60 °C in an accelerated photo-ageing device (based on SEPAP 14–24 described elsewhere (Lemaire et al., 1988). This polychromatic set-up was equipped with four medium pressure mercury sources filtered through a borosilicate envelope (Mazda type MA 400), the device irradiance is 90 W/m2 in the wavelength interval 290–400 nm. Sources are located along the focal axis of a cylinder with an elliptical base. Sample films, fixed on aluminum holders, turned around the other focal axis. The inside of the chamber is made of highly reflective aluminum. The temperature of samples is controlled by a thermocouple connected with a temperature regulator device which controls a fan. All experiments were carried out at 60 °C. The films were analyzed after various exposure times.

2.3. Rheology

Melt rheology experiments were carried out on an ARES TA Instruments mechanical spectrometer. Oscillatory frequency sweep tests were conducted in the plate–plate geometry. (8 mm diameter). In all cases, the stress was checked with respect to the strain amplitude to be sure to remain in the linear viscoelastic region.

2.4. Gel content

Gel content was determined after dissolution in chloroform and collection of the undissolved part on pre-weighed filter. After thorough washing and drying in an oven at 50 °C the undissolved residue was weighed. All was done in triplicate.

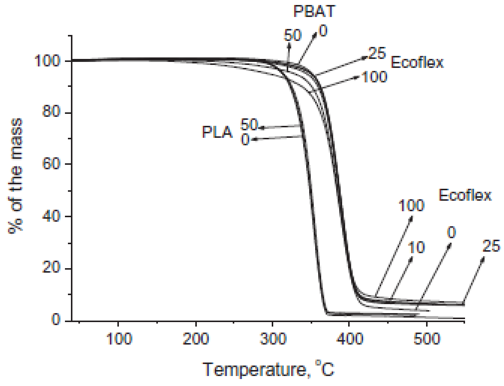

Fig. 3. Non-isothermal thermogravimetry runs in nitrogen for Ecoflex and PLA. The rate of heating 5 °C min^{-1}. Numbers denote the duration of photooxidation in days.

Table 1
Evolution of gel fraction during photooxidation.

Sample	Photooxidation (h)	Gel fraction (% ± SD)
Ecoflex	0	0
	10	8 ± 0.28
	25	54 ± 0.14
	100	78 ± 0.52
PBAT	0	0
	10	8 ± 0.06
	40	34 ± 0.12
	50	37 ± 1.21
PLA	0	0
	10	0
	50	0

SD, standard deviation (n = 3).

2.5. Thermogravimetry

The change of the sample mass with temperature was measured using a Mettler-Toledo TGA/SDTA 851e instrument in nitrogen flow (30 mL min^{-1}) using a heating rate of 5 °C min^{-1} in a temperature range from room temperature up to 550 °C. Indium and aluminum were used for temperature calibration. The amount of samples applied ranged between 2 and 3 mg.

2.6. Biodegradation under the composting condition

The method was based on previously published protocol by Dřímal et al. (2007) with some modifications. Polymer films were cut into 2 mm pieces, then 100 mg of polymer, 5 g of perlite and 2.5 g of dry weight of compost were weighed into each 500 mL biometric flask. To prepare the sample form with increased specific surface area, 5 mL of chloroform was added to 100 mg of polymer in a biometric flask and left to dissolve and/or swell for 5 h on a rotary shaker, then 5 g of perlite was added and the content was mixed so that the polymer solution was deposited on the surface of the inert material; the organic solvent was then stripped out with air overnight and compost was added. The flasks were sealed with stoppers equipped with septa and incubated at 58 °C. Headspace gas was sampled at appropriate intervals through the septum with a gas-tight syringe and then injected manually into a GC instrument (Agilent 7890), equipped with Porapak Q (1.829 m length, 80/100 MESH) and molecular sieve 5A (1.829 m length, 60/80 MESH) packed columns connected in series and thermal conductivity detector (carrier gas helium, flow 53 mL min^{-1}, column temperature 60 °C). Sampling intervals were operatively adapted to actual CO_2 production and O_2 consumption. Concentrations of CO_2 and O_2 were derived from the calibration curve obtained using the calibration gas mixture with declared composition (Linde). Endogenous production of CO_2 by soil or compost in blank incubations was always subtracted to obtain values representing net sample mineralization. From the concentration found, the percentage of mineralization with respect to the initial carbon content of the sample was calculated $M_{\%} = m_{gc}/(m_s w_c)$, where $M_{\%}$ is the percentage of mineralization, m_{gc} is the mass of carbon evolved as CO_2 and obtained form GC analysis, m_s is the weight of a polymer sample, and w_c is the percentage (w/w) of carbon in a polymer investigated. Values of w_c for the given polymers (62.5%, 62.4%, and 50.0%, for PBAT, Ecoflex, and PLA, respectively) were determined in Flash Elemental Analyzer 1112 (Thermo). In parallel, oxygen concentration was also monitored to provide a control mechanism so as to ensure samples did not suffer from hypoxia. Three parallel flasks were run for each sample, along with four blanks.

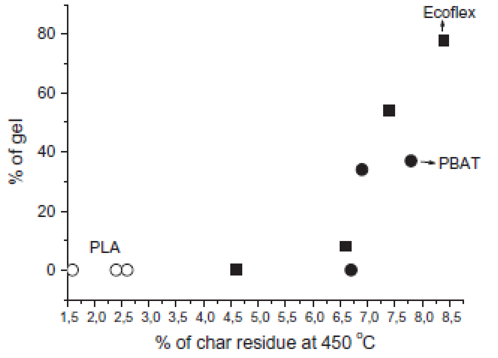

Fig. 4. Correlation of the gel fraction formed in Ecoflex, PLA and PBAT during photooxidation with the amount of char residue obtained at 450 °C in non-isothermal thermogravimetry experiments.

Table 2
Cole–Cole parameters versus irradiation times for Ecoflex.

Irradiation time (h)	Eta-zero, (Pa s)	h	Slope
0	1596	0.50	–
5	5382	0.54	0.0884 ± 0.0136
10			0.808 ± 0.00920
20			3.19 ± 0.0270
25			1.50 ± 0.000622
40			8.19 ± 0.0980
50			18.7 ± 0.275
100			19.6 ± 0.871

3. Results and discussion

3.1. Photooxidation and characterization of resulting samples

One of the main aims of the study was to investigate whether photooxidation processes, taking place in the polymeric materials exposed to sunlight during the time they remain on the field, can affect the biodegradability of said materials in compost. In order to simulate the outdoor exposure under fully controlled conditions compression molded 100 μm thick films made from the materials under investigation were photooxidized in SEPAP 14–24 weathering chamber. Approximately, one hour of incubation in this instrument is equivalent to one day of outdoor exposure in a mild climatic zone

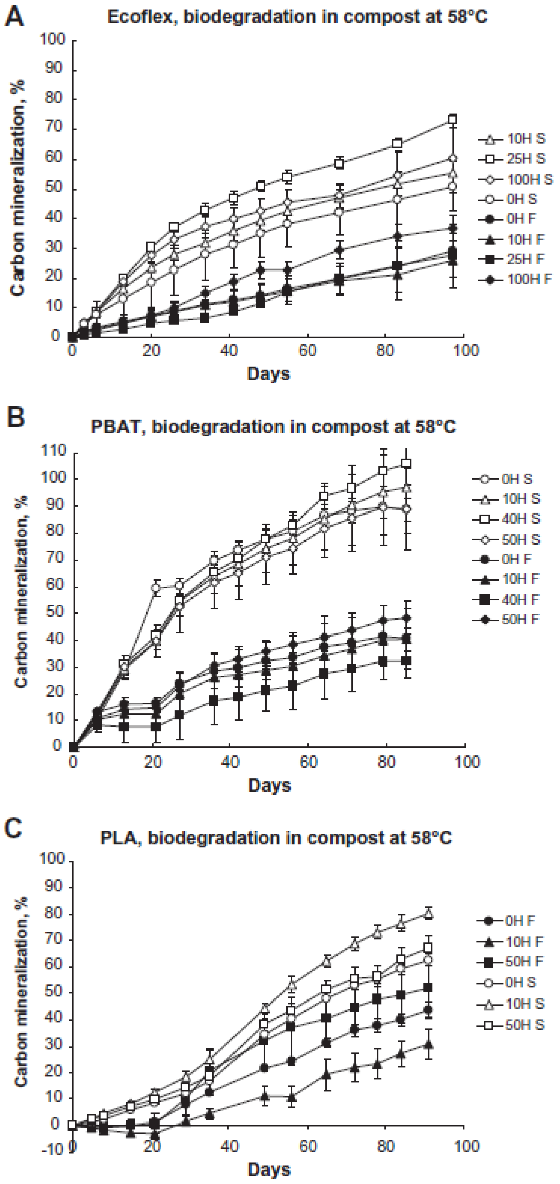

Fig. 5. Biodegradation of investigated materials under composting conditions. In the data description the number with "H" denotes the time of photooxidation of the sample in hours; "F", sample in the form of 100 μm film, "S" sample in the form with an increased specific surface area.

during the summer months for the given materials (Akanian, 2011). A preliminary photooxidation experiment, followed by rheological measurements, helped the authors to rationally adjust a limited number of time points necessary to follow up the evolution of photooxidation in each material, and for these time points larger quantities of samples were prepared to fulfill the needs of all the intended characterization methods. The maximal time of exposure in SEPAP was limited by the structural integrity of the materials.

So as to obtain initial information about the extent of eventual crosslinking, the samples receiving different doses of photooxidation were dissolved in chloroform and fractions of insoluble gel portions in samples were determined (Table 1). PBAT and Ecoflex, polymers of very similar chemical composition, exhibited a gradual increase in the gel fraction, whereas in Ecoflex the process appeared to be significantly faster and also reached a higher extent. This probably reflects the higher content of aromatic constituents in Ecoflex in comparison with PBAT, which is apparently important in the chain-recombination events according to the proposed crosslinking mechanism (Kijchavengkul et al., 2008a; Commereuc et al., 2010). PLA lacking any aromatic constituent did not produce a gel fraction.

Various structural changes in the polymeric materials at the molecular level, including changes in molecular weight (MW) distribution, crosslinking, and/or formation of low MW fraction, can be conveniently elucidated in advanced rheological experiments.

Oscillatory frequency sweeps consist of applying a sinusoidal strain $\gamma^*_{(\omega)}$ and measuring the resulting sinusoidal stress $\sigma^*_{(\omega)}$. The stress/strain ratio is then the complex mechanical modulus $G^*_{(\omega)} = G'_{(\omega)} + iG''_{(\omega)}$, with i the imaginary number ($i^2 = -1$). The ratio of stress over the strain rate is the complex viscosity:

$$\eta^*_{(\omega)} = \eta'_{(\omega)}, -i\eta''_{(\omega)} \quad \text{then} \quad G^*_{(\omega)} = i.\omega.\eta^*_{(\omega)}$$

It is well-known that zero shear viscosity η_0 depends on the molecular weight and adheres to a power law (Ferry, 1980): $\eta_0 \propto Mw^{\alpha}$. Zero shear viscosity η_0 can be obtained from the complex viscosity $\eta^*_{(\omega)}$:

$$\eta^* = G^*_{(\omega)}/i\omega = \eta' - i\eta'' \quad \text{and} \quad |\eta^*|_{\omega\to 0} = |\eta'|_{\omega\to 0} = \eta_0$$

An empirical rheological model used to fit dynamic data is Cole–Cole distribution expressed by (Montfort et al., 1984; Verney and Michel, 1989; Vega et al., 1996):

$$\eta^*_{(\omega)} = \eta_0/[1 + (i\omega\lambda_0)^{1-h}]$$

where λ_0 is the average relaxation time and h the parameter of the relaxation-time distribution. This model predicts the variation of the viscosity components (η'' versus η') as an arc of a circle in the complex plane. From this representation it is easy to determine the parameters of the distribution: η_0 is obtained through the extrapolation of the arc of the circle on the real axis, while distribution parameter h is found through measuring of the angle $\Phi = h\Pi/2$ between the real axis and the radius going from the origin of the axis to the center of the arc of the circle. Therefore, this representation can be a useful one to exemplify molecular weight and molecular distribution changes as they may appear during photoxidation.

Fig. 1 shows the Cole–Cole plots for Ecoflex polymer (η'' versus η') after different periods of photooxidation. From this representation it is obvious that even for short irradiation times, both the viscosity and elasticity of Ecoflex increase sharply, and one can observe a linear variation in both components following 10 h of irradiation. This is characteristic for gel behavior. Then oxidation led to chain recombination until a three dimensional network was obtained. This was in accordance with gel measurements which showed that after 10 h of irradiation it is possible to observe the gel fraction. For longer irradiation times we always obtained a linear variation but with a much steeper slope indicating a densification of the obtained network. The results are listed in the following table (Table 2).

The same results were obtained for PBAT but with a slightly lower crosslinking rate.

PLA exhibits different behavior. For short irradiation times (0 → 10 h), we mainly observed chain scission processes and then a reduction in molecular weight (Fig. 2). After 10 h of photooxidation, Newtonian viscosity increased again and reached approximately the same value as for non-aged PLA, but with the addition of a linear section for the low frequency region, characteristic for chain recombination (Fig. 2). Thus it can be concluded that both chain scissions and chain recombination exists during PLA UV photodegradation. Over short times the first one prevailed, while it was the second one which prevailed over long irradiation times.

Independently, the evolution of materials at molecular level was also monitored by non-isothermal thermogravimetry in a nitrogen atmosphere. After heating samples differing in the duration of their photooxidation we could register in the case of Ecoflex and PBAT an increase in the content of char residue at the end of experiments (Fig. 3), which may be taken as evidence of some crosslinking at lower temperatures or some crosslinks already present in the photooxidated samples.

By plotting the percentage of the gel (Table 1) versus char residue at 450 °C in nitrogen it is possible to see a distinct difference between both copolyesters (Ecoflex and PBAT) and PLA where the latter did not formed any gel whatsoever (Fig. 4).

3.2. Biodegradation under composting conditions

Samples of the studied materials subjected to different degree of photooxidation and containing the above-mentioned contents of a gel fraction underwent biodegradation tests simulating the decomposition of materials under composting conditions by the action of thermophilic microorganisms. Such tests could verify one of the possible ways of the final disposal of the materials. In this case, however, they were primarily used to compare the biodegradability of the samples. It is known that decomposition of these materials in the soil environment is often very slow, therefore, so as to compare their biodegradability, composting tests proven more suitable for practical reasons. In preliminary experiments performed with the studied materials, it was found that the morphology of samples exhibited a significant influence on the rate of biodegradation (Stloukal et al., 2010). It was highly likely that the specific surface area of a sample was the crucial factor. Given that photooxidation was carried out on samples in the form of relatively thick 100 μm films, it was assumed that biodegradation could be delayed and the differences between various samples might not be revealed. Therefore, biodegradation experiments were conducted in parallel with the samples that were modified in a way that increased their specific surface area. A pre-weighed amount of a sample was dissolved in chloroform, the solution was mixed with an inert porous material (perlite) so that the polymer solution remained deposited on the surface of the inert material with a high specific surface area, and then the solvent was thoroughly stripped out with a stream of air. Samples containing a gel fraction were not completely dissolved, the gel fraction, however, was markedly swollen and after removal of the solvent remained in a sponge-like form, consequently, the active surface was increased as well. It should be noted that, in the case of copolyesters, it proved impossible to grind samples in liquid nitrogen, the materials were too tough.

For both copolyesters, the photooxidation led to significant crosslinking of polymer chains, where this tendency was even more pronounced for Ecoflex, which contained a larger proportion of the aromatic component. The initial hypothesis that crosslinking would cause a restraint in biodegradation was not confirmed. For both copolyesters, the non-irradiated samples and those exposed to various degrees of photooxidation were comparable (Fig. 5A and B). Also any clear trend of biodegradability reduction occurring in parallel with an increase in the gel fraction in the samples, or with the duration of photooxidation, was not observed. Even for the most photooxidized highly crosslinked Ecoflex sample, where the gel fraction reached 78% of its weight, biodegradation was not noticeably restrained. The important factors for polyester biodegradability are accessibility of hydrolysable bonds, electron density at the attacked carbon, steric effects, the number of hydrolysable bonds and chain mobility, which can improve the accessibility of bonds to enzymes. From the listed factors crosslinking can mainly affect the mobility of chains. The frequency of bonds and their reactivity is not altered. It is then possible that the number of crosslinking bridges is low in comparison with the total number of hydrolysable bonds, thus the accessibility of hydrolysable bonds is not reduced significantly. The copolyester PBAT, with a lower fraction of the aromatic component, reached a higher percentage of mineralization during the experimental period, which confirmed earlier findings that the content of aromatic components, while improving the mechanical properties of the material, can negatively affect the biodegradability of the material (Witt et al., 1996a,b; Marten et al., 2005). Significant differences were found in both materials between samples in the form of 100 μm film and the form with increased specific surface area. Once again, the previous experience was confirmed that this factor has a significant influence on the rate of biodegradation (Stloukal et al., 2010). Furthermore, comparing the mineralization curves of initial samples with those containing the highest gel fractions, it may be concluded that the curves possess an identical shape, therefore, biodegradation of the gel fraction is not retarded with respect to the non-crosslinked fraction in a sample.

PLA, contrary to the copolyesters, experienced an important level of chain scissions and no gel fraction was detected at any stage of photooxidation. The biodegradation of samples differing in the level of photooxidation was shown to be slightly more varied than in the case of copolyesters but also here no trend could be observed (Fig. 5C). Samples with increased specific surface area biodegraded faster than samples in the form of 100 μm films. A distinct lag-phase at the beginning of the biodegradation experiments was actually recognized with PLA film samples whereas it disappeared for samples with an increased specific surface area. Once more, such behavior can be explained by the specific surface area of the material, where degrading organisms on the relatively smooth and thick film only have a small specific surface area available at the beginning. Gradually, during the course of biodegradation, the specific surface area is increased by microbial erosion, which can lead to acceleration of the process creating the characteristic autocatalytic effect visible on the mineralization curves. In parallel, the effect can be amplified by the multiplication of microorganisms.

4. Conclusions

In the course of photooxidation simulating the exposure of materials to sun irradiation all three of the biodegradable polymers investigated underwent deep changes at the molecular level. The processes resulted in rearrangement of the polymeric chains, while in the case of both copolyesters (Ecoflex, PBAT) the events led to the polymeric chain crosslinking and formation of an insoluble polymeric gel. The tendency was significantly more pronounced for Ecoflex, probably reflecting the higher content of the aromatic component which should play an important role in the mechanism of crosslinking (Kijchavengkul et al., 2008a; Commereuc et al., 2010). In PLA, contrary to the former, photochemical reactions were not accompanied with crosslinking but instead provoked chain scissions. A biodegradation experiment proved that the extend of photooxidation was not the decisive factor significantly modifying the rate of biodegradation in all three investigated materials, despite pronounced changes in their structure. The specific surface area of the sample specimens was shown to be more important. As a consequence, this means, for example, that fragmentation of agricultural films might be an important feature promoting their biodegradability. Based on the above findings we can conclude that photooxidation of agriculture mulching films, made from the investigated materials and caused by sun irradiation during their field application, should not represent a problem for their subsequent biodegradation.

Acknowledgements

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PAPER III

Biodegradation of High Molecular Weight Polylactic Acid

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Abstract. Polylactid acid seems to be an appropriate replacement of conventional non-biodegradable synthetic polymer primarily due to comparable mechanical, thermal and processing properties in its high molecular weight form. Biodegradation of high molecular PLA was studied in compost for various forms differing in their specific surface area. The material proved its good biodegradability under composting conditions and all investigated forms showed to be acceptable for industrial composting. Despite expectations, no significant differences in resulting mineralizations were observed for fiber, film and powder sample forms with different specific surface areas. The clearly faster biodegradation was detected only for the thin coating on porous material with high specific surface area.

Keywords: biodegradation, polylactide acid, gas chromatography, specific surface
PACS: 82.35.Pq

INTRODUCTION

During the last century, there was a gradual increase in utilization of synthetic polymers in many areas of human life. Their dominant status in some areas was reached mainly due to the ever-improving physical and chemical properties that have led to the improvement of their resistance thus prolongation of their lifetime. Along with the growing world production of these materials the omnipresence of the plastic litter has become a serious environmental problem, which arised many actions and strategies to prevent and solve it.

Promising solutions seems to be the use of biodegradable polymers that could be able to replace conventional synthetic polymers in many applications [1,2]. One of the particularly perspective materials belonging to this family of polymers are aliphatic polyesters such as polylactic acid (PLA) due to its low toxicity and biodegradability [3,4]. Previously, the applications of PLA were limited to the biomedical area because of its high cost and relatively low molecular weight [5]. Recently, new techniques which allow economical production of high molecular weight (MW) PLA (greater than 100,000 Da) with relatively good mechanical, thermal and processing properties have brought further expansion of PLA utilization [5, 6]. Therefore nowadays, this material can be used in a wide spectrum of products including packaging materials, mulching films and bottles and for preparation of fibres for nonwovens, textiles and carpets [7]. One of the possibilities for the treatment of biodegradable polymer waste appears to be the industrial composting [8].

Biodegradation of PLA has been already studied in number of research work [9,10,11] However, the biodegradation of high MW PLA, which is most commonly used in many common products, was not studied extensively enough especially regarding to the decomposition of different forms of final products in compost conditions.

In the present study, biodegradation of one particular example of high MW PLA in compost was investigated and its acceptability in an industrial composting plant regarding the rate of decomposition evaluated. Since the rate of biodegradation can be greatly affected by the form of the sample, various forms of the sample with different specific surface areas were tested.

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MATERIALS AND METHODS

Materials

The polymer used throughout the study was high molecular polylactic acid (PLA, M_w = 160000 $g.mol^{-1}$, M_w/M_n = 1.9) in the form of fibres.

Polymer Processing

Original form of the polymer was processed to obtain three other forms of the polymer with different specific surface areas.

Powder. Chloroform solution of polymer (12,5 mg/ml) was added gradually into four volumes of ethanol and the mixture was vigorously stirred. Obtained precipitate was filtered out and dried on air. As a result the fine powder with the high specific surface was obtained.

Films. Films 100 µm thick were prepared by compression moulding at 160°C from obtained precipitated powder.

Thin coating on inert surface. Calculated volume (10 ml) of the chloroform solution of polymer powder (100 mg/ml) was applied on the surface of pre-weighed porous inert material (perlite, 5 g) and stirred. Then the solvent was stripped out with air leaving the polymer coating on the surface of perlite.

Biodegradation Experiment

The biodegradation tests were performed in 500 ml biometric flasks equipped with septa on stoppers. Inside flasks three components were weighed: polymer samples (50 mg), mature compost (5 g of dry weight) and perlite 5g. Sample flasks were incubated at 58°C to establish composting conditions. Head space gas was sampled at appropriate intervals through the septum with a gastight syringe and then injected manually into a GC instrument (Agilent 7890). Sampling was operatively adapted to the actual CO_2 production and O_2 consumption. From the CO_2 concentrations found, the percentages of mineralization with respect to the initial sample carbon content were calculated. The endogenous production of CO_2 by compost in blank incubations was always subtracted to obtain values representing net sample mineralizations. Three parallel flasks were run for each sample form (original fibre, film, powder and thin coating), along with four blanks without sample and positive control flasks with microcrystalic cellulose.

RESULTS

High MW PLA was designed to be biodegradable material hence it should be readily decomposed in compost. As it was already mentioned PLA can be utilized as a material for the production of many various products including agriculture films, fibres in textile industries, powder for special applications. However, all these products differ considerably in their surface area, which influence the rate and course of biodegradation proceeding apparently as a surface erosion process (12,13). In order to evaluate this phenomenon with high MW PLA, several sample forms: film and fibres with relatively low specific surface and two other forms of the polymer with higher specific surfaces, solvent precipitated powder and deposition of the polymer on high specific surface inert material (perlite) were tested.

The resulted biodegradation curves in compost experiment are presented in Figure 1. Despite the fact that sample in form of fibres had the longest induction phase, its biodegradation reached surprisingly the same mineralization (about 65 % after more than 90 days of incubation) as the powder sample with significantly higher specific surface area. The film form with low specific surface, which had the identical induction phase and behaved initially almost equally as the powder, reached, after a slight retardation in the middle of the observed period about 60% of mineralization. Despite significant differences in specific surface areas, the resulting final mineralizations of these three forms were approximately the same.

The fastest biodegradation was observed with the polymer coating on the porous perlit material, where it reached almost 90% at the end of the incubation period and was comparable with the biodegradability of microcrystaline cellulose powder used as a reference material. This result can be explained by the high specific surface of porous perlit, where the polymer was deposited as a thin layer.

Final values of biodegradation for all sample forms could be evaluated as adequate and acceptable for the industrial composting.

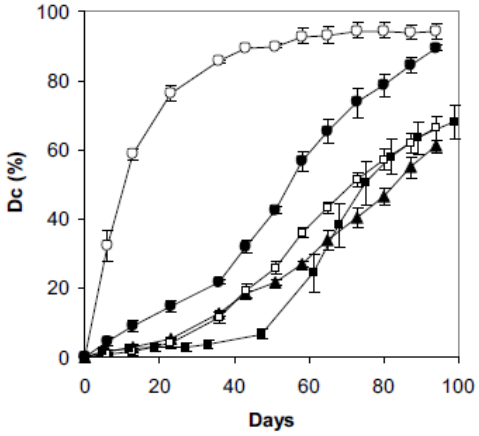

FIGURE 1. Biodegradation of different forms of the polymer in compost experiments. ■, fibre; ▲ film (100 µm) □, powder; ●, thin coating; ○, cellulose (reference compound). Error bars correspond to twice standard deviation (n=3).

CONCLUSION

The biodegradation tests of high molecular PLA in various forms: fibres, powder, film and thin coating on inert material with different surface areas, in compost were performed. The material proved its good biodegradability under composting conditions and all investigated forms were evaluated as adequate and acceptable for the industrial composting. Contrary to the expectations, no significant differences in the final degree of mineralization were observed considering the various active surface areas of fiber, film and powder sample forms. However, the surface area have contributed to the different courses of biodegradation for the individual forms. Only for the thin coating on porous material with high specific surface area significantly faster biodegradation with no apparent initial lag-phase was observed.

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PAPER IV

Title Page

Interrelations of molecular weight and sample form in the biodegradation of poly(L-lactic acid)

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Abstract

The biodegradation of four polylactic acid (PLA) samples with molecular weights (MW) in the interval from about 34 to about 160 kg mol^{-1} was investigated under composting conditions. Biodegradation rate decreased and initial retardation was discernible with increasing MW of investigated samples. Specific surface area was also identified as the important factor promoting biodegradation. Abiotic hydrolysis was also investigated and its courses found quasi identical with biodegradation of all four PLA samples investigated. This suggests that the abiotic hydrolysis represented a rate limiting in the biodegradation process and the organisms present were not able to promote depolymerization by the action of their enzymes significantly.

Highlights

- Biodegradation of PLA was influenced by molecular weight and specific surface area.
- Abiotic hydrolys was the rate limiting step in PLA biodegradation.

Key words

Polylactic acid; Molecular weight; Compost; Biodegradation; Abiotic hydrolysis.

1. Introduction

Plastic litter represents a serious environmental problem, provoking increasing concern among the broader public. The situation appears to be particularly alarming in developing countries without fully efficient waste management systems. Biodegradable polymeric materials represent a promising alternative to conventional polymers, at least for some applications (Rudnik, 2008; Naira and Laurencin, 2007). Polylactid acid is a particularly promising member of this family of polymers (Fukushima, et al., 2009; Fukushima et al., 2011). Previously, applications of PLA were limited to the biomedical sector because of its high cost and relatively low molecular weight (Hamad et al., 2010). Recently, new techniques which allow for economical production of high molecular weight (MW) PLA (greater than 100,000 Da), with relatively good mechanical, thermal and processing properties, have brought about further diversity of PLA utilization (Hamad et al., 2010; Al-Mulla et al., 2011). Presently, this material can be used in a wide spectrum of products as a consequence, including packaging materials, mulching films and bottles, as well as in manufacturing fibers for nonwoven items, textiles and carpets (Lim et al., 2008.). PLA also represents a material that can be produced from renewable resources, due to its monomer being obtained via the fermentation of various plant materials.

During its environmental degradation ester bonds of PLA must be cleaved either hydrolytically or by extracellular enzymes to enable the PLA monomers or oligomers to be assimilated. Both parallel processes can be influenced by several factors. The enzymatically catalyzed process depends on the presence of specific microorganisms, which seems to be sparsely distributed in some environments (Rudnik, E., 2008) and presumably by optical purity (Hideto Tsuji, Shinya Miyauchi, 2001) and crystallinity of the material (Tsuji et al, 2006). It was proposed that amorphous regions are more vulnerable towards hydrolysis by model enzymes (Hideto Tsuji and Shinya Miyauchi, 2001). Abiotic hydrolysis is critically influenced by temperature (Lyu et al, 2007), where its rates differ considerably in the interval between 20 and 60 °C. Crystallinity with the preference of amorphous regions seems to play a role as well (Tsuji et al, 2000).

In the compost environment PLA biodegradation proceeds relatively easily and was rather extensively documented (Ghorpade et al., 2001; Kale et al, 2007). Conditions, especially during the thermophilic phase of the composting process, appear favorable both in spite of the presence of degrading microorganisms (Tokiwa, Y. and Jarerat, 2004) and the promotion of hydrolysis under elevated temperature (Lyu et al, 2007).

It was suggested that abiotic hydrolysis represent a first step in PLA biodegradation though the data documenting such statement are not readily available (Lunt, 1998). On the other hand, experimental data proved very fast and efficient decomposition of PLA by some enzymes (Tokiwa and Jarerat (2004); Oda et al. (2000)) and the presence of hydrolytic enzymes produced by PLA utilizing microorganisms (Pranamuda et al. (2001) Akutsu-Shigeno et al., 2003). Finaly, the specific surface of the testing specimen can have an important role probably especially during first phases of the biodegradation process (Kunioka et al., 2006 ; Stloukal et al., 2010).

As it was outlined, during composting biodegradation of a particular PLA product can be influenced by many conflicting factors. Here we attempted to test four available PLA grades processed each into three different sample forms under identical experimental conditions and monitored and evaluated parallel processes of hydrolysis and microbial decomposition by comparing data from different experimental techniques employed.

2. Materials and methods

2.1 Materials

Polylactic acid samples PLA1 and PLA2, were synthesized through direct melt polycondensation (Sedlarik et al., 2010; Kucharczyk et al., 2012); PLA3, PLA4, were purchased from NatureWorks® Ingeo™, USA.

2.2 Polymer processing

The original polymers were processed to obtain three other forms of polymer samples with different specific surface areas.

Powder. PLA solution in chloroform (12.5 mg.mL^{-1}) was gradually dispensed into 2.5 volumes of ethanol and the mixture was vigorously stirred. Subsequently, the precipitate obtained was decanted and rinsed twice with ethanol to remove chloroform. Finally, the precipitated PLA was filtered out and dried on air for 24 hours to evaporate ethanol and the residues of chloroform. As a result, a fine powder with a high specific surface was obtained.

Films. PLA films 100 μm thick were compression molded via heating for 1 minute to the processing temperature of 180 °C, then molded for 2 minutes and cooled to laboratory temperature under pressure.

Thin coating on an inert surface. The exact amount of PLA (50 mg) was applied as a chloroform solution (5 mg mL^{-1}) to the surface of a pre-weighed porous inert material (perlite, 5 g) in biometric flasks and stirred. The solvent was then stripped out via air flow for 24 hours, leaving the polymer deposited as a thin coating on a high specific surface area of the perlite material.

2.3 Gel permeation chromatography

Molecular weight and distributions were determined by gel permeation chromatography, carried out on the Breeze chromatographic system (Waters, Milford, MA) equipped with a PLgel Mixed-D column (300 × 7.8 mm, 5 µm; Polymer Laboratories, Ltd.) and detected with the Waters 2487 dual-absorbance detector at 239 nm. Data was processed via Waters Breeze GPC software (Waters), and the weight average molar mass (M_w), number average molar mass (M_n) and subsequently polydispersity indexes (M_w/M_n) were calculated.

2.4 Thermal properties and crystallinity

Determining the thermal properties of particles was performed by differential scanning calorimetry (DSC) on the Mettler Toledo DSC1 STAR System. All measurements were carried out in nitrogen flow (20 cm^3 min^{-1}). The samples were heated from 0 °C to 180 °C at the rate of 10 $°C.min^{-1}$), followed by annealing at 180 °C for 1 min, followed by a cooling scan from 180 to 0 °C (at 10 $°C.min^{-1}$), plus an isothermal step at 0 °C for 1 minute, and finally the second heating scan from 0 to 180 °C (at 10 $°C.min^{-1}$). Melting point temperature (T_m) as well as the exothermal response, relating to crystallization temperature (T_c), were obtained from the first heating cycle. From the second heating scan the glass transition temperature region (T_g) was determined. The degree of crystallinity, χ_c, was calculated from the measured heat of fusion (ΔH_f) and crystallization (ΔH_c) according to the following equation:

$$\chi_C = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0} \times 100\% ,$$

where ΔH^0_m is the enthalpy of fusion for 100 % crystalline PLA (93.1 J.g^{-1}) (Lim et al., 2008.).

2.5 Content of D- and L- monomers.

Ratios of both monomers were determined with Kruss P1000 polarimeter at 25 °C using the procedure and data found in Feng et al. (2010).

2.6 Biodegradation under composting conditions

The method utilized was based on a previously published protocol by Dřímal et al. (2007) with some modifications. Polymer films were cut into 2 mm pieces, then 50 mg of polymer, 5 g of perlite and 2.5 g of dry weight of compost were weighed into each 500 mL biometric flask. The flasks were sealed with stoppers equipped with septa and incubated at 58 °C. Headspace gas was sampled at appropriate intervals through the septum with a gas tight syringe and then injected manually into a GC instrument (Agilent 7890), equipped with Porapak Q (1.829 m length, 80/100 MESH) and 5A molecular sieve (1.829 m length, 60/80 MESH) packed columns connected in series, and a thermal conductivity detector (carrier gas helium, flow 53 mL.min^{-1}, column temperature 60 °C). Sampling intervals were operatively adapted to actual CO_2 production and O_2 consumption. Concentrations of CO_2 and O_2 were derived from the calibration curve obtained using a calibration gas mixture with declared composition (Linde). The endogenous production of CO_2 by soil or compost in blank incubations was always subtracted to obtain values representing net sample mineralization. From the concentration found, the percentage of mineralization with respect to the initial carbon content of the sample was calculated $M_{\%} = m_{gc}/(m_s w_c)$, where $M_{\%}$ is the percentage of mineralization, m_{gc} is the mass of carbon evolved as CO_2 and obtained from GC analysis, m_s is the weight of a polymer sample, and w_c is the percentage (w/w) of carbon in the polymer investigated. The value of w_c for the given polymer (50.0 %) was determined on a Flash Elemental Analyzer 1112 (Thermo). In parallel, oxygen concentration was also monitored to provide a control mechanism so as to ensure samples did not suffer from hypoxia. Three parallel flasks were run for each sample, along with four blanks.

2.7 Abiotic hydrolysis

Three sets of experiments at different temperatures were performed to determine the extent of PLA hydrolysis in the aquatic environment. Purified PLA powder (80 mg) was suspended in 40 ml of phosphate buffer (0.1 mol.L^{-1}, pH 7) in triplicate for each temperature (25, 37, and 58 °C). 1.5 ml aliquots were taken in regular time intervals, centrifuged (10 000 g, 10 min), and the supernatants were analyzed for dissolved organic carbon (TOC 5000A Analyser, Shimadzu).

3. Results and discussion

3.1. Characterization and processing of PLA samples

For all four PLA samples used in this study, properties that could be critical for the interpretation of relatively difficult and time-consuming biodegradation experiments were investigated. Measurements with gel chromatography proved that selected materials had their MW well distributed over a relevant interval and the polydispersities of their MW distribution curves were comparable for three of the four materials but higher in the case of PLA2 (Table 1). Thermal analysis can be efficiently used to estimate the crystalline part of polymer materials. It is believed that the amorphous regions are biodegraded preferentially and some authors have tended to interpret an increase in crystallinity during incubation as proof of biodegradation (Badía et al., 2010; Marten et al., 2005). DSC measurements were performed for all polymer samples and all their forms and degrees of crystallinity were deduced (Table 1), with the exception of the thin coating sample form, for which the analysis was not feasible. In principle, crystallinity could be significantly influenced by the processing of samples. For the investigated sample forms, crystallinities were roughly comparable and the crystallinity of powder forms were always lower than film forms, reflecting the probable shorter time available for crystallization during powder form preparation. Crystallinities of PLA1, PLA3 and PLA4 were roughly comparable with somewhat higher values found with PLA2.

The properties of PLA are influenced by the content of D- monomer. A low content of the D monomer could markedly decrease crystallinity thus promote the abiotic hydrolysis (Tsuji et al, 2000). Subsequently it should not directly inhibit the enzymatic hydrolysis which can further profit from the increased content of the amorphous regions as well (Reeve et al, 1994). To control this aspect the stereochemistry of incorporated monomers were determined by polarimetry (Table 1), and it was found that the D monomer contents were comparable in all PLA samples.

TABLE 1. Properties of various PLA samples and the thermal properties of their individual sample forms

Sample	M_w g.mol^{-1}	M_w/M_n	L-lactide %	Form	T_m °C	T_g °C	χ_c %
PLA 1	34000	2	95.5	Film	144.4	34.4	39.5
				Powder	151.5	50.9	29.7
PLA 2	61000	2.9	94.6	Film	165.3	50.8	58.5
				Powder	162.8	54.3	41.1
PLA 3	109000	2.3	95.8	Film	168.3	55.8	51.6
				Powder	168.0	55.5	35.4
PLA 4	160000	1.9	96.0	Film	168.3	54.5	40.9
				Powder	167.3	55.1	33.9

M_w, weight average molecular weight; M_w/M_n, polydisperzity; T_m, melting point temperature; T_g, glass transition temperature; χ_c, degree of crystallinity.

3.2. Biodegradation of PLA samples in compost

The described PLA samples were incubated for about 100 days under composting conditions at 58 °C (Fig. 1). The lag phase of about 20 days duration at the beginning of biodegradation could be clearly distinguished for two higher MW samples, whereas it was apparently missing with the lowest MW PLA. For PLA2, the lag phase could not be clearly observed but the curves exhibited some level of acceleration at about day 25. It could be concluded that, for PLA with a MW of over approximately 60 kg mol^{-1}, this higher MW caused significant retardation of biodegradation onset. The curves presented also reflected the type of the sample. Whereas for a low MW sample all the curves are perfectly parallel and the specific surface area of the film sample did not limit biodegradation, for higher MW film samples biodegradation was markedly retarded. Interestingly, in the case of the highest MW sample, the higher specific surface area of the powder sample did not represent a significant advantage and biodegradation was almost identical to that of the film form. Somewhat specific was the behavior of samples prepared as a thin coating on the inert porous material perlite. The biodegradation of this particular sample type was the fastest of all MWs tested, and even for a higher MW, it was significantly less retarded at the beginning and the lag-phase was replaced with a kind of initial retardation. One can speculate that the thin coating form provides an additional surface of highly porous perlite for microbial colonization, and thus promote faster biodegradation, and/or this form has a significantly higher specific surface even over the powder form.

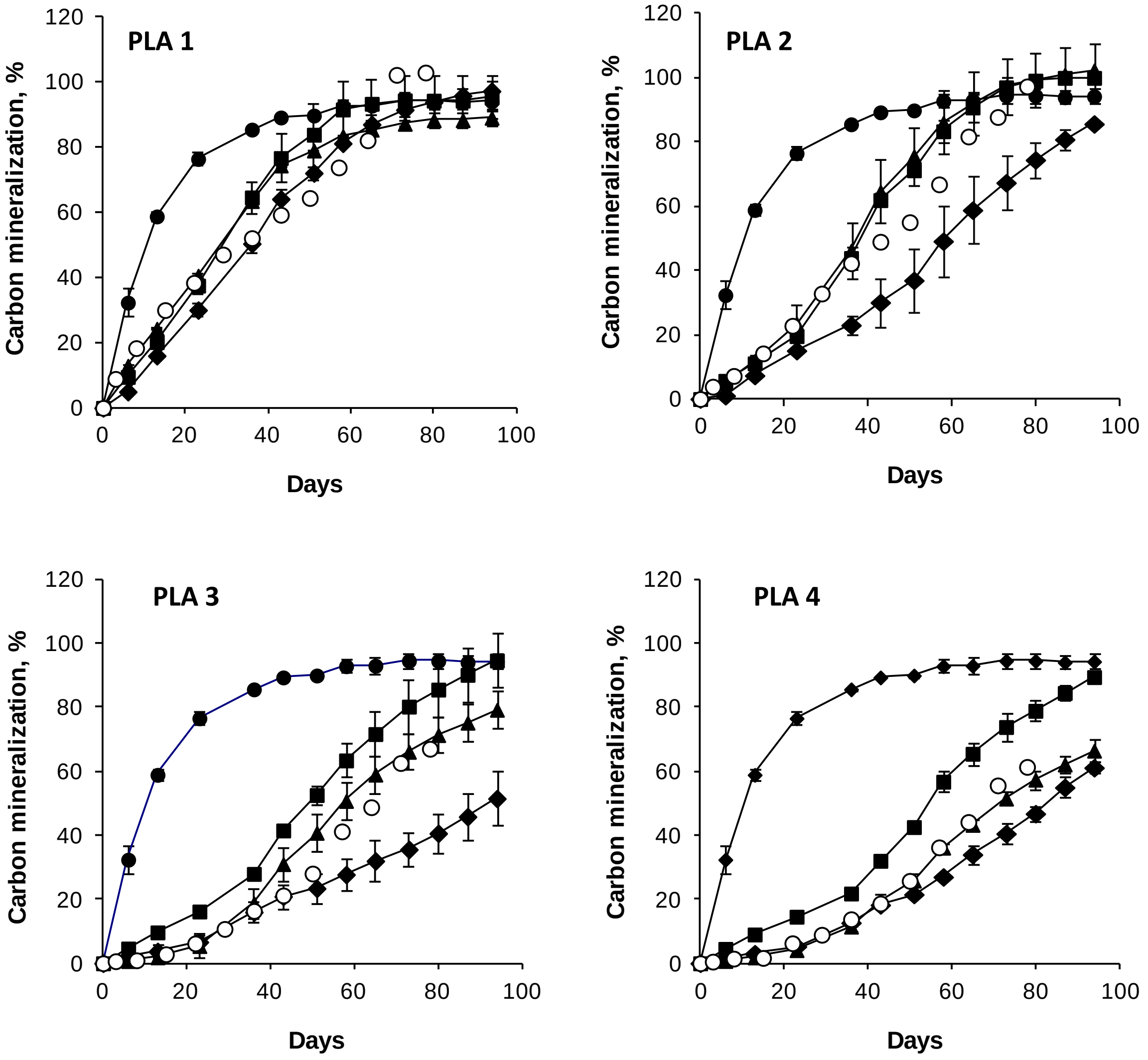

Figure 1. Biodegradation of PLA with various MWs in the compost experiment, comparison with the abiotic hydrolysis of powder form PLA. Comparison of sample forms with different specific surface areas. ■ thin coating; ▲ powder; ♦ film (100 μm); ● cellulose (reference compound); ○ abiotic hydrolysis of the powder form sample. Error bars correspond to twice

The same data as in Fig. 1 are also represented in Fig. 2 ABC, but via a different arrangement that makes it possible to compare PLA samples with different MWs for individual sample forms. It is evident that the biodegradation rate was dependent on MW for all sample forms, and that higher MW samples exhibited retardation during the initial phase, which could be described as the lag phase of biodegradation. In later phases, biodegradation rates decreased with increasing MW for all sample forms. As has already been mentioned, the thin coating sample form always exhibited the highest biodegradation rates, which is even more evident in this representation of the data. The initial retardation appeared gradually more pronounced with increasing MW for all sample forms.

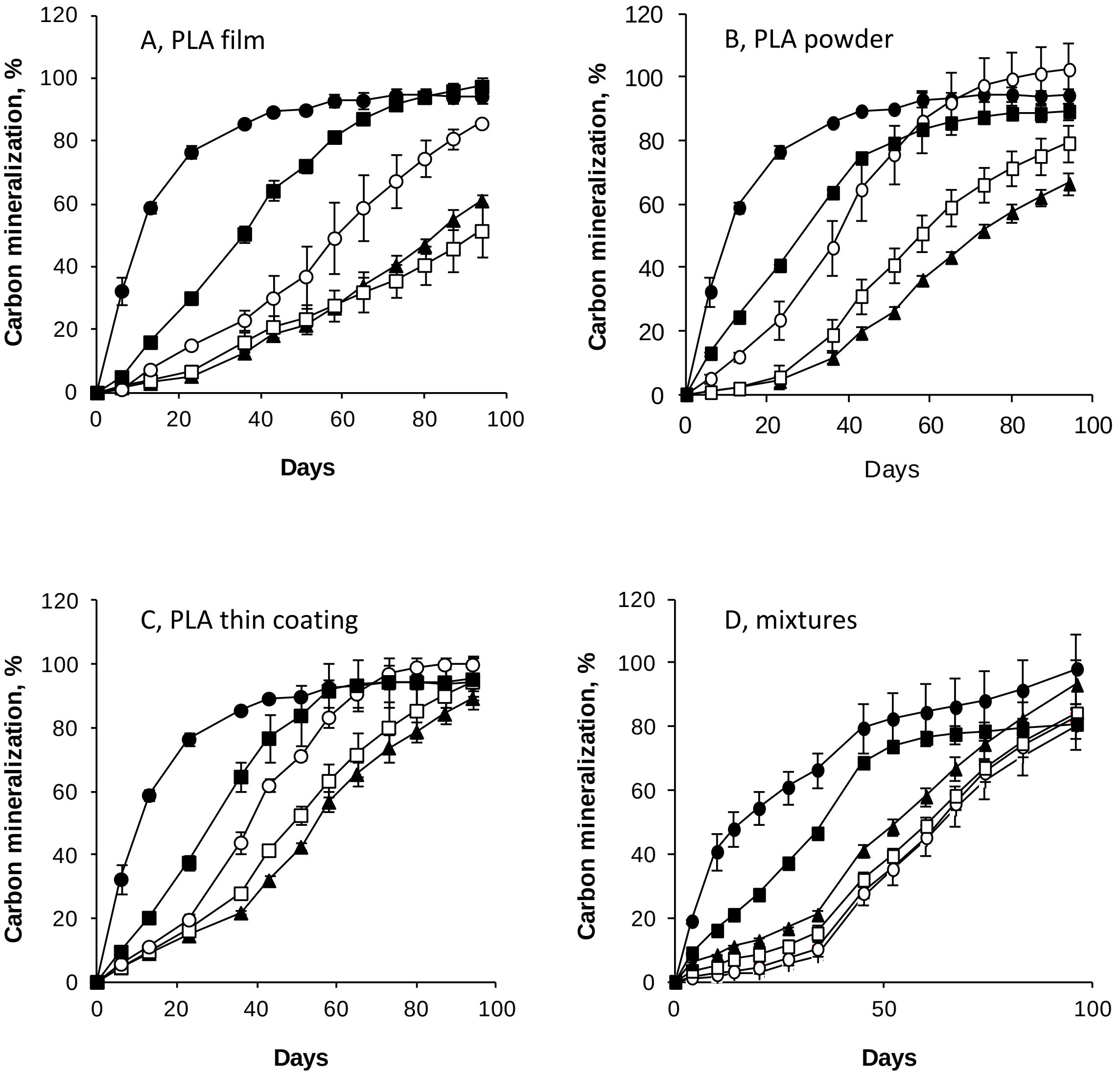

Figure 2. Panels A, B, and C: Biodegradation of PLA sample forms with different specific surface areas in the compost experiment. Comparison of PLA samples with various MWs. ■, PLA1; ○, PLA2; □, PLA3; ▲, PLA4. Panel D: Biodegradation of PLA1 and PLA4 powder mixtures in various ratios in the compost experiment. ■, 100% PLA1; ▲, 20% PLA1 + 80% PLA4; □, 10% PLA1 + 90% PLA4 ; ○, 5% PLA1 + 95% PLA4; + 100% PLA4. ●, cellulose (reference compound). Error bars correspond to twice standard deviation (n=3).

The retardation of higher MW PLA biodegradation could be caused by several factors. One such possible explanation comprises the idea that low MW PLA better supports the growth of degrading microorganisms, and eventually the induction of necessary specific enzyme systems. Not possessing this potency, high MW PLA biodegradation could suffer from the slower onset of enzyme activities and initially only a slowly growing number of specific degraders. In such a case, the relatively limited addition of low MW PLA could induce the enzymes and the growth of the degraders, as well as significantly supporting the biodegradation of high MW PLA. It was decided to test this hypothesis in a separate experiment (Fig. 2D). Mixtures of PLA1 and PLA4, from 5/95 to 20/80 weight ratios, respectively, were followed in the composting experiment along with pure PLA1 and PLA4 samples, all in powder form. The additions up to 20% of PLA1 did not significantly enhance the biodegradation of high MW PLA4; the acceleration during the first 30 days corresponded to the added fraction of low MW PLA1 only. Based on these results, the above hypothesis that retardation is caused by the low number of initially slowly growing degrading microorganisms seemed less likely. Material factors like the lower number of free mobile chains on the high MW PLA surface were probably more important.

3.3 Abiotic hydrolysis of PLA

Both abiotic and enzymatic hydrolysis can participate on PLA biodegradation. To estimate the importance of the abiotic hydrolysis for the particular PLA samples in the study an experiment was set up where the hydrolysis in aqueous environment was followed in the presence of a microbial growth inhibiting substance (NaN_3). The effect of elevated temperature on PLA hydrolysis could be crucial for the good biodegradability of PLA under composting conditions (Lyu et al, 2007) and was tested as well (Fig. 3). It is evident that the hydrolysis of all PLA samples was markedly accelerated with temperature whereas at individual temperatures hydrolysis rates followed MW of PLA samples with lowest MW PLA as the fastest.

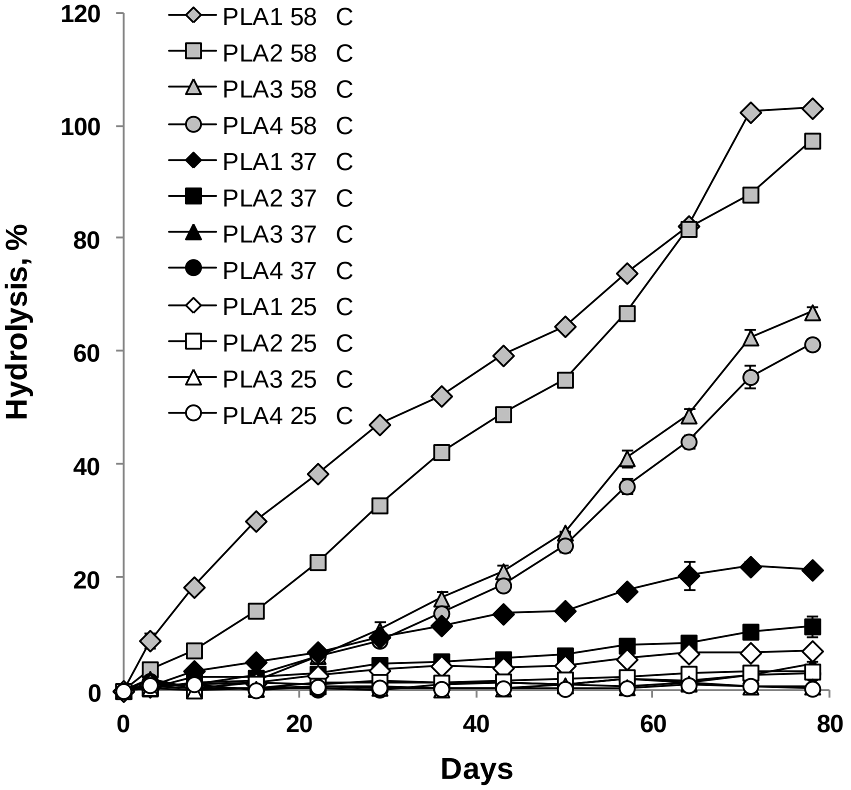

Fig. 3. Abiotic hydrolysis of different PLA grades in form of powder aqueous environment at various temperatures.

Interesting information comes from the comparison of data from biodegradation in compost and from hydrolysis at 58 °C (Fig. 1). It is evident that the curve of the material powder form hydrolysis almost copies the same material form biodegradation curve. In composting experiment at 100% relative humidity hydrolysis rate should be comparable. Under such assumption the results suggest that the abiotic hydrolysis controlled the rate of biodegradation in the system described.

4. Conclusions

Among PLA materials the rate of biodegradation followed the pattern of increasing MW with characteristic lag-phase or autocatalytic shape of the curves for higher MW samples. The specific surface area also proved to be an important factor promoting fast biodegradation especially for with higher MW samples. The course of the biodegradation was quasi identical with the course of abiotic hydrolysis for all PLA grades investigated so the abiotic hydrolysis appeared to be the major depolymeration mechanism and the rate controlling step of the biodegradation process.

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PAPER V

Preparation of submicroparticles based on biodegradable co-polyester

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Abstract**—** The preparation of submicro particles from biodegradable copolyesther by the method of emulsification and solvent evaporation at reduced pressure was investigated. During the study the influence of various process parameters and conditions such as stirring speed, amplitude of sonication and concentration of polymer on the particle diameter and particles size distribution was observed. It was found that it is not possible to achieve a diameter lower than 1 µm without sonication at a relatively high concentration of copolyesther. Without sonication the typical mean diameter ranged from 1-10 µm. On the other hand, sonication enabled to prepare particles with diameter lower than 1 µm. A significant influence of the concentration of polymer on final diameter was found, too.

Keywords**—**microparticles, Oil-in-water (O/W) emulsion solvent evaporation method, biodegradable copolymer

I. INTRODUCTION

THE increasing interest in the area of polymer microparticles is due to a number of promising applications in pharmacology [1], [2], agriculture [3], [4], and environmental engineering. One of such applications is filling polymer microparticles with bioactive a low molecular weight compound which is subsequently, under suitable conditions, released in a controlled way. This application has been extensively studied in recent years. A central issue is the releasing rate of active agents, which is influenced by various parameters, for instance particle diameters or particles size distribution (PSD), as described in [5]. The size of particles and PSD depends on the chosen method of preparation and the process parameters and conditions.

Micro and submicroparicles can be prepared in several ways. However, in the literature, two preparation processes predominate. The first one is the Oil-in-water (O/W) emulsion solvent evaporation method [6], [7]. It is a popular way to get microparticles, which is described in a number of research papers in details and can also be readily realized in the laboratory without the need for specialized equipment [8]. On the other hand, the second method, which is based on the utilization of supercritical fluids [9], [10], requires more sophisticated laboratory equipment.

Regarding the availability of the O/W method and the lack of sufficient data taking into account the influence of process parameters and conditions on the resulting particles diameter/PSD, the present work is focused on investigation and evaluation of these effects.

In some of research works [2], [3], [5], [7] which are oriented primarily on the release of bioactive compounds, the influence of the concentration of stabilizer in suspension, stirring speed [5], [7] and solvent [7] were observed. However, after closer study of the O/W emulsion solvent evaporation method, it is possible to find that the particles diameter and PSD are influenced by other process parameters and conditions, such as concentration of polymer in solvent, solvent/water ratio in emulsion or type of stabilizer. Particles which were investigated in all of these studies comprised encapsulated bioactive compound. It is presumable that even such low molecular weight compounds can have a certain influence on the size of particles.

In some papers [11]-[13] ultrasonication, which can considerably help to achieve smaller particles in the resulting suspension, was used during preparation. Although it is an indispensable factor in the preparation by the O/W method, the impact of process parameters such as time and amplitude on the resulting diameter and PSD has not been satisfactorily investigated and described yet.

In this study the possibilities of preparation of micro and submiscroparticles from commercially available biodegradable copolyester were tested. The influence of stirring speed of the homogenizer and sonication amplitude on the particle diameters and PSD is evaluated and compared with the literature.

II. MATERIALS AND METHODS

A. Materials

Polymer used throughout the study was aromatic-aliphatic copolyesther Ecoflex purchased from BASF (Germany). Airvol 205 - polyvinylalcohol (JVP, Japan) was applied as an emulsion stabilizer in the form of 0.5 % aqueous solution.

B. Preparation of particles

Micro and submicroparticles were prepared by the

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oil-in-water emulsion solvent evaporation technique. This method was carried out in the following way.

Firstly, the polymer (2, 1.5, 1, 0.5, 0.25 g) was dissolved in 10 ml of chloroform. The resulting concentration of polymer in chloroform (P/C concentration) was 200, 150, 100, 50, 25 mg ml^{-1}. Chloroform was primarily used because it has the higher boiling point in comparison with similar solvents such as dichloromethane. The relatively high value of boiling point ensures that chloroform will not evaporate from the mixture when the emulsion is prepared by vigorous stirring.

Then, the polymer solution was emulsified into 0.5 % (w/v) water solution of PVA (emulsifying agent) and dispersed under the continuous stirring by the homogenizer Ultraturax (IKA T18, Germany) at stirring speeds of 6000, 10000, 14000, 18000, 22000 and 24000 rpm. The mixture was stirred for 10 min. and during this period the produced emulsion was cooled with ice. The ratio between water solution of PVA and chloroform solution of Ecoflex was the same in all experiments (4:1).

Individual series of samples were marked S, U, A, C. For samples S ultrasonication was not used in second step of the procedure.

For labelings U, A, C, the obtained emulsion was ultrasonicated by ultrasonic probe (Hielscher UP 400S, Germany) for 5 min, when the cycle of sonication was 0.5 and amplitude 20, 25, 30, 35, 50, 70 %.

At last, the prepared emulsion (for all labels) was placed into the erlenmayer flask and stirred. Subsequently the organic solvent was evaporated from the emulsion under reduced pressure and the suspension of microparticles was formed. Reduced pressure was created by a membrane pump. The samples were stored in freezer at -20 °C for analyses.

C. *Particle characterization*

Particle parameters were measured with the help of optical microscope. During the microscopic investigation, the samples were placed on a glass slide with a graduated grid.

Where the diameter of particles allowed (particles smaller than 5 µm), the particle distribution was measured with Zetasizer (Zetasizer Nano ZS, Malvern Instruments, UK).

D. *Evaluation of images from the optical microscopy*

The length of the edge of a square in graduated field was 50 µm. From each image, six squares were randomly chosen for evaluation. The number of particles was counted in each square for all predetermined size intervals.

III. RESULTS AND DISCUSSION

Table I shows the conditions used to prepare the micro and submicroparticles and the obtained mean diameter (weighted average relative to the intensity of scattering). As can be seen, except for samples of S1-S6, the mean diameter of particles below 1 µm was achieved due to sonication.

In comparison with literature [5]-[7], where preparation was

TABLE I
THE CONDITIONS USED TO PREPARE THE MICRO AND SUBMICROPARTICLES AND THE OBTAINED DIAMETER

Sample	Stirring speed (rpm)	Polymer concentration (mg ml^{-1})	Ultrasonication	Amplitude (%)	Mean diameter of particles (µm)
S1	6000	100	no	-	9.44
S2	10000	100	no	-	4.74
S3	14000	100	no	-	2.60
S4	18000	100	no	-	2.64
S5	22000	100	no	-	1.94
S6	24000	100	no	-	1.74
U1	6000	100	yes	35	not determined
U2	10000	100	yes	35	0.94
U3	14000	100	yes	35	0.79
U4	18000	100	yes	35	0.94
U5	22000	100	yes	35	0.87
U6	24000	100	yes	35	0.57
A1	24000	100	yes	20	0.88
A2	24000	100	yes	25	0.79
A3	24000	100	yes	30	0.89
A4	24000	100	yes	35	0.57
A5	24000	100	yes	50	0.59
A6	24000	100	yes	70	0.72
C1	24000	25	yes	35	0.40
C2	24000	50	yes	35	0.61
C3	24000	100	yes	35	0.57
C4	24000	150	yes	35	0.95

performed without sonication, the diameter of the obtained particles was always in order of micrometers, whereas in research papers [12]-[14], where sonication was used, particles smaller than 1 μm were achieved.

The results are discussed in details in further paragraphs. It is necessary to say that the comparison with the mentioned research papers has some limitations, which will be discussed, too.

A. Influence of stirring speed on particle distribution

The mean diameters of particles for samples S1-S6 (described in Table 1) are shown in Figure 1.

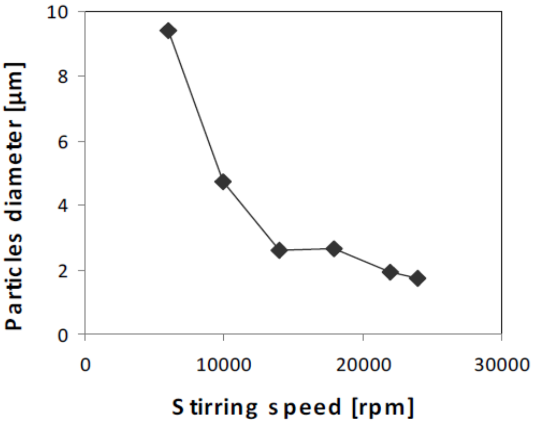

Fig. 1 Dependence of the mean particle size on stirring speed without ultrasonication

As can be seen, whereas in the interval from 6000 rpm to 14000 rpm the particle size is strongly affected by the stirring speed, for speeds over 14000 rpm the medium particle size remains almost constant. The application of higher stirring speed than 14000 rpm is not very efficient for the further reduction of mean diameter. As mentioned before, with stirring only we did not succeed to prepare particles under diameter 1 μm. In samples which were stirred at speed 14000 rpm and higher, the largest particles were reduced and mean diameter over 3 μm was achieved. However, the certain number of particles was bigger than 5 μm, therefore z-sizer could not be used to measure their diameters.

In the research work [6], where the same method and similar process conditions were used, the diameter of particles in the order of micrometer was also obtained. The authors observed polymer with the molecular weight higher than 100,000, which corresponds with ours (polymer molecular weight higher than 100,000).

It is presumable that the achievement of the diameter under 1 μm, when the similar polymer (molecular weigh higher than 100,000) and process conditions are used, is impossible without sonication. However, the particles with the diameter lower than 1 μm could be successfully obtained if some process parameters, such as initial concentration of polymer in the organic solvent, are changed. Unfortunately, there is a lack of information from the literature, to prove our presumptions.

The particles diameter obtained with each stirring speed was calculated from the data in Figure 2. In this figure, the comparison of particles size distributions for different stirring speeds is presented. The overall shift and the decrease of particle size distributions with the increasing stirring can be observed. The larger particles disappear gradually and ,practically, only smaller ones remain. The shift of PSD to the lower values of particle diameters can be probably achieved by using longer time of stirring. However, the mentioned assumption should be verified by further investigation.

Figure 3 shows the significant difference between particles prepared by the lowest stirring speed (6000 rpm) and the highest speed (24000 rpm). The diameter of all particles was approximately determined with the help of calibrated grid, which is perceptible in the figure.

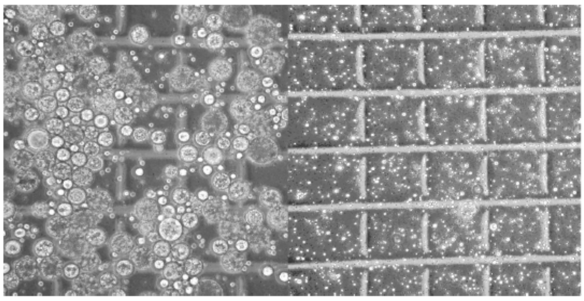

Fig. 3. Particles prepared at 6000 rpm (left) and 24000 rpm (right) the size of calibration chamber squares is 50 μm

As can be seen, the number of particles increased and size decreased with higher of stirring speed. This trend results in the inaccuracies of the measurement of the particle diameter and subsequent calculations for higher stirring speeds.

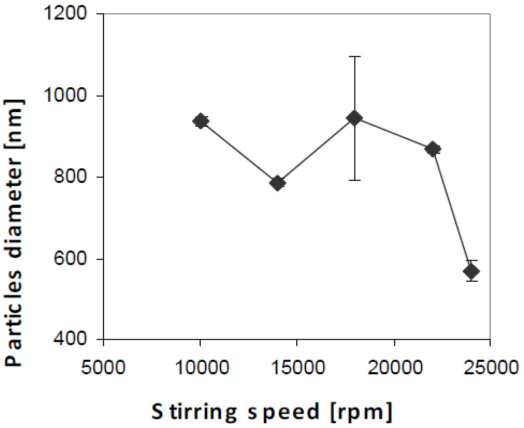

Fig. 4 Dependence of the mean particle size on stirring speed with ultrasonication, points are averages from 3 measurements. Error bars represent twice standard deviations.

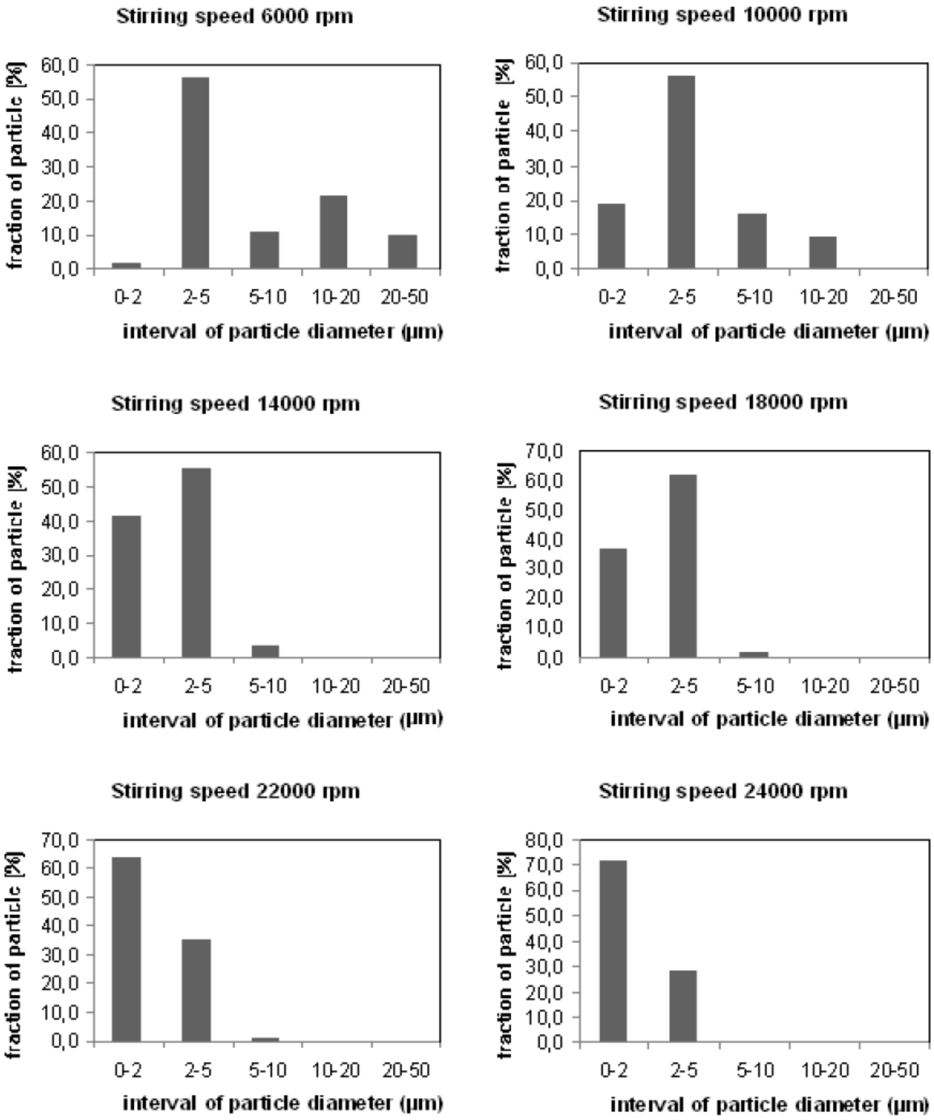

Fig. 2 Comparison of particles size distributions for different stirring speeds.

Figure 4 shows the dependence of particles diameters on stirring speeds. In this case ultrasonication was used during the preparation. The process parameters for samples U1- U6 are described in Table 1.

The mean particle diameter was reduced under 1 μm. After ultrasonication, PSD were shifted significantly toward lower diameters, which made possible to use z-sizer for their characterization. As can be seen in figure 4, the measured values of particles diameter for stirring speed 6000 rpm were not used because they seem to be unreliable. The wider standard deviation of particle diameter for stirring speed 18000 rpm is probably caused by non-homogeneity of the sample. It is evident that the particle diameter after ultrasonication is not so strongly dependent on the stirring speed. For almost all stirring speeds the particles diameters are very similar. However, for the highest homogenization stirring speed (24000 rpm) significantly smaller particles were obtained.

In literature [12]-[14], much smaller particle diameter was achieved. Main reason seems to be the use of considerably lower concentrations of polymers, which had, together with amount of stabilizer in suspension, important influence on the final mean diameters and PSD.

In [13] lower particle diameter was achieved even with electromagnetic stirring only (stirring speed 500 rpm). It is possible that ultrasonication has bigger influences on particles diameter and PSD than stirring speed. However, in all of these research papers the polymers with the molecular weight lower than 100,000 were used in contrast with Ecoflex which has the molecular weight higher than 100,000.

B. Optimization of ultrasonication procedure

Figure 5 presents the dependence of particle size on the amplitude of ultrasonication for the series of samples A1 – A6. As can be seen, the amplitude was in the range of 20 % to 70 %. A sharp critical value close to 35% amplitude is apparent from the figure. It is very interesting that larger particles are formed for the amplitude 70 % than for the 35 %.

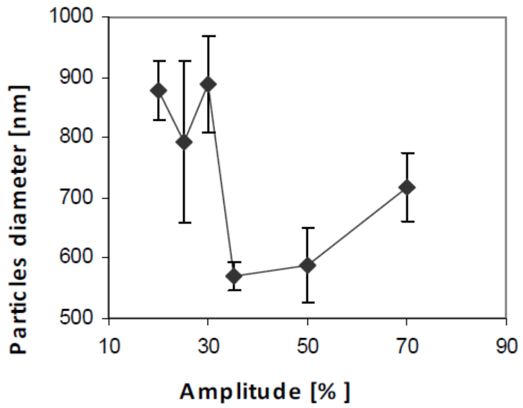

Fig. 5 Dependence of mean particle size on the amplitude of ultrasonication parameter. Points are averages from 3 measurements. Error bars represent twice standard deviations.

It could be explained by the partial agglomeration of particles instead of their reduction, which is caused by the great amount of energy delivered into the emulsion and possibly the local increase of temperature using higher amplitudes.

C. Influence of the polymer concentration

Finally, the measurement was performed for the series of samples C1-C5. The influence of polymer concentration was investigated.

As can be seen from Figure 6, the particle diameter increased rising the concentrations. The trend of increasing particle diameters with rising concentrations was also detected in [12] and [14] and could be explained in various ways, for example too small volume of the water phase, too short time of stirring and ultrasonication for higher concentrations of the polymer. The concentration of emulsion stabilizer (PVA) in emulsions has probably a crucial influence. For a large quantity of Ecoflex in the emulsion, the amount of stabilizer is unable to cover the surface of all of the small particles formed.

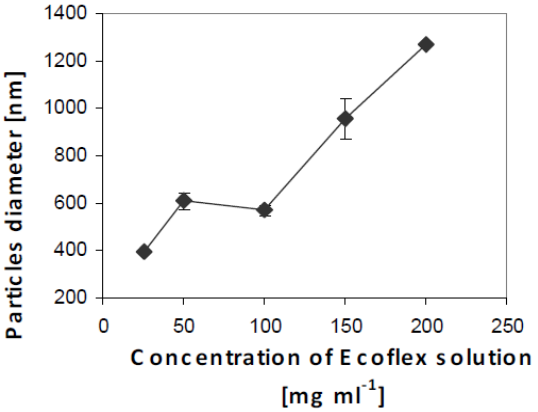

Fig. 6 Dependence of the mean particle size on the concentration of Ecoflex solution. Points are means from 3 measurements. Error bars represents twice standard deviations.

In the paper [14] authors presumed that the enlargement of nanoparticles is probably caused by the increasing viscosity of dispersed phase (more concentrated polymer solution), resulting of a poorer dispersability of the polymer solution into the aqueous phase caused by a high viscous resistance against the shear forces during emulsification. From which it follows that it is possible to obtain smaller particles from more concentrated polymer solutions by using either longer time of stirring or ultrasonication or both.

In our case, the linear dependence of the particle diameter on the concentration of Ecoflex solution can be observed. In [14], where three different but rather low concentrations were investigated the values of particles diameters for the two lowest concentrations were almost identical. It is possible, that lower diameters are not achieved by further reduction of the concentration under some limit.

IV. CONCLUSION

The study has proved that it is possible to prepare micro and submicro polymer particles in the procedure comprising emulsification and organic solvent evaporation at reduced pressure. Among the investigated processing parameters, sonication was found to be the crucial step in particle preparation that makes possible to prepare particles of diameters under 1 μm.

Further research should be focused on the investigation of the influence of other process parameters, namely the nature of the emulsifying agent and concentrations of all components used throughout the procedure and their influence on the particle size.

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PAPER VI

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Article

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Low Molecular Weight Poly(lactic acid) Microparticles for Controlled Release of the Herbicide Metazachlor: Preparation, Morphology, and Release Kinetics

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ABSTRACT: The preemergence chloroacetamide herbicide metazachlor was encapsulated in biodegradable low molecular weight poly(lactic acid) micro- and submicroparticles, and its release to the water environment was investigated. Three series of particles, S, M, and L, varying in their size (from 0.6 to 8 μm) and with various initial amounts of the active agent (5%, 10%, 20%, 30% w/w) were prepared by the oil-in-water solvent evaporation technique with gelatin as biodegradable surfactant. The encapsulation efficiencies reached were about 60% and appeared to be lower for smaller particles. Generally, it was found that the rate of herbicide release decreased with increasing size of particles. After 30 days the portions of the herbicide released for its highest loading (30% w/w) were 92%, 56%, and 34% for about 0.6, 0.8, and 8 μm particles, respectively. The release rates were also lower for lower herbicide loadings. Metazachlor release from larger particles tended to be a diffusion-controlled process, while for smaller particles the kinetics was strongly influenced by an initial burst release.

KEYWORDS: *poly(lactic acid), controlled release, metazachlor, oil-in-water solvent evaporation technique, microparticles, kinetics*

INTRODUCTION

In the last three decades, bioactive compound delivery systems have attracted increasing interest in the pharmaceutical industry[1,2] and agriculture and environmental engineering[3,4] because they are able to reduce undesirable effects of some of these bioactive molecules considerably. In agriculture, such controlled release formulations, including particles loaded with various agrochemicals, could prevent the unwanted phenomena associated with conventional applications of agrochemicals such as leaching through the soil, volatility, and degradation (photolytic, hydrolytic, and microbial),[5] and simultaneously they could extend their activity in soil, improve their stability, and reduce unwanted toxicity and thus safety for the environment and for operators along with easier handling and application.

Suitable materials meeting the demands for low impact on the environment could particularly be found among biodegradable polymers capable of gradual decomposition either during or after the release of an active agent.[6] In the area of microparticle formulations, one of the materials used regularly appears to be poly(lactic acid) (PLA), belonging to the family of biodegradable polyesters. The utilization of PLA as a matrix in microparticle release systems has been widely investigated through various techniques of encapsulation including emulsification−diffusion method,[7] nanoprecipitation,[8] supercritical antisolvent coprecipitation,[9] etc. Among these, the oil-in-water solvent evaporation technique, which has been already described in detail in a number of research papers,[10−14] proved to be the most preferred encapsulation method due to its relative simplicity, with no need for specialized equipment. In addition, this method is frequently used for encapsulation of lipophilic drugs with low water solubility and high efficiencies of encapsulation are achieved.[15]

PLA particles as a matrix were tested with a wide spectrum of drugs with various targeting in the field of pharmaceutical industry[16,17] and with some agrochemicals.[18,19] Almost uniformly, researchers used relatively high molecular weight (MW) PLA, with the exception of Takei et al.,[18] where low MW PLA was used in a granulate formulation prepared through melting and blending of the polymer and the bioactive compound at rather high temperature. In our laboratory we ran a series of preliminary experiments with high MW PLA (MW about 100 000) and low MW PLA (MW about 30 000) and blends of both materials, and we found that high MW PLA and the blends of both materials tended to form rather large aggregates in the relatively broad interval of processing conditions, while low MW PLA produced fine and homogeneous suspensions of particles tunable by processing properties. Utilization of low MW PLA can bring some further advantages over high MW PLA because of its faster biodegradation in the environment and possibly some other advantageous features that could arise during particle preparation and a bioactive agent encapsulation. We decided to investigate this eventuality.

Metazachlor (MTZ) is a common preemergence chloroacetamide herbicide frequently used to control grass and some dicotyledonous weeds in rape, soybean, potatoes, and other crops.[20] The agent is usually used in water suspension formulations, where one of the drawbacks is that the crystals

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(triclinic form) tend to form agglomerates, which can lead to clogging of the spray nozzles during application.[21]

The aim here was to study the encapsulation of herbicide metazachlor in micro- and submicroparticles made from low molecular PLA. The oil-in-water solvent evaporation technique was selected as the preferred method of particle preparation. Particle characteristics and active agent release were examined for various particle formulations. Fully biodegradable gelatin proved to be a suitable emulsion stabilizer,[21,22] and was applied through this work as the principal surface-active agent. Compared with common commercial surfactants used in most research works,[23−25] gelatin is remarkable for its low price and biodegradability, and hence could be considered as a potentially good emulsion stabilizer for environmental applications.

■ MATERIALS AND METHODS

Materials. The polymer used throughout the study was poly(lactic acid) (PLA, M_w = 34 000 g·mol^{-1}, M_w/M_n = 2) prepared in our laboratory. PLA was synthesized through direct melt polycondensation of lactic acid. At the beginning, 50 g of 80% L-lactic acid (optical purity 99.9%, Penta) was placed into a two-neck flask equipped with a condenser. The contents were stirred and heated for 4 h at 160 °C under reduced pressure of 20 kPa. After 4 h 0.5% (w/w) of catalyst tin(II) octoate was added, the pressure was reduced to 0.1 kPa, and reaction continued for a further 24 h. Finally, the product was cooled, dissolved in acetone, precipitated from water/methanol (1:1) solution, filtered, and washed with distilled water several times. The obtained powder was dried at 50 °C under vacuum for 24 h and subsequently analyzed by GPC.[26]

The bioactive agent used for the encapsulation was the herbicide metazachlor [2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl)acetamide], purchased from Chemos (Czech Republic), in the monoclinic and triclinic form, off-white powder with molecular weight 277.75, water solubility 430 mg·L^{-1}, purity >98%, melting point 74−78 °C, and density 1.19 g.cm^{-3}.

Surfactants used in the oil-in-water evaporation technique were gelatin from porcine skin type A (Sigma Aldrich) and Tween20 (Sigma Aldrich). Gelatin was in form of yellowish powder, with water solubility 67 mg.mL^{-1} at 50 °C. Tween20 (polyoxyethylenesorbitan monolaurate) was in the form of clear, yellow to yellow-green viscous liquid, with M_w = 1225 Da and water solubility 100 g·L^{-1}.

Preparation of PLA Micro- and Submicroparticles. PLA (0.4 g) was dissolved in 4 mL of chloroform. The exact amount of MTZ solution was then used to reach the required final mass fraction in a formulation (from 5% to 40%) and emulsified in water solution of selected surfactant system. Gelatin A (0.5%) and Tween20 (0.5%) were used in combination to produce larger size particles marked L, or gelatin A alone (0.5%) was used for smaller or medium particles marked S or M, respectively. The mixture was dispersed under continuous stirring at speed from 11 500 to 18 000 rpm for 5 min (homogenizer DI 18 basic, Yellow Line by IKA, Belgium), while the mixture was cooled on ice. The ratio between water and oil phases was kept the same in all experiments (10:1). To obtain smaller and medium size particles marked S or M, the emulsion was further sonicated with an ultrasonic probe (Hielscher UP 400S, Germany) for 5 min with amplitude 20% or 35%, respectively. In the last step, the prepared emulsion was placed into an Erlenmeyer flask, the organic solvent was evaporated under reduced pressure with stirring, and the suspension of microparticles was formed. The blank samples for all series were prepared in the same way without MTZ.

Characterization of Particles. *Metazachlor Encapsulation.* Efficiency of the encapsulation process was characterized by two parameters: encapsulation efficiency (EE) and herbicide loading (HL). The EE (%) was defined as a ratio between the weight of MTZ encapsulated and its total weight at the beginning of the process:

$$\text{EE (\%)} = \frac{\text{weight of encapsulated metazachlor}}{\text{initial weight of metazachlor}} \times 100$$

One milliliter of the obtained suspension was centrifuged (Hettich Universal 320) at 10 000 rpm for 10 min to separate water phase from particles, then each of them was handled in a different way. Water phase was decanted and analyzed later. Distilled water (40 mL) was then added and the mixture was stirred to resuspend particles. The total time of contact of particles with fresh water phase was exactly 60 s. It should represent a sufficient time to dissolve nonencapsulated MTZ on particle surface but short time for the release of encapsulated MTZ from the particles.[15] The mixture was then immediately filtered through a 0.22 μm poly(tetrafluoroethylene) (PTFE) syringe filter.

MTZ concentrations in both separated water phases were analyzed by HPLC on a Reprosil 5 μm C_{18} end-capped reversed-phase column (Watrex, Czech Republic), isocratic acetonitrile/water (60:40) mobile phase, 0.6 mL/min flow and dual UV detection (Waters 2487) at 220 and 266 nm.

Weight of encapsulated MTZ was calculated as the difference between its initial amount and combined amounts of MTZ in the initial suspension water phase and the readily soluble fraction on the surface of particles.

Herbicide loading (HL, %) was defined as the amount of MTZ encapsulated divided by the final weight of particles with encapsulated MTZ at the end of the process:

$$\text{HL (\%)} = \frac{\text{weight of encapsulated metazachlor}}{\text{weight of particles}} \times 100$$

Size of Particles. Size and morphology of large particles were evaluated by optical microscopy on a glass slide with a calibrated grid. Evaluation of particle size distribution (PSD) and *z*-average (Z_D) was performed with the help of ImageJ software. For particles smaller than 5 μm, PSD and Z_D were measured with Zetasizer (Zetasizer Nano ZS, Malvern Instruments) following manufacturer instructions. In both cases, PSD was expressed in the form of polydispersity (Pd), also called the coefficient of variation, calculated as the quotient of standard deviation σ and Z_D:

$$\text{Pd (\%)} = \frac{\sigma}{Z_D} \times 100$$

Particles Morphology by Scanning Electron Microscopy. Size and morphology of particles were also investigated by scanning electron microscopy (SEM; VEGA-II, Tescan). Particles were washed with distilled water three times to remove soluble substances from their surface, dried at laboratory temperature, and coated with a gold−platinum layer. Electron accelerating voltage was 10 kV.

Thermal Properties. Determination of thermal properties of particles were measured by differential scanning calorimetry (DSC) on Mettler Toledo DSC1 STAR system under nitrogen atmosphere (20 cm^3·min^{-1}) according to the following program: (i) first heating scan 0−170 °C (10 °C·min^{-1}); (ii) annealing at 170 °C for 1 min; (iii) cooling scan 170−0 °C (10 °C·min^{-1}); (iv) annealing at 0 °C for 1 min; (v) second heating scan 0−170 °C (10 °C·min^{-1}). Melting point temperature (T_m) as well as the exothermal response relating to cold crystallization temperature (T_c) was obtained from the first heating cycle. From the second heating scan, the region of glass transition temperature (T_g) was determined. Both onset and midpoint were recorded. The degree of crystallinity χ_c was calculated from the measured heat of fusion (ΔH_m) and crystallization (ΔH_c) according to the following equation:

$$\chi_c = \frac{\Delta H_m - \Delta H_c}{\Delta H_m{}^0} \times 100$$

where $\Delta H^0{}_m$ is the enthalpy of fusion for 100% crystalline PLA (93.1 J·g^{-1}).[27]

Fourier Transform Infrared Spectroscopy. Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectra were recorded on a Nicolet iS10 instrument equipped with a diamond crystal (Thermo Fisher Scientific, Waltham, MA). Total light reflection

Table 1. Conditions Used in the Preparation of Particles: Size, Surfactant Incorporation, and EE and HL Values

sample	MTZ[a] (%)	St[b] (rpm)	Amp[c] (%)	Z_D[d] (μm)	Pd[e] (%)	surf[f] (%)	EE[g] (%)	HL[h] (%)
S0	0	18 000	35	0.603	48.8	5.5	0	0
S5	5	18 000	35	0.592	52.1	4.4	35.1	1.8
S10	10	18 000	35	0.626	51.7	4.2	51.4	5.3
S20	20	18 000	35	0.587	56.2	5.3	59.1	11.1
S30	30	18 000	35	0.607	51.6	3.8	64.7	18.0
M0	0	11 500	20	0.752	50.7	3.2	0	0
M5	5	11 500	20	0.737	48.2	2.2	64.2	3.7
M10	10	11 500	20	0.760	51.5	2.6	62.5	6.8
M20	20	11 500	20	0.778	55.9	2.2	57.7	11.1
M30	30	11 500	20	0.803	49.5	3.0	61.9	17.8
L0	0	18 000	no	5.25	63.9	0.5/0.9	0	0
L5	5	18 000	no	6.72	36.4	0.6/1.8	73.0	3.7
L10	10	18 000	no	5.66	35.3	1.0/0.8	70.2	7.9
L20	20	18 000	no	4.94	27.6	0.4/1.4	64.8	13.5
L30	30	18 000	no	8.09	30.7	1.0/0.3	65.2	22.9

[a] Percentage of MTZ in a formulation at the beginning of the process. [b] Stirring speed. [c] Amplitude of sonication. [d] Z-average diameter. [e] Polydispersity. [f] Surfactant incorporation in particles (gelatin for S and M series, gelatin/Tween20 for L series). [g] Encapsulation efficiency. [h] Herbicide loading.

from surface was measured in the wavenumber range 500–4000 cm^{-1}. The collected spectra represented the average of 32 scans at a spectral resolution of 2 cm^{-1}. Spectra of formulations before and after herbicide release tests were recorded.

Surfactant Incorporation. The amount of surface-active agents encapsulated in particles was determined by elemental analysis in Thermo Electron Corp. Flash EA 1112 series analyzer in a configuration CHNS/O. Particles were washed with distilled water three times to remove soluble substances from their surface. Contents of individual compounds (PLA, MTZ, and surfactants) in formulations were calculated from the system of linear equations, which was based on known percentages of measured elements (C, H, N, S, and O) in pure compounds and in a given sample.

Release of Herbicide. Aliquots of particle suspensions containing exactly 5 mg of MTZ were centrifuged. Separated particles were resuspended in 100 mL of phosphate buffer (20 mmol·L^{-1}, pH 7) containing 0.2% sodium azide to prevent undesirable microbial degradation. All was done in triplicate. Suspensions were shaken (120 rpm) at 25 °C. Subsamples of 1.5 mL were taken in time intervals, centrifuged at 14 000 rpm for 10 min, and filtered through a 0.22 μm syringe PTFE filter to remove remaining particles. MTZ in samples was determined by the HPLC method described above.

Hydrolysis of PLA. Three sets of experiments at different pH were performed to determine the extent of PLA hydrolysis in the aquatic environment. Purified PLA powder (1.6 g) was suspended in 80 mL of phosphate buffer (0.1 mol·L^{-1}, pH 3, 7, and 9), in triplicate for each pH and kept at 25 °C. Samples (1.5 mL) were taken at intervals and centrifuged, and supernatants were analyzed for dissolved organic carbon (TOC 5000A analyzer, Shimadzu).

RESULTS AND DISCUSSION

Particle Preparation and Herbicide Encapsulation. PLA particles containing MTZ were prepared by the above-described oil-in-water solvent evaporation technique. The size of particles was influenced by some of the procedure parameters. These process parameters and characteristics of the resulting particles are listed in Table 1. The study approach included preparation of three series of particles marked S, M, and L, which were intended to differ mainly in size. In order to achieve the maximal amount of encapsulated MTZ, its initial ratio in a formulation was increased up to 40% limit, where PLA particles tended to precipitate. Similar phenomenon has been already observed with a lipophilic drug encapsulated in PLA matrix.[18]

Particle diameters were controlled by ultrasonication parameters, stirring speed, and use of auxiliary surfactant Tween20 in the case of L series. Finally, S series of particles differed from M series by only about 200 nm, whereas L series particles were about 10 times larger. In contrast to the expectations derived from literature data, no significant increase in particle size with herbicide loading was detected in comparison with empty particles (S0, M0, L0);[16,17] in S series the values fluctuated close to 600 nm, in M series a slightly growing trend could be detected, and in L series a significantly higher *z*-average was achieved for 30% MTZ loading only. No significant differences in PSD represented by polydispersity (Pd) could be observed, either between various formulations in S and M series or between the individual series. Pd values seem to be more variable in L series, with the broadest PSD found for blank sample and PSD of loaded formulations considerably narrower in comparison with other formulations. It could be concluded that in the described case the process conditions, mainly the use of ultrasonication, have a crucial impact on particle size and PSD, unlike MTZ loading, which appeared to have almost no effect.

The amount of surfactants enclosed in particles was determined to evaluate its transfer with particles and potential influence on the herbicide release process. In the case of L series, the appropriate amount of Tween20 had to be added along with gelatin, used as the main stabilizer, in the process of submicroparticle preparation, because PLA precipitation occurred when gelatin alone was used. The decrease of surfactant incorporation with increasing size of particles can be observed in Table 1. Since a surfactant is expected to be present near the particle surface, the trend can be attributed to the reduction of surface area related to particle volume with increasing diameter of particles. There is no clear trend related to MTZ loads.

The encapsulation efficiencies obtained were about 60%, which are lower values in comparison with the study describing encapsulation of another herbicide, tubeconazole,[14] where EE values over 95% were achieved. An explanation could be found in about 10 times lower water solubility of tubeconazole (0.032 $g \cdot L^{-1}$) in comparison with MTZ (0.45 $g \cdot L^{-1}$). Higher EE are easily reached with less water-soluble agents. EE values were significantly lower for smaller particles (S and M series). Again this could be explained by higher specific surface area of smaller particles.

As expected according to a number of research papers,[5,17] HL values increased with increasing initial amounts of MTZ in all series. The highest HL values with respect to initial MTZ amounts were achieved in the L series, which is in agreement with the highest obtained EE values for this series.

Morphology of Particles. SEM micrographs representing a comparison between blank particles without MTZ and maximally loaded particles containing 30% MTZ are assembled in Figures 1 and 2 for S, M, and L series of particles. No marked differences in the size and the surface morphology of particles were observed between empty (Figures 1A,C and 2A) and maximally filled particles (Figures 1B,D and 2B) for S, M, and L series. All types of particles from S and M series appears to be spherical (Figure 1); however, in L series, significant deviations from spherical shape of 30% MTZ loaded particles (Figure 2A) in comparison with empty particles (Figure 2B) are quite evident.

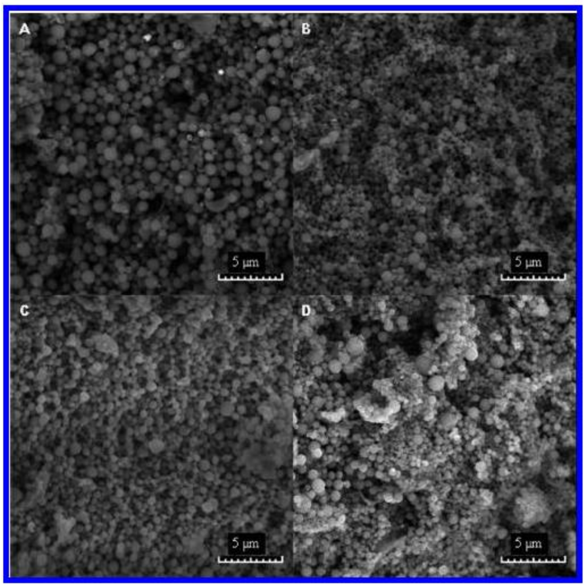

Figure 1. Scanning electron microscopy of polymer submicroparticles (10000×): (A) S0, (B) S30, (C) M0, and (D) M30 formulations.

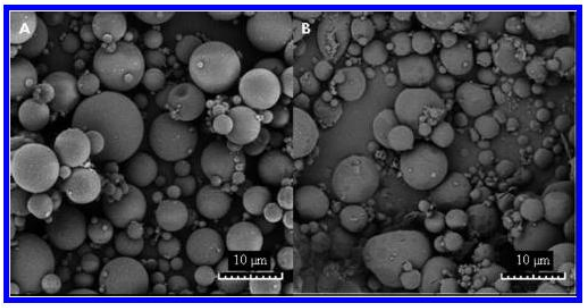

Figure 2. Scanning electron microscopy of polymer submicroparticles (5000×): (A) L0 and (B) L30 formulations.

Differential Scanning Calorimetry. Thermal analysis of particles was performed to clarify the effect of polymer properties, especially crystallinity, on MTZ release. Thermal properties of loaded and unloaded microparticles investigated are summarized in Table 2. It can be seen that both T_g and T_m decreased with increasing particle loading. For S and M particles, the glass transition temperature dropped by nearly 12 °C, while for L particles the decrease was 9.7 °C. This could be explained by an assumption that MTZ acted as a plasticizer in PLA matrix and increased free volume of polymer chains. Similarly, melting temperature declined, probably because MTZ limited crystal development in PLA matrix and decrease of crystallinity χ_c is a logical consequence.

Table 2. Thermal Properties of Microparticle Formulations

sample	T_m[a] (°C)	T_c[b] (°C)	T_g, midpoint[c] (°C)	T_g, onset[d] (°C)	χ_c[e] (%)
S0	149.8	85.2	46.6	42.9	58.9
S5	147.0	80.0	43.8	39.7	50.1
S10	143.5	80.7	39.4	36.2	49.9
S20	141.3	79.7	35.2	31.2	44.4
S30	139.8	79.7	34.6	31.1	40.3
M0	148.7	85.7	46.3	44.2	64.9
M5	147.7	88.1	45.2	42.3	63.9
M10	145.5	86.5	42.0	39.3	61.0
M20	142.7	85.0	39.2	36.1	55.9
M30	140.0	83.3	35.3	32.3	55.0
L0	149.3	90.4	42.4	37.8	58.7
L5	148.5	90.6	36.5	32.8	64.3
L10	146.6	86.4	40.1	36.3	59.6
L20	142.5	85.0	36.5	32.8	51.1
L30	140.0	81.7	32.3	28.1	43.9

[a] Melting temperature. [b] Crystallization temperature. [c] Glass-transition temperature at midpoint. [d] Glass-transition temperature at onset. [e] Degree of crystallinity.

Herbicide Release Study. The influence of herbicide loadings in micro- (L, Figure 5) and submicroparticles (S, Figure 3; M, Figure 4) on the cumulative release of herbicide

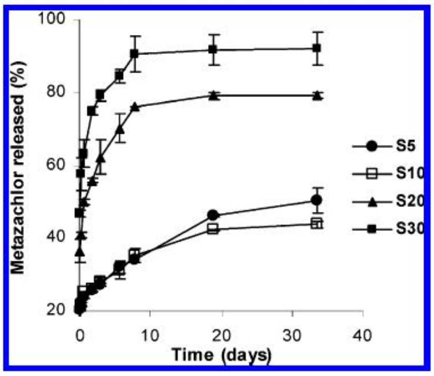

Figure 3. MTZ release profiles from PLA particles for S formulations. Error bars correspond to twice the standard deviation ($n = 3$).

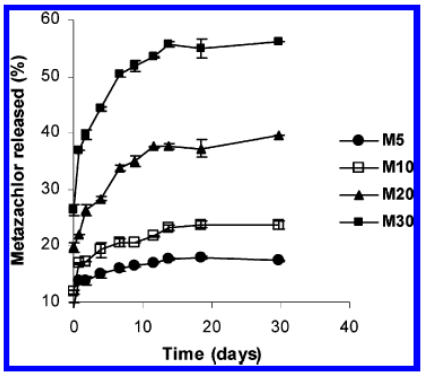

Figure 4. MTZ release profiles from PLA particles for M formulations. Error bars correspond to twice the standard deviation ($n = 3$).

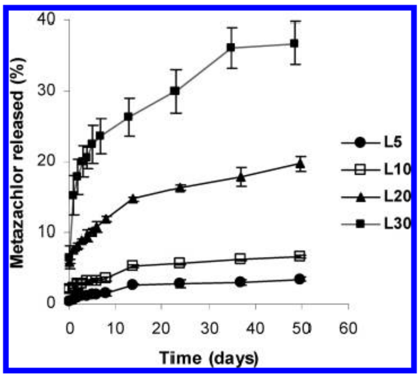

Figure 5. MTZ release profiles from PLA particles for L formulations. Error bars correspond to twice the standard deviation ($n = 3$).

MTZ for various particle sizes was investigated. As can be seen, for all formulations the release of MTZ could be characterized by a nonlinear biphasic profile, where the initial rapid release was followed by a considerably slower phase. The initial fast phase might be interpreted as the release of MTZ situated close to the surface.[5] Although a certain portion of MTZ was lost during a relatively fast initial period, the principal part of the agent was being released gradually during days and weeks as required for a controlled release formulation.

Results in Figures 3−5show that the percentage of released MTZ in the initial phase increased significantly with increasing particle loadings. Such behavior corresponds well with previously published observations made with another polymer matrix and herbicide[5,15] and might primarily be caused by the increased percentage of the herbicide close to the surface and the consequent shortening of the diffusion pathway from particles for this portion of the active agent. Described trends, however, could also be potentiated by changes in thermal properties of particles. It is well-known that the release of agents from polymer matrix changes dramatically with the glass transition of the polymer matrix, because of the pronounced shift of diffusion coefficients and other parameters. Generally, above T_g the release is expected to be faster than below T_g.[16,28,29] In our experiments the glass transition region of highly loaded formulations (Table 2) was relatively close to the temperature in release experiments (25 °C), which can, at least partially, explain higher MTZ release rates from highly loaded particles.

By comparison of formulations with identical MTZ loadings, it is apparent that the size of particles affects drug release significantly. The diminution of herbicide cumulative release with increasing *z*-averages of particles can be observed for all formulations. For instance, after 30 days the percentage of released herbicide for the highest loading was 92% for S30 (Figure 3), 56% for M30 (Figure 4), and 34% for L30 (Figure 5) formulations. Similar behavior was already observed for the same technique of particle preparation[25,30] and can be explained by the prolongation of an active agent diffusion pathway in larger particles. However, as can be detected from Figures 3−5, this trend is also strongly influenced by the magnitude of the release in the initial burst phase. As an illustration of this fact, the decrease of herbicide released from the above-mentioned formulations (47% from S30, 27% from M30, and 7% from L30) at the beginning of tests can be used. Since the initial burst relates to the release of the agent situated close to the surface of particles, the effect can probably be attributed to the reduction of specific surface area with increasing particle diameters.

For largest and maximally loaded particles (L30), their morphology before and after releasing test can be compared in Figure 6 as an example. The SEM microphotographs suggest that before testing the particles have somewhat rougher surface, in comparison with a rather smooth surface after the release test. The roughness could be a consequence of some portion of MTZ deposited on the surface. However, despite the release of a considerable amount of MTZ, surprisingly, no surface porosity was detected after the test.

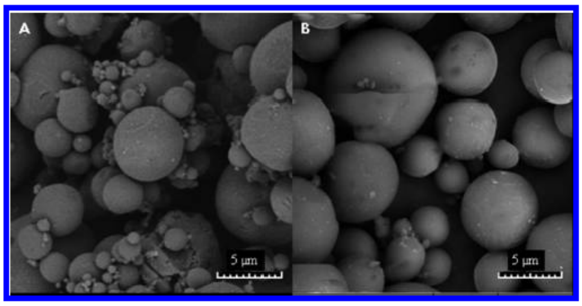

Figure 6. Scanning electron microscopy of polymer submicroparticles (10000×): L30 formulation (A) before and (B) after release tests.

As supporting evidence of MTZ incorporation and its subsequent release, FTIR-ATR spectra of pure MTZ and PLA particles without MTZ, as well as loaded particles before and after release tests, were recorded (Figure 7). The spectrum of pure PLA can be characterized by signals at 1751 cm^{-1} (attributed to C═O bond), 1452 cm^{-1} (typical for CH_3 bending), 1366 cm^{-1} (asymmetric −CH− bending), and the region between 1300 and 1040 cm^{-1} with four intensive absorption peaks at 1185, 1126, 1088 (C−O−C stretching), and 1046 cm^{-1} (−OH bending).[25,30,31] The spectrum of MTZ is more complex[20] but could be characterized by a distinctive signal at 1685 cm^{-1} of C═O stretching in the amide group, clearly visible also in the background of PLA spectra.[32] As can be seen for loaded particles, signals at 1685 cm^{-1} were clearly reduced after release tests, witnessing a drop in MTZ concentrations and its release from particles. The fact that the signal did not disappear completely also confirmed above-presented data, obtained by HPLC, that some herbicide

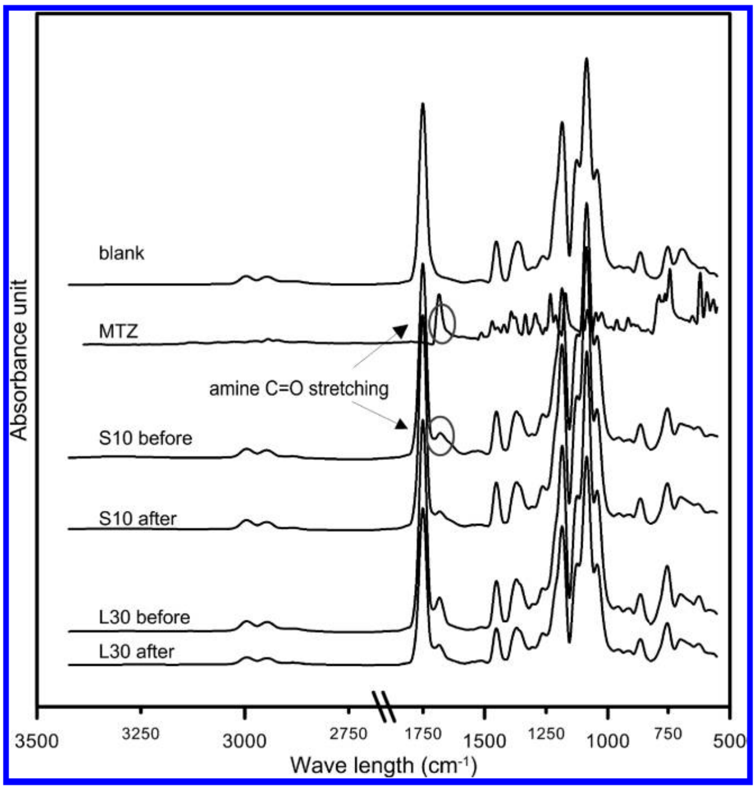

Figure 7. FTIR-ATR spectra of MTZ, unloaded particles, and MTZ-loaded particles.

remained in particles at the end of the release test period. This residual MTZ probably would be released during a longer time period and/or along with the degradation of particles.

PLA Hydrolysis. PLA hydrolysis, if taking place to a significant extent, could have an important influence on the kinetics of MTZ release from PLA particles. Cumulative amount of hydrolyzed PLA was calculated from the measured release of dissolved organic carbon. Three pH values (3, 7, and 9) corresponding to the theoretical interval of common soil pH were investigated (Figure 8). Results showed that the rate of PLA hydrolysis increased with increasing pH value. However, during the recorded period, corresponding with the period of MTZ release tests, percentages of the released carbon hydrolyzed from the polymer were rather low for all pH values and especially for pH 7 used in MTZ release experiments. It could be concluded then that PLA hydrolysis should not influence considerably MTZ release from PLA particles under given conditions.

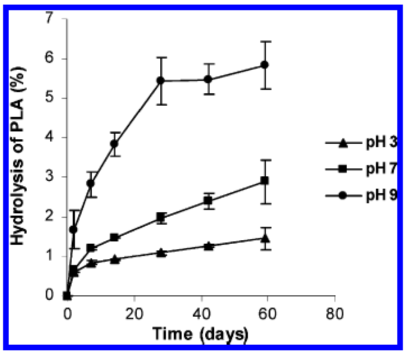

Figure 8. PLA hydrolysis at various pH values. Error bars correspond to twice the standard deviation ($n = 3$).

Metazachlor Release Kinetics. Obtained data from MTZ release experiments were analyzed by applying the common semiempirical exponential equation to describe and assess the influence of size and herbicide loading on the release kinetics:[15]

$$\frac{M_t}{M_\infty} = Kt^n + C$$

where M_t is the amount of active agent released at time t, M_∞ is the total amount of agent in particles. K is a release constant, C is a constant that takes into account the initial burst effect, and n is a diffusional exponent describing the mechanism of release depending on the geometry of the system.[33] For a system described by Fickian diffusion, where the concentration gradient of a released compound is the dominant driving force, the diffusion parameter is equal to 0.50; for a degradation controlled-release system, where the erosion of particles is the major force, the diffusion parameter is close to 1.0. In the case of spheres, n should be corrected to 0.43 and 0.85, respectively.[33,34]

Parameters of the equation along with the correlation coefficient R and the time needed for 50% release of MTZ were obtained by nonlinear regression and are listed in Table 3. High R values indicate good fitting of exponential equations on measured MTZ release profiles from all particle formulations.

Table 3. Parameters Characterizing Fitting of the Model Equation on MTZ Release Data

sample	K^a (10^{-2})	n^b	C^c (10^{-2})	R^d	$t_{50}{}^e$ (days)
S5	1.374	0.459	19.360	0.986	36.35
S10	2.820	0.347	16.750	0.993	50.82
S20	9.183	0.255	33.910	0.971	0.38
S30	13.660	0.196	45.170	0.968	0.000 21
M5	4.612	0.199	9.188	0.986	2362.09
M10	5.125	0.271	11.820	0.989	69.44
M20	6.554	0.361	19.060	0.980	3.08
M30	13.330	0.269	25.880	0.988	0.38
L5	0.112	0.483	0.262	0.974	12 634.83
L10	0.126	0.521	1.951	0.977	3730.47
L20	0.510	0.471	5.684	0.994	541.04
L30	3.557	0.306	6.619	0.996	149.33

[a] Release constant. [b] Initial burst constant. [c] Diffusional exponent. [d] Correlation coefficient. [e] Time corresponding to 50% agent release.

As can be seen, the release exponent $n < 0.52$ obtained in all experiments indicate that particle degradation was not the crucial driving force of the release. This observation is in agreement with the data from PLA hydrolysis at pH 7, when negligible hydrolysis was detected. In formulations with n close to 0.43, the release was probably controlled by diffusion; however, n values lower than 0.43 indicate another mechanism governing the herbicide release. There the high C values suggested that the release kinetics was probably influenced by "burst release",[15] which could be regarded as an undesirable effect.[35]

The resulting relationships of MTZ release half-times (t_{50}) to z-averages and herbicide loadings in three series of preparations are shown in Figures 9 and 10.

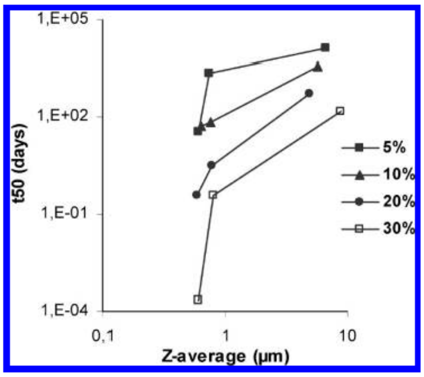

Figure 9. Dependence of t_{50} on particle z-averages for various initial percentages of MTZ .

These trends calculated from nonlinear regression analysis confirmed our previous observations that generally the rate of MTZ release decreased with increasing z-average of particles and increased with herbicide loading. Steep initial phases of t_{50} versus z-average dependences are probably a consequence of the fact that for the smallest particles the release kinetics is most strongly influenced by the initial burst release.

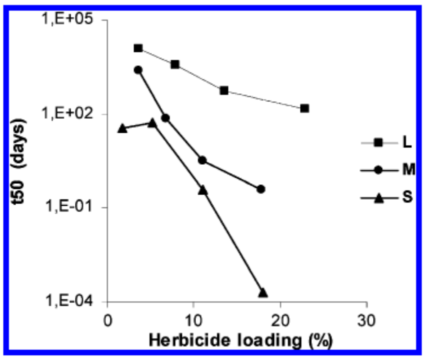

Figure 10. Dependence of t_{50} on herbicide loading for various formulations.

Three series of polymer micro- and submicroparticles varying in size were prepared from low molecular PLA by oil-in-water solvent evaporation technique and loaded with various initial amounts of the herbicide metazachlor. Particle loadings up to 30% of the theoretical herbicide content were investigated: for higher loadings, polymer precipitation occurred during solvent evaporation. Encapsulation efficiency reached about 60% and was lower for smaller particles (S and M series). Consequently, the highest actual herbicide loading values (HL) were found in L series, which was in agreement with encapsulation efficiency (EE) results. Ultrasonication was found to have a crucial impact on particle size and particle size distribution (PSD), unlike MTZ loading, which had almost no effect. All formulations appeared to be spherical and not affected by the amount of MTZ incorporated, with the exception of the highest loaded formulations in L series, which exhibited deviations from spherical shape. MTZ release was characterized by the typical nonlinear biphasic profile, where the release from formulations with diffusional exponent n close to 0.43 was found to be diffusion-controlled, while for formulations with n lower than 0.43 it was significantly influenced by the initial burst release. Low degree of PLA hydrolysis means that particle erosion has not considerably influenced MTZ release from PLA particles under given conditions. Generally, it was found that the rate of release depends inversely on the z-average and directly on herbicide loading. Since MTZ acted as a plasticizer in PLA matrix and decreased T_g, MTZ release rate could have been enhanced by this effect in highly loaded formulations, whose T_g was relatively close to the temperature employed in release experiments. Our study proved that preparation of a controlled-release formulation of the herbicide metazachlor with relatively high water solubility based on biodegradable PLA microparticles is possible in principle. By combination of different formulations, a particular release profile could be tuned to fit desired parameters.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

DSC, differential scanning calorimetry; EE, encapsulation efficiency; GPC, FTIR- ATR, Fourier transform infrared attenuated total reflectance; GPC, gel-permeation chromatography; HL, herbicide loading; HPLC, high-performance liquid chromatography; M_n, number-average molecular mass; MTZ, metazachlor; MW, molecular weight; M_w, weight-average molecular mass; Pd, polydispersity; PLA, poly(lactic acid); PSD, particle size distribution; SEM, scanning electron microscopy; T_c, cold crystallization temperature; T_g, glass-transition temperature; T_m, melting point temperature; Z_D, z-average; ΔH_c, heat of crystallization; ΔH_m, heat of fusion; σ, standard deviation; χ_c, degree of crystallinity

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