Resources, Conservation & Recycling 198 (2023) 107202

Contents lists available at ScienceDirect

Resources, Conservation & Recycling

journal homepage: www.elsevier.com/locate/resconrec

Degradation of poly-L-lactic acid biopolymer films in $Ca(OH)_2$-dosed fresh human urine collected in source-separating sanitation systems

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ARTICLE INFO

Keywords:
Polymer
Decentralised sanitation
Nutrient recovery
Source separation
Wastewater treatment

ABSTRACT

Alkaline dehydration is a promising technology for producing organo-mineral fertiliser from source-separated human urine, but potential risks to users from handling alkaline earth hydroxides at source and carbonation of hydroxides during dehydration limit its implementation in real-life settings. These issues could be addressed by enclosing chemicals in bio-derived polymers. In this study, we investigated degradation and erosion of poly-L-lactic acid (PLLA) biopolymer films of different thicknesses (0.05, 0.1 and 0.25 mm) in $Ca(OH)_2$-dosed fresh urine and Milli-Q water at two temperatures (20 °C and 45 °C). Evaluation of degraded films by XRD, GPC and SEM showed changes in crystallinity, reduction in molecular weights and change in surface morphology, respectively. SEM/EDX, FTIR and ICP-OES data indicated that L-CaL_2, L-lactides and lactic acid were formed in urine. Overall, we found that thinner films, higher temperature and higher pH accelerated PLLA degradation in urine.

1. Introduction

Human urine contributes 80% of the nitrogen (N) and 50% of the phosphorus (P) and potassium (K) found in domestic wastewater (Jönsson et al., 1997). The majority of the N and K in conventional wastewater (blackwater) is not recovered in centralised treatment plants (Simha et al., 2020). A different approach to treating urine is to collect it separately from other domestic wastewater, e.g. in urine-diverting toilets (Tilley et al., 2014). However, source-separated urine mainly consists of water and for recycling as fertiliser the water needs to be separated from plant-essential nutrients (Simha et al., 2018). If freshly excreted source-separated urine is immediately dosed with alkaline earth chemicals such as $Mg(OH)_2$ (Simha et al., 2022) or $Ca(OH)_2$ (Simha et al., 2020), enzymatic degradation of urea, the major N-containing metabolite in urine, to ammonia is prevented, avoiding losses of N during dehydration. Further treatment of alkalised urine by dehydration allows complete recovery of all nutrients and produces a solid fertiliser containing >15% N, >2% P and >5% K (Simha et al., 2020). However, there are two issues with the use of alkaline earth chemicals for treating urine. First, alkalised urine absorbs carbon dioxide (CO_2) during evaporation, causing carbonation of hydroxides (Aguilar, 2012) that can form an insoluble layer over unused hydroxides and prevent their further dissolution (Vasiljev et al., 2022). Second, there can be health risks to humans during handling and dosing of urine with alkaline earth chemicals. For example, $Ca(OH)_2$ can irritate the eyes, nose, lips and tongue if inhaled, while prolonged exposure to $Ca(OH)_2$ can lead to breathing difficulties and swelling of the throat (New Jersey Department of Health and Senior, 2005). One way to prevent these issues could be to enclose the alkaline earth chemicals used for dosing with a bioderived polymer such as polylactic acid (PLA) so that the chemicals are dosed passively and released at specific time intervals. This could also help maintain a stable pH throughout the treatment of urine by alkaline dehydration.

Water-soluble polymers have been used widely in pharmaceuticals and biomedicine, including the development of novel drug delivery systems (Kadajji and Betageri, 2011). Bio-based polymers such as starch and cellulose have also been used previously in wastewater treatment, with applications including oil-water separation and heavy metal removal (Gao et al., 2019). However, the use of bio-based polymers for wrapping, delivering and releasing chemicals required to stabilize nutrients excreted in source-separated domestic wastewater such as human urine has not been investigated before.

PLA is one of the most widely used bioplastics and is a biodegradable and recyclable polyester derived from renewable feedstocks such as

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https://doi.org/10.1016/j.resconrec.2023.107202
Received 4 August 2023; Received in revised form 2 September 2023; Accepted 4 September 2023
Available online 8 September 2023

starch (Chen et al., 2016). It exists in three enantiomeric forms, PLLA (l-PLA), PDLA (d-PLA) and PDLLA (mixture of D-PLA and L-PLA) (Jamshidian et al., 2010), and possesses various properties such as good mechanical strength, transparency and good processability (Murariu and Dubois, 2016). It is used widely in biomedical applications such as grafts, implants and other types of equipment (Singhvi et al., 2019). It is also the most commonly used plastic for the production of filaments for 3D printers (Subramaniam et al., 2019).

The biodegradability of PLA has been the subject of various investigations. PLA degrades into lactic acid, lactates, lactides or CO_2 and water depending on degradation conditions and the breakdown pathway (Vink et al., 2003). At alkaline pH, PLA degrades via surface erosion and random chain scission, while at neutral pH it degrades via bulk erosion and chain end-scission (Vink et al., 2003). Degradation is the process of cleavage of a polymer chain to form oligomers and monomers, while erosion is the loss of mass from the polymer bulk/-structure (Göpferich, 1996). Existing literature only discusses the biodegradation pathways of PLA in marine, soil and compost environments, where degradation occurs via hydrolytic, photo, microbial or enzymatic means (Karamanlioglu et al., 2017; Malinconico, 2017; Qi et al., 2017). In industrial settings, PLA can be degraded by thermolysis, hydrolysis and alcoholysis (transesterification). These processes are faster than biodegradation, but require specialist conditions such as high temperature and pressure, as well as close monitoring of the optimal conditions (Zaaba and Jaafar, 2020).

To our knowledge, erosion and/or degradation of PLA in human urine has not been studied to date and the concept of enclosing chemicals for treating source-separated human urine in bio-derived polymers has not been proposed previously. These research gaps are addressed in the present study, the overall aim of which was to investigate the feasibility of using PLA biopolymer films for encasing and dosing alkaline earth chemicals to fresh human urine collected in source-separating sanitation systems. Of the three enantiomers of PLA, we chose to investigate PLLA since it has the highest crystallinity and would take the longest time to degrade in human urine. Specific objectives of our study were to evaluate: (i) the effects of PLLA film thickness and urine temperature on degradation of PLLA in fresh urine dosed with $Ca(OH)_2$; (ii) changes in physical and chemical properties of urine due to PLLA degradation; and (iii) changes in surface properties, morphology and chemical properties of PLLA during degradation.

2. Methods

2.1. Materials

Fresh human urine was collected in 500-mL polypropylene bottles from 16 anonymous urine donors, both male and female, aged between 20 and 65 years. The urine donations ($n = 27$) were pooled, dosed with 10 g $Ca(OH)_2$ L^{-1} to prevent enzymatic hydrolysis of urea to ammonia (Simha et al., 2020), and mixed at 300 rpm for 30 min using a propeller (R 1345, 4-bladed, Ø100 mm, 540 mm) connected to a digital overhead stirrer (OHS 60, Velp Scientifica, Italy). The treated urine was then allowed to stand undisturbed overnight to settle out precipitates.

Biaxially oriented PLLA biopolymer film (Goodfellow Cambridge, UK) with initial thickness of 0.05 mm was used. The biopolymer was heat-pressed in an oven at 160 °C for 1 h and fabricated into square films (30 mm × 30 mm) with different thicknesses (0.05, 0.1 and 0.25 mm). No additives were used during film fabrication.

2.2. Experimental procedure

The experiment evaluated degradation over an eight-day period of PLLA films of different thicknesses in $Ca(OH)_2$-dosed fresh urine at two temperatures (20 °C and 45 °C). First, 100 mL of $Ca(OH)_2$-dosed urine and a PLLA film sample were added to air-tight clear glass vessels. The vessels were then placed in closed opaque boxes at 20 °C or in an incubator (Heratherm IGS400, Thermo Scientific, USA) set to 45 °C. After two, four and eight days of storage in these conditions, the urine and PLLA films were sampled destructively. As a control, the same procedure was performed using Milli-Q water instead of human urine.

The urine collected was subjected to filtration, using filter paper with pore size of 3–5 μm (Grade 390, Ahlstrom Munksjö, Sweden), and stored at room temperature for further analysis. Any particles that remained in the glass vessel were collected by flushing with 50 mL of Milli-Q water and filtered through the same filter paper which was used for the main sample. The filtered urine and the solid residue collected on the filter paper were dried at 40 °C for 24 h.

2.3. Characterisation

Urine pH was measured using a glass electrode (Metrohm iUnitrode with Pt1000, 6.0278.300, Switzerland) and electrical conductivity (EC) was determined using a measuring cell (Metrohm, 6.0917.080, Switzerland) connected to a 914 pH/Conductometer (2.914.0020, Metrohm, Switzerland). Fourier-transformed infrared spectra (FT-IR) were recorded on a Nicolet FT-IR Impact 410 spectrometer (Madison, USA) in absorbance mode. The detector covered the range from 400 to 4000 cm^{-1} with a spectral resolution of 8 cm^{-1}. The PLLA films were milled and made into small pellets with potassium bromide (KBr) using a laboratory KBr FTIR Hydraulic Press. Powder X-ray diffraction (PXRD) analysis was performed at 25 °C using a SPECTRUM 100 spectrometer (Perkin Elmer, USA) over the range $2\Theta = 10–70°$. The PLLA films were further prepared for gel permeation chromatography (GPC) by grinding to a fine powder using a mortar and pestle. Then 0.1 mg of PLLA film was dispersed in 1 mL tetrahydrofuran and ultra-sonicated for 15 min, after which 50 μL of the solution was injected into the GPC setup (HPLC PUMP-515; Waters Corporation, USA) with a run time of 15 min per cycle. The results were analysed using EMPOWER-2 software (Waters Corporation, USA). Some films were not soluble in tetrahydrofuran and GPC data for those films could not be obtained. Scanning electron microscopy (SEM) images and energy-dispersive X-ray (EDX) spectrograms were obtained using a JSM 6390LV (JEOL, Japan). An area of 4000 μm^2 was selected in the SEM images, with 2000x magnification. The concentration of calcium (Ca) and sodium (Na) in urine was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES), using an ICP Avio 200 (Thermo Fisher Scientific, USA).

2.4. Calculations

Degree of crystallinity (X_c%) is the ratio of the crystalline fraction of a polymer to its amorphous fraction, where the higher the degree of crystallinity, the more organised the polymeric chains). X_c was calculated as (B. Aziz et al., 2020):

$$X_c = \frac{A_c}{A_t} \times 100 \quad (1)$$

where A_c is area under peaks in an XRD curve and A_t is total area under the XRD curve.

Poly dispersity index (*PDI*) is the ratio of weight-average molecular weight to number-average molecular weight of a polymer, where a PDI value of 1 indicates that the weight average molecular weight is similar to the number-average molecular weight of a polymer (Kissin, 1995). PDI was calculated as (Viéville et al., 2011):

$$PDI = \frac{M_w}{M_n} \quad (2)$$

where M_w is weight-average molecular weight and M_n is number-average molecular weight.

Number-average molecular weight (M_n) is calculated from the mole fraction distribution of different-sized molecules in a sample, weight-average molecular weight (M_w) is calculated from the weight fraction

distribution of different-sized molecules and peak molecular weight (M_p) is the molecular weight at the peak of the molecular weight distribution curve of the polymer. Based on molecular weights (number-average, weight-average and peak) of the biopolymer obtained by GPC, the percentage degradation of the PLLA films was calculated as:

$$\%D = \frac{M_i - M_f}{M_i} \times 100 \quad (3)$$

where M_i is initial molecular weight and M_f is final molecular weight of the degraded film.

2.5. Statistical analyses

All experiments were performed in triplicate and values reported are mean of triplicates and standard deviation. Analysis of variance (ANOVA) followed by Tukey's multiple comparison test (Honest Significance Difference, HSD) was carried out to check for significant changes in pH and EC of urine in relation to PLLA film thickness or urine temperature. All statistical analyses were performed using RStudio version 1.2.5042 and R version 4.0.0 (RStudio Team, 2016), with the R-packages *car* (Fox and Weisberg, 2018) and *agricolae* (De Mendiburu, 2020).

3. Results

3.1. Structural changes and degradation of PLLA films

Degradation of PLLA films in $Ca(OH)_2$-dosed fresh urine was faster at 45 °C than at 20 °C (Fig. 1). By day 4, the 0.05 mm and 0.1 mm thick films in urine had broken down structurally at 45 °C, whereas the 0.25 mm films in urine degraded to a lesser extent and broke down into fragments only during the drying process. However, by day 8 the 0.25 mm films at 45 °C had degraded completely. At 20 °C, only the 0.05 mm films had broken down structurally by day 8. Degradation of PLLA in Milli-Q water was slow in all cases, irrespective of film thickness and temperature (Fig. 2).

3.2. Physicochemical changes in urine due to PLLA degradation

At both storage temperatures studied, there was no change in pH and EC of the urine samples that did not contain PLLA films throughout the experiment. For 0.05 mm films in $Ca(OH)_2$-dosed fresh urine stored at 20 °C, there was a non-significant decline in pH from day 2 to day 4 ($p < 0.05$), but a significant decline from day 4 to day 8 ($p < 0.001$) (Fig. 2). The decline in pH was significant at every sampling day ($p < 0.001$) for the 0.05 mm thick PLLA film samples at 45 °C, and for the 0.1 mm and 0.25 mm thick films at both 20 °C and 45 °C. The decrease in EC of the urine was significant at both temperatures (20 °C and 45 °C) on every sampling day and for all three thicknesses of PLLA film ($p < 0.001$). In Milli-Q water, the presence of PLLA film caused the pH and EC of Milli-Q water to fluctuate considerably, but there was no clear or apparent trend (Fig. 3).

3.3. Fourier transform infrared spectroscopy

The FTIR spectra of the virgin PLLA showed all the characteristic peaks of PLLA, whereas new peaks were detected in the FTIR spectra of PLLA films stored in $Ca(OH)_2$-dosed fresh urine for two days (Fig. 4a), indicating the formation of new functional groups. For instance, the sharp peak around 850 cm^{-1} is attributable to C—C stretching of the lactate group and the broad peaks around 1500–1300 cm^{-1} and 800–650 cm^{-1} indicate the formation of lactate salts. The FTIR spectra of the PLLA films stored in Milli-Q water for eight days also showed various new peaks, but there were no visible structural changes in the films (Fig. 4b). Broad peaks emerged around 1500–1000 cm^{-1}, indicating formation of a carboxylate (-COO-) group due to hydrolytic scission of the ester, and two peaks appeared around 3600–3500 cm^{-1}, indicating that the number of –COOH and –OH bonds had increased and implying that lactic acid had formed.

3.4. Powder X-ray diffraction

The *s* value of the virgin PLLA film was 44.6% (Table S1 in Supplementary Information (SI)). For the 0.05 mm PLLA films stored in Milli-Q water for eight days, there was no change in X_c at either of the temperatures studied (Fig. 5). For the 0.05 mm PLLA films stored in $Ca(OH)_2$-dosed fresh urine for two days, X_c decreased to 33.4% at 20 °C and 33.9% at 45 °C. For the 0.1 mm PLLA films stored in $Ca(OH)_2$-dosed fresh urine for two days, X_c increased to 59.3% at 20 °C and decreased to 39% at 45 °C. For the 0.25 mm thick films stored in $Ca(OH)_2$-dosed fresh urine for two days, X_c was 45.7% at 20 °C and 43.3% at 45 °C (Table S1 in SI).

3.5. Gel permeation chromatography

As PLLA starts to degrade and forms oligomers and monomers, all the molecular weights (M_n, M_w *and* M_p) would be expected to decrease. The molecular weights of the virgin PLLA film were 131,568 Da (M_n), 188,622 Da (M_w) and 203,368 Da (M_p) (Table S2 in SI). When the PLLA films were stored in $Ca(OH)_2$-dosed fresh urine at 20 °C for two days, the molecular weights decreased to 126,617 Da (M_n), 151,757 Da (M_w) and

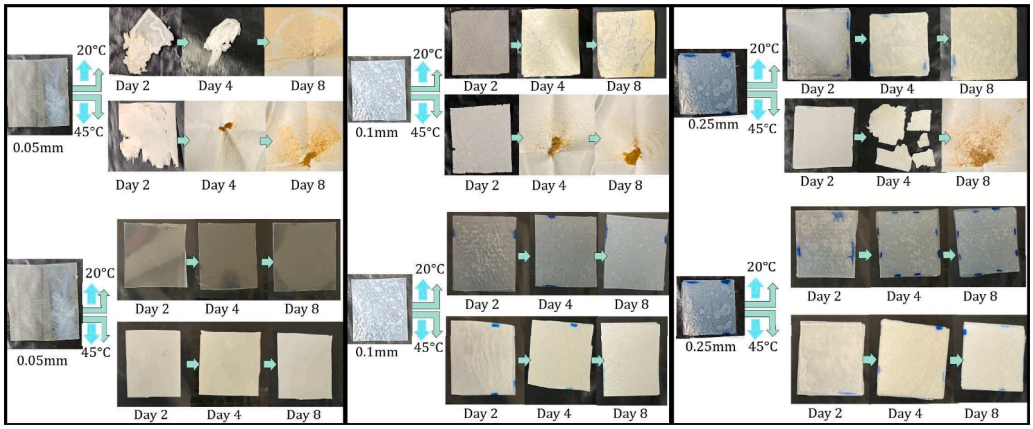

Fig. 1. Degradation of PLLA films stored in $Ca(OH)_2$-dosed fresh urine (top row) and Milli-Q water (bottom row) and stored for different periods (day 2, 4 and 8) and at different temperatures (20 °C and 45 °C). The films were collected after passage through filter paper with pore size 3–5 μm, dried at 40 °C for 24 h and stored at room temperature.

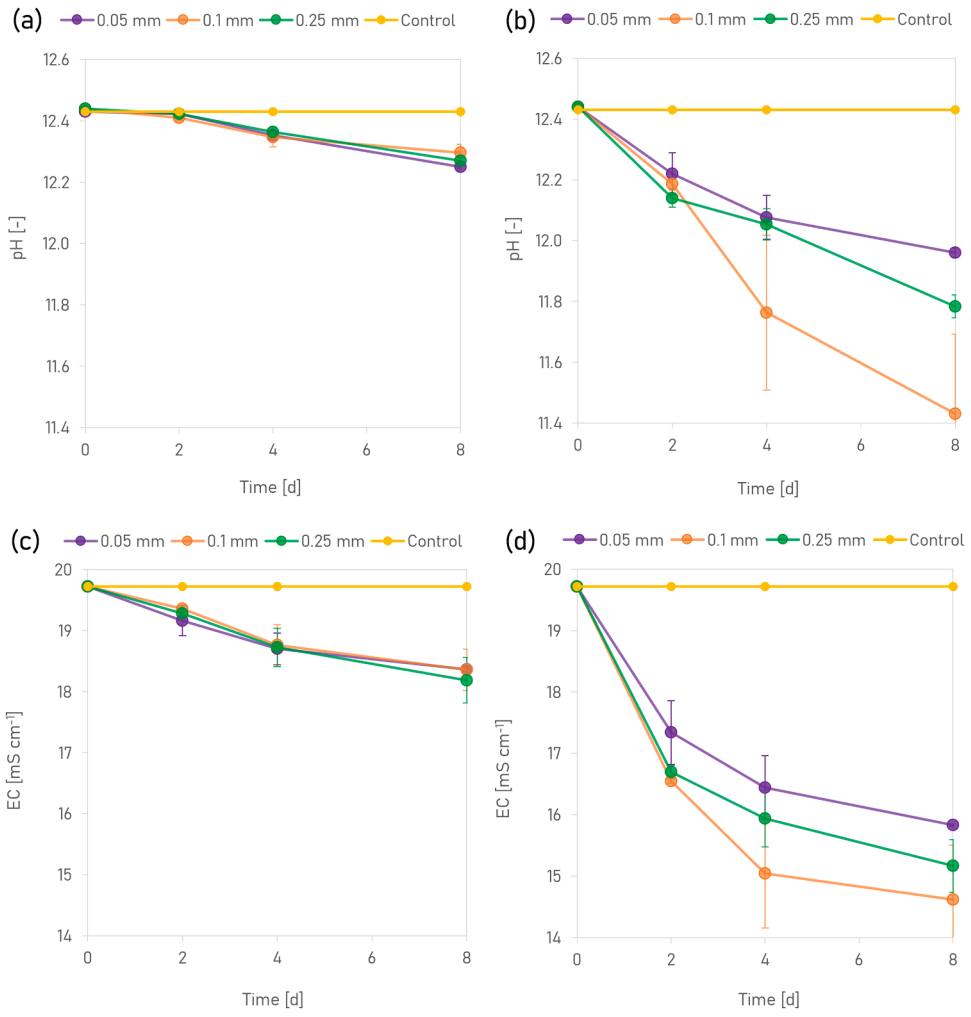

Fig. 2. Change in pH and EC of $Ca(OH)_2$-dosed fresh urine over time in the presence of PLLA films of different thicknesses (0.05, 0.1, and 0.25 mm) at 20 °C (a, c) and 45 °C (b, d).

170,323 Da (M_p) (Table S2 in SI). The decrease in molecular weights was greater at 45 °C, where the average values were 98,614 Da (M_n), 163,657 Da (M_w) and 151,893 Da (M_p) (Table S2 in SI). When the PLLA films were stored in Milli-Q water for eight days at 20 °C, the molecular weights were 161,975 Da (M_n), 199,801 Da (M_w) and 194,549 (M_p) (Table S2 in SI). Based on M_n of the PLLA films in $Ca(OH)_2$-dosed fresh urine, the degradation was 3.8% at 20 °C and 25% at 45 °C (Table S3 in SI). Based on M_w, the degradation was 19.5% at 20 °C and 13.2% at 45 °C. Based on M_p, the degradation was 16.2% at 20 °C and 25.3% at 45 °C (Table S3 in SI). The PDI of the samples also changed, a further indication of degradation of the PLLA films. The PDI of the virgin PLLA film was 1.43. After being stored in $Ca(OH)_2$-dosed fresh urine for two days, the PDI of 0.05 mm thick film decreased to 1.2 at 20 °C and increased to 1.66 at 45 °C. For the 0.05 mm films stored in Milli-Q water at 20 °C for eight days, the PDI decreased to 1.23 (Table S2 in SI). For all GPC curves, see Fig. S1 in SI.

3.6. Scanning electron microscopy, energy-dispersive X-ray spectroscopy and inductively coupled plasma optical emission spectroscopy

The virgin PLLA film had smooth morphology and negligible defects. After storage in $Ca(OH)_2$-dosed fresh urine at 20 °C for two days, the film was structurally broken down into small fragments and was full of deposits, which were likely a mixture of degraded polymer and precipitates formed in the urine (Fig. 6). When the films were stored in urine at 45 °C for two days, the fragments become smaller and more deposits were detected (Fig. 6). No deposits were observed on the PLLA films kept in Milli-Q water for eight days (Fig. 7). However, structural imperfections and erosion were apparent in films kept in Milli-Q at 20 °C for eight days, and there was significant fragmentation in films kept in Milli-Q at 45 °C (Fig. 7). According to the ICP-OES results, the initial concentration of Ca and Na in the $Ca(OH)_2$-dosed fresh urine was 968 mg L^{-1} and 1800 mg L^{-1}, respectively. After eight days, the concentration of Ca and Na in urine with 0.05 mm thick PLA film at 20 °C decreased to 549 mg L^{-1} and 1665 mg L^{-1}, respectively (Fig. S2 in SI). The EDX results showed that various elements were introduced into the PLLA films stored in $Ca(OH)_2$-dosed fresh urine, while the virgin film only contained carbon and oxygen (Fig. S3 in SI). The most notable new element was Ca, which was present in films degraded at 20 °C (12.4% of total weight of the degraded film) and 45 °C (28.7% of total weight of the degraded film). No new elements were introduced in PLLA films that were stored in Milli-Q water (Fig. S4 in SI).

4. Discussion

4.1. Degradation pathway of PLLA in fresh urine dosed with $Ca(OH)_2$

Degradation of PLLA in alkaline conditions occurs via hydrolysis of ester bonds. This results in formation of carboxylic acid and alcohol (Södergård and Stolt, 2002), while the PLLA breaks down into soluble oligomers and monomers (Karamanlioglu et al., 2017). The acid and alcohol that form are retained on the material or in the reaction medium,

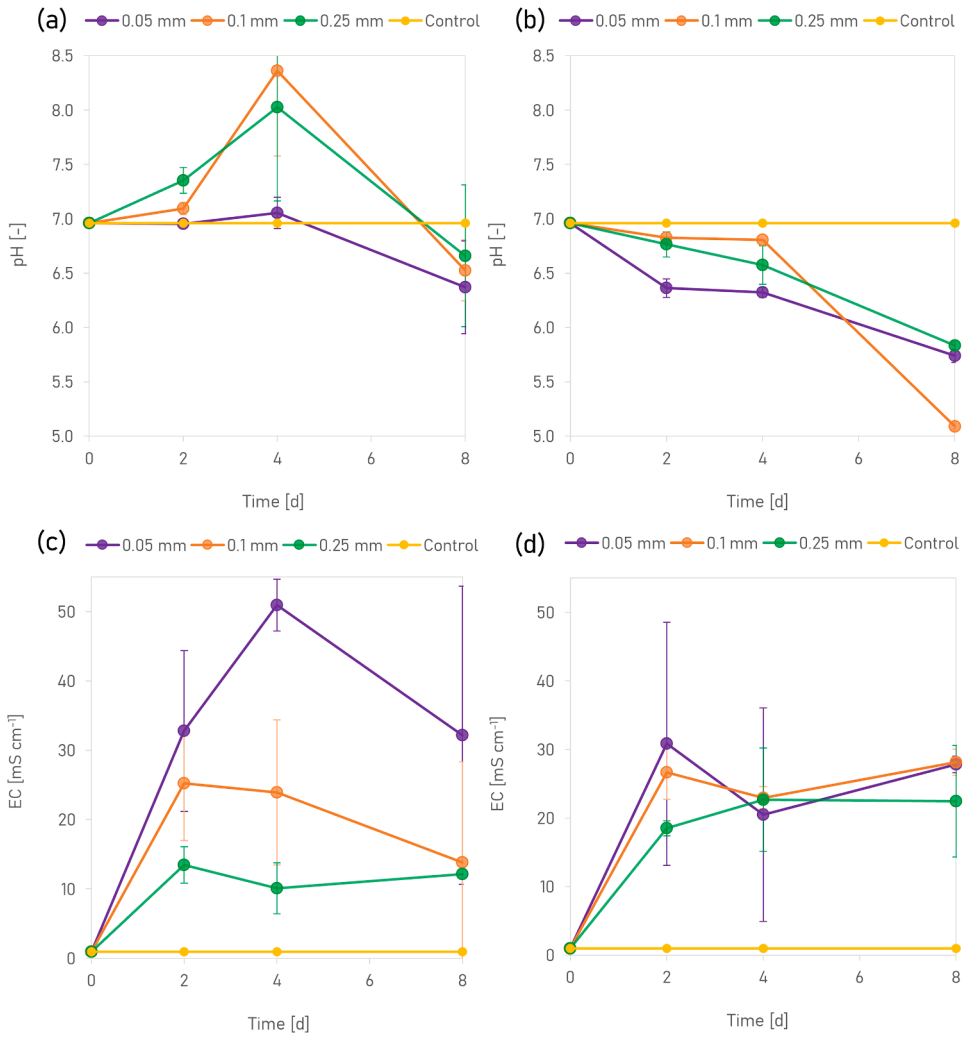

Fig. 3. Change in pH and EC of Milli-Q water over time in the presence of PLLA films of different thicknesses (0.05, 0.1, and 0.25 mm) at 20 °C (a, c) and 45 °C (b, d).

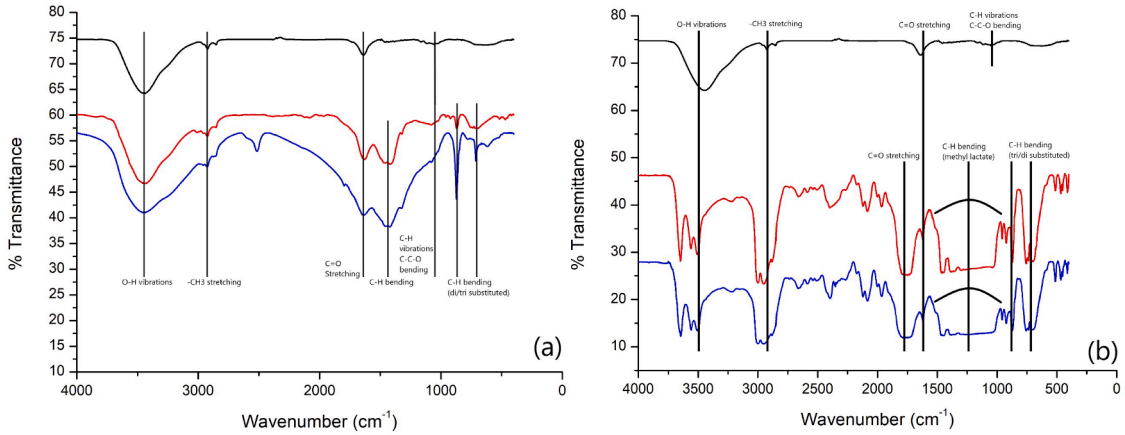

Fig. 4. FTIR spectra of 0.05 mm PLLA film collected after (a) two days of storage in $Ca(OH)_2$-dosed fresh urine and (b) eight days of storage in Milli-Q water. The black line shows the spectrum for virgin PLLA, while the red and blue lines show the spectra for PLLA film stored at 20 °C and 45 °C, respectively.

which results in a decrease in pH (Zaaba and Jaafar, 2020). This explains the decline in pH of alkalised fresh urine that occurred over time in the present study (Fig. 2). Since hydrolysis is self-catalysed, the rate of degradation increased with time, as did the subsequent drop in pH (Fig. 2).

Degradation of polymers into oligomers and monomers is always accompanied by reductions in chain length and molecular weight (M_n, M_w *and* M_p) (Hakkarainen et al., 1996). During degradation of PLLA film in urine, molecular weights and chain length will continue to decrease until all the oligomers present have been converted to lactic acid. The GPC results obtained in the present study indicated that this occurred. After two days, the 0.05 mm thick film in $Ca(OH)_2$-dosed fresh urine

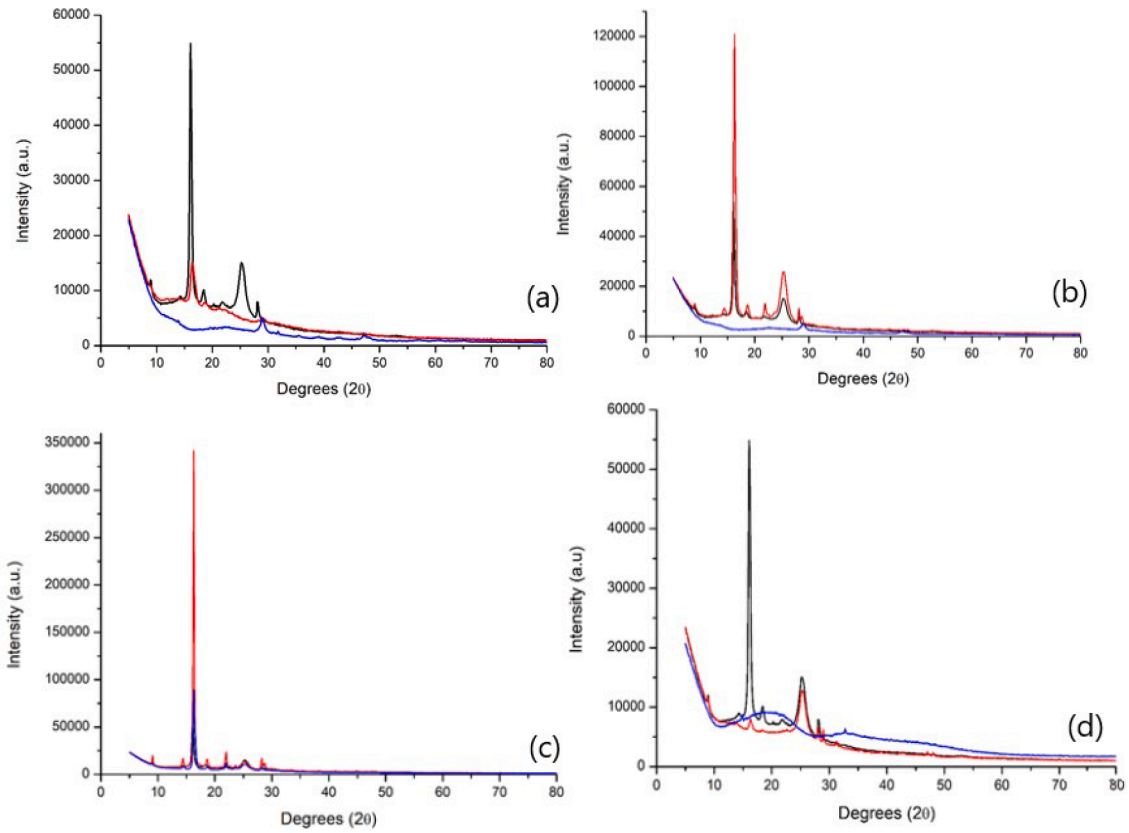

Fig. 5. PXRD spectra of PLLA films of (a) 0.05 mm (b) 0.1 mm and (c) 0.25 mm thickness collected after two days of storage in $Ca(OH)_2$-dosed fresh urine and (d) PXRD spectra of 0.05 mm PLLA film collected after eight days of storage in Milli-Q water. The black line shows the spectrum for virgin PLLA, while the red and blue lines show the spectra for PLLA stored at 20 °C and 45 °C, respectively.

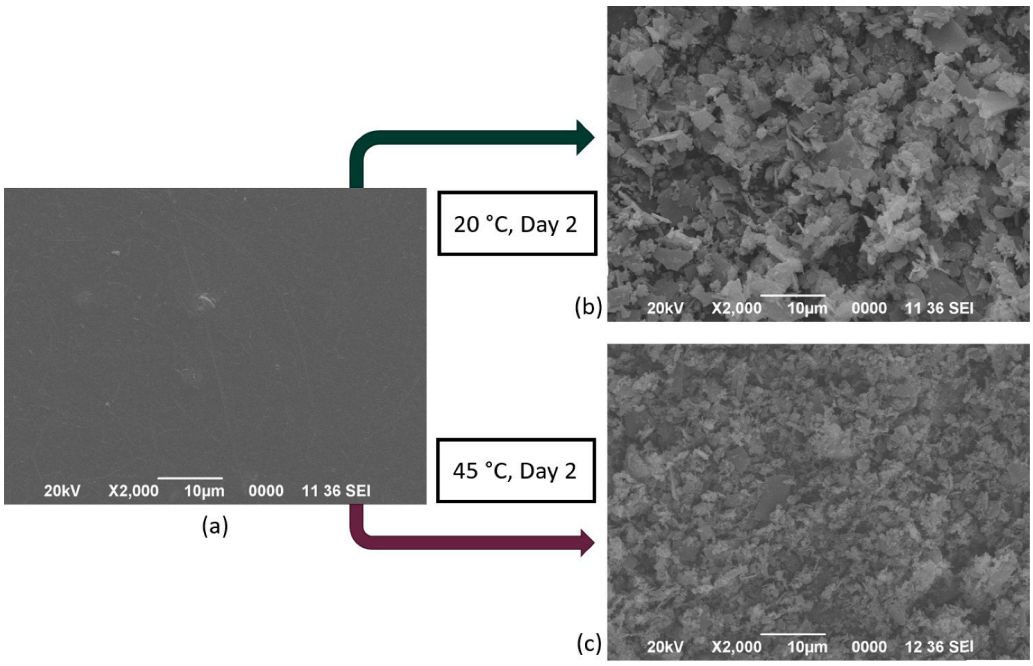

Fig. 6. SEM images at 2000x magnification of (a) virgin PLLA film and (b,c) PLLA film stored in $Ca(OH)_2$-dosed fresh urine at (b) 20 °C and (c) 45 °C.

stored at 20 °C showed a 3.8% reduction in M_n, 19.5% reduction in M_w and 16.3% reduction in M_p. At 45 °C, the 0.05 mm film showed a 25% reduction in M_n, 13.3% reduction in M_w and 25.4% reduction in M_p.

In the presence of cations or bases, degradation of PLLA results in the formation of lactate salts, L-lactides and L-methyl lactate (Avgoustakis, 2005). In the presence of $Ca(OH)_2$, L-methyl lactate can form calcium lactate (CaL_2) (Alberti et al., 2020). Formation of CaL_2 likely occurred in our study because we made the following three observations from the

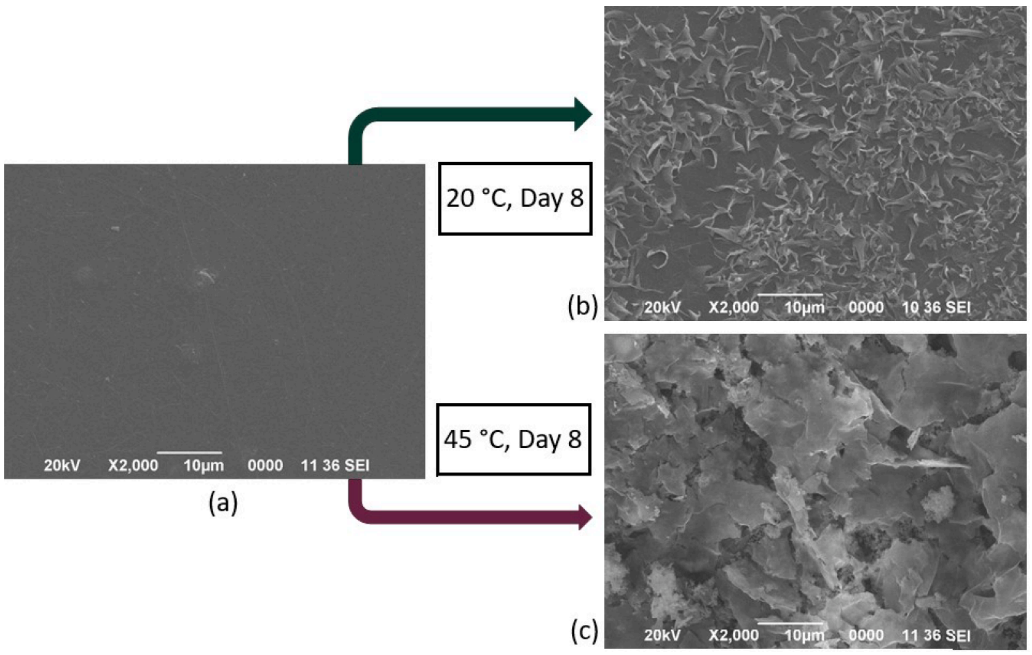

Fig. 7. SEM images at 2000x magnification of (a) virgin PLLA and (b,c) PLLA film stored in Milli-Q water at (b) 20 °C and (c) 45 °C.

results obtained during characterisation of the polymer films. First, the concentration of Ca in the urine decreased significantly over time, e.g. there was a 43% reduction in the Ca concentration in urine containing 0.05 mm PLLA thick film at 20 °C (Fig. S2 in SI). Second, the chemical composition of the films measured during EDX analysis showed an increase in Ca content (Fig. S3 in SI), whereas no Ca was detected in PLLA films that were stored in Milli-Q water (Fig. S4 in SI). The SEM images also showed that PLLA films in urine contained a large number of solid deposits and precipitates (Fig. 6), whereas no deposits were formed in PLLA films in Milli-Q water (Fig. 7). Third, formation of lactate salts was confirmed in the FTIR spectra of PLLA films that were stored in Ca $(OH)_2$-dosed urine (Fig. 4a), where broad peaks appeared within the range characteristic of the lactate group (1500–1300 cm^{-1} and 800–650 cm^{-1}; (Cabrini et al., 2015)). These peaks were absent in the FTIR spectra of the PLLA films kept in Milli-Q water (Fig. 4b).

4.2. Effect of temperature on PLLA degradation

The higher the temperature, the higher the molecular motion of PLLA chains and hydroxide ions, which leads to more frequent collisions between these and a faster hydrolysis reaction (Tsuji and Nakahara, 2002). This was evident from the images of the PLLA films taken on the different sampling days in this study (Fig. 1). On day 4, the degradation of all films was greater at 45 °C than at 20 °C, and the pH decrease was significantly greater in urine kept at 45 °C than in urine kept at 20 °C ($p < 0.001$). This could only have occurred if more had PLLA degraded, as there was no change in the pH of urine controls (without PLLA) kept at 45 °C (Fig. 2). The reduction in molecular weights was also greater for PLLA films kept at 45 °C compared with 20 °C.

Virgin PLLA is semicrystalline and its degradation occurs in two stages (Fischer et al., 1973). The first stage starts with cleavage of ester bonds in amorphous regions of the polymer. These bonds are cleaved more rapidly because they are more prone to hydrolysis, as it is relatively easy for water molecules to penetrate amorphous regions and attack ester bonds when there is no distinct pattern or orientation of the polymer chains. Thus, on hydrolysis, the crystallinity of PLLA films increases as the amorphous region has already broken down. The second phase starts with cleavage of ester bonds in the crystalline phase of PLLA films. The degradation starts from the edges, moving towards the core of the film. The PXRD results obtained in this study are in line with this explanation. For the 0.05 mm and 0.1 mm PLLA films, the number of crystalline peaks decreased at 45 °C ($n = 3$ for 0.05 mm, $n = 6$ for 0.1 mm) compared with 20 °C ($n = 4$ for 0.05 mm, $n = 7$ for 0.1 mm) (Fig. 5). Degree of crystallinity also increased for the 0.1 mm (59.3%) and 0.25 mm thick films (45.7%) at 20 °C compared with the virgin film (44.6%) (Fig. S5 in SI). There was a decline in crystallinity of the 0.1 mm (38.9%) and 0.25 mm thick films (43.3%) at 45 °C, which is an indication that the amorphous regions of those films were completely degraded and that degradation of the crystalline phase had started. These results confirm that the rate of degradation of PLLA increases with increasing urine temperature.

4.3. Effect of pH and matrix on the degradation of PLLA: urine versus water

In neutral and acidic media, bulk erosion of PLLA occurs via a slow chain end-scission pathway (Teixeira et al., 2021). Degradation of PLLA is faster in alkaline medium because it undergoes surface erosion via random ester bond cleavage, which in turn accelerates the overall degradation process (Karamanlioglu et al., 2017). In alkaline conditions, the hydroxide ions also act as catalysts and increase the rate of degradation of PLLA (Tsuji and Nakahara, 2002). The hydroxide ion acts as a nucleophile, attacking the carbonyl carbon of the ester group, which initiates cleavage of the bond. The effect of this was visible in the images of films taken on the different sampling days in this study (Fig. 1). The films in Ca(OH)2-dosed fresh urine showed more visible signs of degradation than their counterparts in Milli-Q water.

Several new peaks that were absent in the FTIR spectra of the control film appeared in the FTIR spectra of the 0.05 mm PLLA films kept in Milli-Q water for eight days (Fig. 4b). A new broad peak occurred from 1500 to 1000 cm^{-1}, a region associated with the carboxylate (-COO-) group formed due to hydrolytic scission of the ester bond (Granados-Hernández et al., 2018). The formation of two peaks around 3600–3500 cm^{-1} suggests that the number of –COOH and –OH bonds increased and indicates that lactic acid was formed (Choksi and Desai, 2017). The increase in intensity of the peaks around 3000 cm^{-1} indicates that more methyl groups (methyl lactide) were also present.

Degree of crystallinity (X_c) increased in the PLLA film stored in Milli-

Q water at 45 °C for eight days (Table S1 in SI), but did not change in the PLLA film stored in Milli-Q water at 20 °C. This implies that only the amorphous phase of the film at 45 °C had started to degrade (Duarah et al., 2020). It reflected the fact that the rate of degradation of PLLA was faster at higher pH, as the second phase of degradation had started for similar samples in $Ca(OH)_2$-dosed fresh urine.

The molecular weights (M_n and M_w) of the film stored in Milli-Q water increased relative to those of the virgin film (Table S2 in SI). A possible explanation for this is that calculation of both M_w and M_n is biased towards larger fragments, and that smaller fragments degrade faster than larger fragments. If degradation of fractions with higher molecular weights is much slower than degradation of fractions with smaller fragments, then molecular weight can increase (Makino et al., 1986).

5. Conclusions

Potential risks from handling alkaline earth hydroxides when producing organo-mineral fertiliser from source-separated human urine could be avoided by enclosing these chemicals in bio-derived polymers such as PLLA. This study showed that pH, temperature and solution matrix (water or urine) can significantly influence PLLA degradation rate and degradation pathway. The degradation rate was faster at higher temperature (45 °C versus 20 °C), at higher pH ($Ca(OH)_2$-dosed alkalised fresh urine versus Milli-Q water) and for thin PLLA films (0.05 mm) than for thicker films (0.1 and 0.25 mm). Solution matrix also affected the degradation products formed, where L-CaL_2, L-lactides and lactic acid were the likely degradation products in $Ca(OH)_2$-dosed fresh urine, whereas in Milli-Q water L-lactides and lactic acid were likely formed. These degradation products of PLLA would not directly affect recovery of plant-essential nutrients (such as urea) present in human urine further treated with PLLA-encased chemicals to produce concentrated fertiliser. However, their formation would result in a pH decrease in urine and this could reactivate urease and pose a potential risk of enzymatic urea hydrolysis, which must be considered in practice. Overall, this study demonstrates promise for use of biopolymers to encase chemicals in treatment of source-separated urine.

CRediT authorship contribution statement

Anuron Deka: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Prithvi Simha:** Conceptualization, Methodology, Writing – review & editing, Data curation, Formal analysis, Supervision. **Liudmila Nazarova:** Investigation, Data curation. **Rupam Kataki:** Supervision, Writing – review & editing. **Björn Vinnerås:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Colleagues at SLU are acknowledged for donating urine. The study was funded by a grant from the Swedish Research Council Vetenskapsrådet for the project NECESSITY (Nästa generations källsorterande avloppssystem) (Grant number 2022-04188). Sophisticated Analytical Instrumentation Centre (SAIC) at Tezpur University is thanked for help with GPC, FTIR, PXRD, SEM and EDX analyses.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.resconrec.2023.107202.

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