Anaerobic digestion of aliphatic polyesters
Pavla Šmejkalová, Veronika Kužníková, Jan Merna and Soňa Hermanová

ABSTRACT

Anaerobic processes for the treatment of plastic materials waste represent versatile and effective approach in environmental protection and solid waste management. In this work, anaerobic biodegradability of model aliphatic polyesters, poly(L-lactic acid) (PLA), and poly(ε-caprolactone) (PCL), in the form of powder and melt-pressed films with varying molar mass, was studied. Biogas production was explored in batch laboratory trials at 55 ± 1 °C under a nitrogen atmosphere. The inoculum used was thermophilic digested sludge (total solids concentration of 2.9%) from operating digesters at the Central Waste Water Treatment Plant in Prague, Czech Republic. Methanogenic biodegradation of PCLs typically yielded from 54 to 60% of the theoretical biogas yield. The biodegradability of PLAs achieved from 56 to 84% of the theoretical value. High biogas yield (up to 677 mL/g TS) with high methane content (more than 60%), comparable with conventionally processed materials, confirmed the potential of polyester samples for anaerobic treatment in the case of their exploitation in agriculture or as a packaging material in the food industry.

Key words | anaerobic digestion, biodegradable polymers, methane, poly(L-lactic acid), poly(ε-caprolactone), sludge

INTRODUCTION

The utilisation of polyester-based materials in packaging and agricultural applications is justified by their susceptibility to the hydrolytic scission and their biodegradation under various environmental conditions (Yavuz & Babaç 2005; Kale et al. 2007; Mensitieri et al. 2011; Gorrasi & Pantani 2013; Karamanlioglu & Robson 2013). Among aliphatic polyesters, poly(L-lactic) acid (PLA), and poly(ε-caprolactone) (PCL), represent compostable and biodegradable thermoplastics, which are decomposed to carbon dioxide and methane along with water and mineral salts during their mineralisation in compost and sludge, respectively (Gartiser et al. 1998; Calil et al. 2006; Funabashi et al. 2009; Yagi et al. 2009).

Polymer particle size, molar mass, and thermal history significantly affect the degradation rate (Murthy et al. 2012). For both polyesters anaerobic biodegradation rate was significantly enhanced in the thermophilic conditions (∼55 °C) in comparison with the mesophilic ones (∼35 °C) (Itiävaara et al. 2002). This accelerating effect of temperature is ascribed to both increased mobility of chains regularly arranged in crystalline phase and those being in disordered glassy state (Martén et al. 2005; Ndazi & Karlsson 2011; Kolstad et al. 2012) and different compositions of thermophilic microbial community. The biodegradability of PLA films under thermophilic anaerobic conditions ranges from 86 to 99% depending on the specimens’ size (Yagi et al. 2012). Smaller sized specimens were disadvantaged due to floating in the solution layer above highly active sludge sediment. Consequently the larger sized specimens degraded faster. The final biodegradability of PCL in the form of the powder decreased from 91 to 81% depending on particle size under thermophilic sludge conditions. Particles with smaller size degraded faster due to the higher accessible specific surface (Yagi et al. 2009). Albertsson et al. reported a decrease in number-average molar mass (Mn) by 80%, accompanied with the crystallinity increase by 45% for film-blown PCL (0.04 mm thickness) degraded in anaerobic sewage sludge for 29 days (Albertsson et al. 1998).

In this study, we investigated the degradation behaviour of PLA and PCL in the powder form and the form of melt-pressed film (10 × 10 × 0.7 mm) under anaerobic sludge conditions at 55 °C in a 150 day timeframe. Water uptake values, molar mass reduction, thermal properties and mineralisation (percentage of the theoretical biogas amount) were...
used as the indicators of the abiotic and enzyme-catalysed hydrolytic scission and biodegradation, respectively. Our objective was also to evaluate aliphatic polyesters in various forms as materials for anaerobic treatment.

**METHODS**

L-Lactic acid (85 wt % aqueous solution) and ε-caprolactone, ε-CL (97%), were purchased from Sigma Aldrich. ε-CL was vacuum distilled from calcium hydride before use. PLA was supplied by Nature Works LLC. The value of ε-tone, time interval (10, 29 and 49 days), the PLA and PCL samples in the form of sludge from operating digesters at Central Waste Water Treatment Plant (CWWTP) in Prague, Czech Republic. The polymer in the form of films and powders was placed into the serum bottle with 80 mL of sludge. After certain time interval (10, 29 and 49 days), the PLA and PCL films were taken out, carefully washed with distilled water to remove solid sludge particles and dried until constant mass loss. Samples in the powder form were incubated without periodical removal and material analysis.

**Biodegradation of polyester powders and films under anaerobic conditions**

Batch test was carried out in 120 mL serum bottles at a temperature of 55 ± 1 °C under the nitrogen atmosphere. The inoculum used in this test was thermophilic digested sludge from operating digesters at Central Waste Water Treatment Plant (CWWTP) in Prague, Czech Republic. The polymer in the form of films and powders was placed into the serum bottle with 80 mL of sludge. After certain time interval (10, 29 and 49 days), the PLA and PCL films were taken out, carefully washed with distilled water to remove solid sludge particles and dried until constant mass loss. Samples in the powder form were incubated without periodical removal and material analysis.

To determine the appropriate ratio of polymer specimen to sludge, the chemical oxygen demand (COD) of the samples was estimated ISO 15705:2002. COD of the model powder samples was in the range of 1.1–2.2 g/g.

The characteristic parameters of inoculum were as follows: TS, total solids (28.6 g/L); VS, volatile solids (14.7 g/L); TSS, total suspended solids (26.7 g/L); and VSS, volatile suspended solids (13.5 g/L), concentrations were determined according to Standard Methods (APHA-AWWA-WPFC 1998).

According to starting mass of polyester melt-pressed specimens the initial organic loading of inoculum was 0.38 g COD/g and 0.15 g COD/g VSS for PCL and PLA, respectively. For model powder materials the initial organic loading of inoculum was in the range from 0.2 to 0.9 COD/g VSS. These values belong to the optimum value range of 0.1–1.0 g COD/g VSS for the system in our study.

The biogas production was measured volumetrically at regular time intervals using a gas-tight burette with NaCl saturated solution as a stop liquid. The bottles were stirred before each measurement. The gas composition was analyzed by a gas chromatograph (Fisons Instruments GC 8000 Top) equipped with a thermal conductivity detector HWD 800 and a Separon AE (200–300 μm) glass column (2.5 m, 3 mm inside diameter). Argon was used as a carrier gas at a pressure of 100 kPa. The test was stopped when the biogas production was close to zero, i.e. after 150 days. Biodegradability is expressed as an achieved percentage of the theoretical biogas yield calculated according to Buswell equation (Buswell & Mueller 1952).

**Abiotic degradation and water uptake of polyester films**

For the purpose of water uptake test and abiotic degradation, the samples in the film form were incubated. The polymer films were placed into the serum bottle with 80 mL of phosphate buffer (pH 7.5) with sodium azide (0.05%) and kept at 55 ± 1 °C for 29 days. Phosphate buffer with pH of 7.5 was selected as incubation medium as this pH value was achieved after 24 hour incubation of all polyester films and persisted up to 49 days. Such slight decrease could be ascribed to the accumulation of degradation products ending with carboxylic groups undergoing a deprotonation or simply metabolic changes and transformations due to anaerobic digestion of these products within the biomass cells.

Water uptake study was carried out independently of biodegradation test in digested sludge and phosphate buffer (pH 7.5)
Polyester characterisation

Sample mass loss (%) and water uptake gain (%) were calculated as follows:

\[
\text{mass loss} (\%) = \frac{(m_0 - m_d)}{m_0} \times 100,
\]

\[
\text{water uptake} (\%) = \frac{(m_w - m_0)}{m_0} \times 100,
\]

where \(m_0\) denotes the initial mass of the film, \(m_d\) denotes the mass of the dried degraded film, and \(m_w\) is the mass of wet sample.

For the differential scanning calorimetry (DSC) experiments, DSC Q100 (TA Instruments) was used. Samples were heated and cooled at a rate of 5 °C/min under a nitrogen flow rate of 50 mL/min. The measurement was (i) 25–200 °C, (ii) 200–25 °C, (iii) 25–200 °C and (iv) 200–25°C for PLA and (i) 0–100 °C, (ii) 100–0 °C, (iii) 0–100 °C and (iv) 100–0 °C for PCL. Crystallinity of films was evaluated according to the following equation, where \(\Delta H_m\) is measured melting enthalpy and \(\Delta H_m^0\) is melting enthalpy for hypothetically 100% crystalline sample. \(\Delta H_m^0\) 93.1 J/g for PLA and 139.5 J/g for PCL. Crystallinity of films was evaluated according to respective calibration using narrow polystyrene (PS) standards (580–3,250,000 g/mol) was used to calculate apparent molar masses. Data were evaluated using Clarity 5.0 data processing software.

Molar mass of oligomer poly(L-lactic acid) out of calibration of SEC was calculated according to respective proton signals in nuclear magnetic resonance spectrum. \(^1\)H NMR spectrum was measured on NMR Spectrometer Bruker Avance DRX 500 at laboratory temperature in CDCl₃.

RESULTS AND DISCUSSION

Anaerobic digestion of polyester films

Both polyester samples, PCL and PLA, underwent the hydrolysis to release degradation products which were rapidly converted to biogas in the first few days of incubation. This was documented by no evident lag phase and correlation between the theoretical amount of produced gas and PCL specimens’ mass loss during 29 days (Supplementary Material, available with the online version of this paper).

Given that in anaerobic digestion the biological hydrolysis is assumed as the rate-limiting step of biogas production, one must consider that different susceptibility of polyester samples to hydrolytic scission would influence their anaerobic digestion profile. In accordance with this assumption lower rate of biogas production was measured for PCL than for PLA (Table 1). Maximal specific biogas production rates were comparable for both PCL (1.01 mL/(g·g·h)) and PLA (1.06 mL/(g·g·h)) samples. Both polyester samples were comparable with solid substrates frequently used in biogas plants for co-digestion, such as straw (0.68 mL/(g·g·h)) at mesophilic conditions, 0.95 mL/(g·g·h) at thermophilic conditions) and maize silage (1.6 mL/(g·g·h)). These natural substrates were evaluated independently of polymers under the same conditions (data not shown).

Biodegradability achieved 60 and 74% of expected value calculated according to Buswell equation (with methane content of 67 and 62%) for PCL and PLA films, respectively despite PCL materials are suggested as almost completely degradable in thermophilic anaerobic digestion process in the literature. Ponsart et al. showed that biodegradation of tritiated PCL proceeded up to the ultimate stage over ca. 72 days, giving tritiated water (80–90%) and biomass

<table>
<thead>
<tr>
<th>Material</th>
<th>Mₙ (0) [kg/mol]</th>
<th>Biodegradability [%]</th>
<th>Biogas yield [L/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL (film)</td>
<td>58.1 (2.3)</td>
<td>60</td>
<td>663</td>
</tr>
<tr>
<td>PCL (powder)</td>
<td>38.0 (2.0)</td>
<td>54</td>
<td>643</td>
</tr>
<tr>
<td>PCL (powder)</td>
<td>13.0 (2.8)</td>
<td>57</td>
<td>676</td>
</tr>
<tr>
<td>PLA (film)</td>
<td>44.5 (2.0)</td>
<td>74</td>
<td>677</td>
</tr>
<tr>
<td>PLA (powder)</td>
<td>3.4 (1.7)</td>
<td>56</td>
<td>520</td>
</tr>
<tr>
<td>PLA (gel)</td>
<td>0.35</td>
<td>84</td>
<td>625</td>
</tr>
</tbody>
</table>

*Biodegradability of the samples is expressed as an achieved percentage of the theoretical biogas yield calculated according to Buswell equation (Buswell & Mueller 1952).

*To sample mass (TS), under standard conditions (STP – standard temperature 273.15 K and pressure 10¹³ Pa).
Albertsson et al. reported on the biodegradation corresponding to theoretical amount of produced gas from the amount of PCL incubated in sewage sludge (Albertsson et al. 1998).

It is worth mentioning that the biodegradability values could be influenced by the sludge concentration; concretely undiluted sludge with TS concentration of 2.9% was used in this study.

The biodegradability concept states that all polymer carbon is converted into gaseous carbon (CO₂, CH₄) when complete mineralisation (100% value calculated according to Buswell equation) occurs. However, the inoculum introduces into the digestion process a wide range of ballast substances. During the decomposition of complex molecules it can be expected that polymer degradation intermediates interact with these substances or are sorbed in the sludge. Consequently they become unavailable for further degradation and mineralisation within cells. Besides, some portion of degraded polyester specimens was assumed to be incorporated into biomass under the incubation conditions of this study. That is why complete mineralisation was not achieved.

To simulate the digestion of the polyester film in the later phase of incubation when it is disintegrated, eroded, and has shorter chains, lower-molar mass samples of both polyester types in the powder form were subjected to anaerobic incubation in sludge. In accordance with the expectation, model samples are more susceptible to biodegradation. Their biodegradation has a faster onset and progression, i.e. biogas production (Figures 1(a) and (b)), due to both shorter chains and also higher-surface area. These factors are supposed to also accelerate the abiotic hydrolysis in comparison with the higher molar mass films.

On the other hand, the total biodegradability of polymer powder does not exceed the value reached for the film form of polyesters (Figures 1(c) and (d)) and similar situation was found for the biogas yield (Table 1). This was probably caused by floating of fine particles on the surface of the liquid phase without their sufficient contact with the sludge. For PLA sample in gel form this problem does not occur as gel sediments readily. So due to good contact with the sludge and low molar mass it reached the highest biodegradability.

Figure 1 | Time of 80% decomposition (dark bars) and maximal specific biogas production rate (light bars) for PCL samples (a) and PLA samples (b) differing in apparent molar mass under thermophilic sludge conditions; specific biogas production related to sample mass (TS) in anaerobic biodegradation test of samples PCL (c) and PLA (d) under thermophilic sludge conditions.
Each type of aliphatic polyester films is assumed to undergo the hydrolytic scission of ester bonds exhibiting particular behaviour due to different chemical structure and related steric hindrance of ester linkages and also different morphology (crystalline versus amorphous). To bring more insight into the course of anaerobic digestion process, incubated films were removed at certain time intervals, analysed and the results were compared with changes induced by incubating in phosphate buffer.

**Water uptake of polyester films**

Water uptake measurement was performed to evaluate the extent of increasing bulk hydrophilicity of polyester specimens during initial 10-day period of incubation in digested sludge and phosphate buffer (Figure 2). Assuming the specimens' thickness (0.7 mm) and the morphology of polymeric materials, the hydrolytic scission is supposed to begin at the film surface generating new hydroxyl and carboxylic groups and then to progress into inner layers depending on the competition between water diffusion and hydrolytic reaction. Hydrolytic degradation under conditions of this study is assumed to be either OH⁻ ions-catalysed random chain scission (sludge and buffer) or extracellular enzymes' catalysed unzipping reaction (sludge), which can occur only at the film surface. Activities of extracellular hydrolases capable of acting on ester bonds’ cleavage in digested sludge are shown in Supplementary Material.

Water uptake values of both polyester films were very low in phosphate buffer, which reflected slow water diffusion into semicrystalline (PCL) and amorphous glassy (PLA) matrix. Water uptake of PCL films incubated in sludge was almost the same as in the abiotic environment which means possible occurrence of hydrolytic scission only at the sample surface. Significantly increasing water uptake values of biotically incubated PLA films reflected pronounced hydrolytic scission starting at the surface and rapidly approaching inner layers up to samples’ disintegration.

**Changes in molar mass of polyester films**

The extent of hydrolytic scission was evaluated according to changes in average molar mass of dried samples taken from digested sludge and the resulting characteristics were compared with those of samples immersed in phosphate buffer. Overall results are summarised in Table 2.

For PCL film, a surface erosion mechanism prevailed during the whole 49-day incubation. Initially enzyme-catalysed chain unzipping is suggested to occur as no

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**Table 2 | Molar masses and thermal properties of polyesters after the incubation in digested sludge (29, 49 days) and phosphate buffer (29 days)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>(M_n) [kg/mol]</th>
<th>D</th>
<th>(T_m^{1\text{st}}) [°C]</th>
<th>(T_m^{2\text{nd}}) [°C]</th>
<th>(\chi^{1\text{st}}) [%]</th>
<th>(\chi^{2\text{nd}}) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL 0</td>
<td>58.1</td>
<td>2.3</td>
<td>n.d. b</td>
<td>68</td>
<td>31</td>
<td>55</td>
</tr>
<tr>
<td>29-buffer</td>
<td>38.4</td>
<td>2.1</td>
<td>n.d.</td>
<td>70</td>
<td>35</td>
<td>55</td>
</tr>
<tr>
<td>29d-sludge</td>
<td>37.1</td>
<td>2.2</td>
<td>n.d.</td>
<td>73</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
<td>49d-sludge</td>
<td>30.0</td>
<td>1.8</td>
<td>n.d.</td>
<td>74</td>
<td>34</td>
<td>61</td>
</tr>
<tr>
<td>PLA-0</td>
<td>44.5</td>
<td>2.0</td>
<td>65</td>
<td>169</td>
<td>n.d.</td>
<td>10</td>
</tr>
<tr>
<td>29d-buffer</td>
<td>5.9</td>
<td>2.6</td>
<td>n.d.</td>
<td>161</td>
<td>109</td>
<td>69</td>
</tr>
<tr>
<td>29d-sludge</td>
<td>1.5</td>
<td>1.5</td>
<td>n.d.</td>
<td>146</td>
<td>100</td>
<td>72</td>
</tr>
<tr>
<td>49d-sludge</td>
<td>0.82</td>
<td>1.8</td>
<td>n.a. c</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Conditions: sludge with initial pH 8.0, phosphate buffer with initial pH 7.5, temperature 55 °C.

aMolar masses determined by SEC according to the PS calibration.

b n.d. not detectable under conditions of DSC analysis due to the high content of crystalline phase.

nc.n. not analysed due to small amount of solid sample residues.
reduction in molar mass (Figure 3) and mass loss up to 2.6% for 10-day biotically incubated PCL film was achieved in comparison with no mass loss of abiotically incubated one. During following incubation OH⁻ ions-catalysed random chain scission took place which is supported by the same extent of the reduction in molar mass of both abiotically and biotically incubated PCL films.

PLA films were found to be more susceptible to random hydrolytic scission in alkaline conditions that PCL ones as evidenced by a significant drop in average molar mass (Mn, Mw) during samples’ incubation in both sludge and buffer (Figure 3). Monomodal character of elution curves supported the homogenous way of proceeding degradation in the film. Pronounced decrease in molar mass of PLA films incubated in sludge is ascribed to extracellular enzymes action on the sample surface making it more hydrophilic and vulnerable to water diffusion into entire specimen which in turn accelerated the random scission.

Thermal properties of polyester films

Thermal properties of polyester films before and after the incubation were determined to understand the changes induced by hydrolytic scission and physical aging (Table 2). DSC analysis of PCL film before the incubation shows that it is a semicrystalline material with a relatively high crystallinity of 55%. The crystallinity of abiotically aged and biodegraded PCL samples remained almost unchanged after 29 days of their incubation and only slightly increased following incubation. The temperature of incubation media, 55°C, was close to melting point of PCL (Tm = 68°C) and so could act as the annealing temperature. Consequently physical aging took place during samples’ incubation as documented by higher melting point measured after the first heating scan and the same thermal characteristics obtained after the second heating scan of starting and incubated sample. Slight increase in crystallinity of samples incubated for 49 days in sludge could be the consequence of the degradation proceeding preferentially in amorphous domains.

According to DSC analysis PLA film showed glass transition temperature of 65°C with the structural relaxation, cold crystallisation and melting temperature of 169°C. During the incubation both in sludge and buffer the sample lost the transparency, became brittle and significantly increased crystalline phase content. Such increase is ascribed to the preferential degradation and release of the amorphous part of the solution, which is evident for biotically incubated PLA films, where the reduction of amorphous content was accompanied by comparable mass loss. The formation of new crystallites during the secondary crystallisation means another possibility to explain this crystallinity increase. The scission-induced crystallisation (Han & Pan 2009; Gleadall et al. 2012) is suggested to occur in PLA films immersed in buffer for 29 days as documented by relevant drop in molar mass, low mass loss (2 wt%), and comparable increased crystallinity calculated according to the first and the second heating scan.

In conclusion, the crystallinity of all incubated polyester films was found to increase due to preferential cleavage and release of amorphous regions, secondary crystallisation of embedded degraded chains and physical aging. Consequently, one may expect that the hydrolysis process of the solid residual material becomes slower and probably also less complete under the conditions of the study.

CONCLUSIONS

The anaerobic biodegradation test, used in this study, enables good evaluation of the polymer materials’ biodegradation, which is very important for understanding plastic waste behaviour under real environmental conditions. Methanogenic biodegradation of PCLs typically yielded from 54 to 60% of the theoretical biogas yield. The biodegradability of PLAs achieved from 56 to 84% of the theoretical value. The biogas yield about 676-677 mL/g TS of original material with satisfactory methane content in excess of 60% confirmed the potential of these materials for anaerobic treatment in the case of their exploitation in agriculture or as a packaging material in the food industry.
Concerning the degradation mechanism, surface erosion pattern was confirmed for PCL melt-pressed films; whereas both surface erosion and bulk degradation occurred in PLA films. Despite general concept of random abiotic hydrolysis, which is assumed to occur at the beginning stage of polymer samples’ incubation, we clearly proved that also enzyme-catalysed unzipping took place. Formed monomeric products were rapidly mineralised yielding biogas (no lag phase). However, during following incubation random hydrolytic scission begin to prevail and the films degradation, i.e. chains scission proceeded more rapidly than biodegradation process itself.

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