


## PAPER

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# Speeding it up: dual effects of biostimulants and iron on the biodegradation of poly(lactic acid) at mesophilic conditions†

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Plastic pollution presents a growing concern, and various solutions have been proposed to address it. One such solution involves the development of new plastics that match the properties of traditional polymers while exhibiting enhanced biodegradability when disposed of in a suitable environment. Poly(lactic acid) (PLA) is a biobased, compostable polymer known for its low environmental impact and ability to break down into harmless components within a specified timeframe. However, its degradation in industrial composting facilities poses challenges, and it cannot degrade in home composting. In this study, we investigated the biodegradability of PLA within a biostimulated compost matrix at mesophilic conditions (37 °C) over 180 days. The compost environment was enhanced with Fe<sub>3</sub>O<sub>4</sub> nanopowder, skim milk, gelatin, and ethyl lactate, individually and in combination, to target different stages of the PLA biodegradation process. We monitored key indicators, CO<sub>2</sub> evolution, number average molecular weight, and crystallinity, to assess the impact of the various biostimulants and iron. The results demonstrated that the most effective treatment for degrading PLA at mesophilic conditions was adding gelatin and Fe<sub>3</sub>O<sub>4</sub>. Gelatin accelerated PLA biodegradation by 25%, Fe<sub>3</sub>O<sub>4</sub> by 17%, and a combination of gelatin and Fe<sub>3</sub>O<sub>4</sub> by 30%. The effect of skim milk and ethyl lactate is also reported. This research introduces novel pathways to enhance PLA biodegradation in home composting scenarios, offering promising solutions to address the plastic pollution challenge.

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## Environmental significance

Plastics are omnipresent in daily life, particularly in food packaging applications, and are often released into the environment due to poorly managed waste disposal practices. It is crucial to consider alternative disposal options for biobased and compostable polymers like poly(lactic acid) (PLA), even on a household scale. Given the low temperatures typically encountered in home or backyard compost settings, the microorganisms present in compost or soil require an additional stimulus for effective decomposition. This study investigates the incorporation of various nutrients, including gelatin, skim milk, ethyl lactate, and iron nanopowder, to enhance the compost environment. The findings reveal that adding these compounds serve to biostimulate the compost environment, facilitating accelerated biodegradation of PLA that would otherwise take an extended period (over two years) to break down naturally. These results open opportunities for considering home composting as a viable alternative to industrial composting to dispose of PLA.

## 1. Introduction

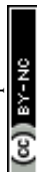
Poly(lactic acid) – PLA – is a biobased, biodegradable polymer that is an eco-friendly substitute for fossil-based polymers for a circular and sustainable economy. PLA is derived from natural resources and quickly degrades and breaks down in suitable waste management environments, such as industrial composting. PLA is a versatile biobased polymer because it has properties comparable to conventional polymers, is cost-effective, and can provide an additional disposal scenario, namely composting, at the end of a contaminated package life

cycle.<sup>1</sup> These benefits, combined with the growing consumer awareness of plastic littering and white pollution, have propelled PLA to the forefront as the face of the green, biobased plastic movement.<sup>2</sup>

Although PLA is industrially compostable, its practical and rapid biodegradation depends on reaching temperatures in the thermophilic range, 45 to 60 °C, to undergo chemical hydrolysis and significantly reduce its molecular weight in a shorter period so that microorganisms present in the compost can use PLA oligomers as a food source. This constraint makes it difficult to degrade PLA at lower and ambient temperatures (*i.e.*, mesophilic range, 20 to 45 °C).<sup>3</sup> Chemical hydrolysis is a crucial step in the PLA degradation mechanism, involving the breakdown of high into low molecular-weight polymer chains, such as oligomers, dimers, and monomers, which are easily assimilated by

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microorganisms.<sup>4</sup> However, at the lower temperatures commonly encountered in the home or backyard composting environments (herein referred to as backyard composting), chemical hydrolysis proceeds at a prolonged pace.<sup>5</sup> Boosting the hydrolytic degradation of PLA, particularly in backyard composting, is extremely difficult due to the limitation of high temperature essential to activate the biodegradation process.<sup>3</sup>

Several research studies have included nanocomposites and metal compounds within the PLA matrix at lower concentrations to enhance its depolymerization and degradation.<sup>6–8</sup> Including these metal oxides and nanoparticles significantly alters physical properties, such as the number average molecular weight ( $M_n$ ), melting temperature ( $T_m$ ), and glass transition temperature ( $T_g$ )<sup>9–11</sup> of the resulting PLA or PLA blends, and may not be practical for food contact or single-use PLA discarded packages.

Enzymatic hydrolysis involving the release of extracellular enzymes is also essential for PLA degradation when low molecular weight PLA chains are available. PLA's degrading enzymes include the hydrolase class of enzymes, primarily proteases.<sup>3</sup> The serine proteases released by the microorganisms in response to the presence of amino acid compounds in compost have been shown to cleave the ester bonds in PLA.<sup>12,13</sup> So, the introduction of enzymes to compost may assist in the biodegradation of PLA.

Adding various components besides enzymes, such as nutrients, electron donor/acceptor compounds, or compounds essential to trigger the biochemical processes of microorganisms in the given environment, is termed biostimulation.<sup>14–16</sup> Biostimulation techniques to enhance PLA biodegradation have been reported.<sup>17</sup> However, most of these studies were conducted in restricted settings where PLA was the only carbon source, in liquid media with specific microbial strains, and usually at higher temperatures, which do not replicate the real conditions encountered during backyard composting.<sup>18,19</sup>

In a previous study, we assessed the change in PLA biodegradation at a mesophilic temperature of 37 °C in a solid composting matrix by adding biostimulants to the compost. The compounds selected as biostimulants in that study were screened through multiple criteria: anticipated to have no toxicity towards the microorganisms present, be able to degrade, be biodegradable in a suitable timeframe, be consumed, be cost-effective, and be readily available. The main goal was to introduce compounds enhancing the biotic degradation stage. Skim milk and gelatin were selected to trigger proteolytic activity. Ethyl lactate, belonging to the lactate esters family, was used to stimulate the lactate-utilizing microbial species in backyard compost. All these compounds effectively enhanced the biodegradation of PLA in simulated backyard composting by at least 15%, as determined by the accelerated reduction of  $M_n$ .<sup>20</sup>

In this study, we focused on evaluating the effect of adding a metal compound to catalyze the chemical hydrolysis of PLA and combine that with our previously work demonstrated biotic enhancement. Metals can be added externally to the compost media rather than within PLA for humification purposes, as previously reported<sup>21,22</sup> and screening through the earlier

criteria. Table 1 presents the permissible limits of metal compounds as derived from regulatory standards for heavy metals in agricultural soils ( $\text{mg kg}^{-1}$ ),<sup>23</sup> since the resulting amended compost may be applied to agricultural land, lawns, or home gardens. The primary standards include EEA 2007,<sup>24</sup> TMS 2007,<sup>25</sup> BPI 2021,<sup>26</sup> GB 15168-2018,<sup>27</sup> OMOE 2011,<sup>28</sup> and NZME 2012.<sup>29</sup>

Considering the criteria mentioned before and the information from Table 1 only a few metals could be selected for further consideration due to limitations placed by permissible limits. The selected compounds were further scrutinized (Table 2) for their antibacterial properties, as reported in the literature, to evaluate their use directly in compost.

Table 2 indicates that iron was an acceptable compound that could be used to target chemical hydrolysis. It was demonstrated that introduction of a Lewis acid,  $\text{FeCl}_3$ , can speed up the hydrolysis of PLA in an alkali solution.<sup>38</sup> However, due to chlorine's antimicrobial behavior, it was impossible to introduce  $\text{FeCl}_3$  into compost. Alternatively, iron oxides, such as  $\text{FeO}$ ,  $\text{FeO}_2$ ,  $\text{Fe}_3\text{O}_4$ , or  $\text{Fe}_2\text{O}_3$ , could be the primary option and are in a form that is not toxic to the microbes in the compost.<sup>32,33</sup> Several of these iron forms are present in soil worldwide.<sup>39</sup>

Thus, this study aimed to investigate the effectiveness of biostimulating the compost environment with compounds that may be able to enhance the chemical hydrolysis ( $\text{Fe}_3\text{O}_4$ ), the enzymatic degradation (skim milk and gelatin), and electroconductivity (ethyl lactate as electron donor compound) during the aerobic biodegradation of PLA in compost at mesophilic conditions. Differences in  $\text{CO}_2$  evolution, changes in  $M_n$ , and the crystallinity ( $X_c$ ) of PLA degradation with and without biostimulants were monitored to account for the activity of biostimulants in compost.

## 2. Materials & methods

### 2.1. Materials

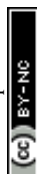
PLA Ingeo™ 2003D resin, with L-lactic acid content of 96%, was obtained from NatureWorks LLC (Minnetonka, MN, USA). Iron oxide nano-powder ( $\text{Fe}_3\text{O}_4$ ) was obtained from US Research Nanomaterials, Inc. (Houston, TX, USA). Skim milk powder was procured from a local store (Walmart, Lansing, MI, USA). Gelatin of the brand McCormick & Co. (Hunt Valley, MD, USA) was purchased on Amazon LLC. Ethyl lactate was procured from Sigma Aldrich™ (St. Louis, MO, USA).

### 2.2. Characterization of PLA and the biostimulants

The carbon, hydrogen, and nitrogen compositions of PLA resin, skim milk, gelatin, and ethyl lactate were determined using elemental analysis, (CHNS/O Elemental Analyzer, PerkinElmer 2400 Series II) (Shelton, CT, USA), and are presented in Table 3.

### 2.3. Biodegradation test in compost

The biodegradation of PLA and the effectiveness of introducing biostimulants in compost on the degradation of PLA were evaluated under aerobic mesophilic conditions using a direct measurement respirometric (DMR) system.<sup>40–42</sup> The system



**Table 1** Critical limits of heavy metals in agricultural soils (mg kg<sup>-1</sup>), adapted from ref. 23

Country	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Australia	20	3	50	100	1	60	300	200
Canada	20	3	250	150	0.8	100	200	500
China	20–40	0.3–0.6	150–300	50–200	0.5–3.4	60–190	70–240	200–300
Germany	50	5	500	200	5	200	1000	600
Tanzania	1	1	100	200	2	100	200	150
Netherlands	76	13	180	190	36	100	530	720
New Zealand	17	3	290	>10 <sup>4</sup>	200	N/A	160	N/A
UK	43	1.8	N/A	N/A	26	230	N/A	N/A
US	6.5	1.5	105	50	0.4	31	75	250

**Table 2** List of elements screened for their antimicrobial properties

Element	Antibacterial nature	References
Zn	Yes	30 and 31
Fe	No	30, 32 and 33
Cu	Yes	30
Ni	Yes	30
Ti	Yes	34 and 35
Cl	Yes	30 and 36
Ag	Yes	36 and 37

**Table 3** Carbon, hydrogen, and nitrogen content (percentage by weight) of the tested materials

Material	% carbon	% hydrogen	% nitrogen
PLA	49.7 ± 0.2	5.7 ± 0.0	0.0 ± 0.0
Skim milk	41.3 ± 0.1	6.3 ± 0.0	5.7 ± 0.1
Gelatin	44.8 ± 0.3	7.0 ± 0.1	16.4 ± 0.1
Ethyl lactate	31.9 ± 1.0	5.5 ± 0.3	0.0 ± 0.0

included a non-dispersive infrared gas analyzer (NDIR) (Li-COR<sup>®</sup> LI-820, Lincoln, NE, USA) which measures the CO<sub>2</sub> concentration. The DMR system chamber was maintained at temperature of 37 ± 2 °C and relative humidity (RH) of 50% ± 5%. A flow rate of CO<sub>2</sub>-free air (concentration <30 ppm) to establish a low baseline) was controlled at 40 ± 2 cm<sup>3</sup> min<sup>-1</sup>. Detailed information about the DMR equipment can be found in other source.<sup>43</sup>

The mature compost obtained from MSU composting facility was sifted using a 10 mm screen to get rid of any huge debris or chunks present, and then conditioned at 37 °C until use. Deionized water was used to adjust the moisture content of compost to 50%. Saturated vermiculite (Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA), was mixed with compost in 1:4 parts (dry weight). Samples of the resulting compost-vermiculite mixture were later sent to the Soil and Plant Laboratory at MSU for determining the physicochemical parameters. Data regarding the nutrient analysis is presented in Table S1 in the ESI.<sup>†</sup> The bioreactors were packed with 400 g of compost, 8 g of PLA sample and the selected biostimulant, and all the samples were tested in triplicate. Blank (only compost) and positive control (cellulose) were also tested.

**2.3.1 Biostimulation.** Fe<sub>3</sub>O<sub>4</sub> nanopowder at 17 g was mixed with the compost matrix in the bioreactor containing PLA to target the hydrolysis step. Ethyl lactate, skim milk, and gelatin were added at 8 g individually per bioreactor and mixed with the compost matrix thoroughly with the PLA pellets in it to ensure uniform distribution.

## 2.4. Size exclusion chromatography (SEC)

The *M<sub>n</sub>* and molecular weight distribution (MWD) of PLA for the control and each treatment with the biostimulants were measured using SEC (Waters Corp., Milford, MA, USA) as described elsewhere.<sup>4</sup> PLA samples weighing approximately 10 mg were retrieved at predetermined time intervals and dissolved in 5 mL of tetrahydrofuran (THF) solvent. A temperature of 35 °C and a 1 mL min<sup>-1</sup> flow rate were maintained during testing. The Mark-Houwink constants of *K* = 0.000174 dL g<sup>-1</sup> and *α* = 0.736 were used to determine *M<sub>n</sub>*, *M<sub>w</sub>*, and MWD of the PLA samples. Data analysis was carried out using Waters Breeze<sup>™</sup>2 software.

## 2.5. Differential scanning calorimetry (DSC)

A DSC model Q100 (TA Instruments, New Castle, DE, USA), was used to determine the *T<sub>g</sub>*, *T<sub>m</sub>*, crystallization temperature (*T<sub>c</sub>*), and crystallinity (*X<sub>c</sub>*) for the PLA samples retrieved from the regular and biostimulated compost. PLA samples weighing between 5–10 mg were packed in aluminum pans and cooled down to -5 °C and then subjected to a heating cycle to reach 210 °C at a ramp rate of 10 °C min<sup>-1</sup>. This helped to evaluate the evolution of *X<sub>c</sub>*. The cooling was achieved using a nitrogen cooling system that maintained the purge flow rate at 70 mL min<sup>-1</sup>. The degree of crystallinity was estimated using equation:<sup>1</sup>

$$\chi_c \% = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0} \times 100 \quad (1)$$

where  $\Delta H_m$  is the heat of fusion,  $\Delta H_c$  is cold crystallization enthalpy, and  $\Delta H_m^0$  is the heat of fusion for 100% crystalline pure PLA (93 J g<sup>-1</sup>).<sup>45</sup>

## 3. Results & discussion

The CO<sub>2</sub> evolution of PLA samples in compost and biostimulated with Fe<sub>3</sub>O<sub>4</sub> nanopowder and the combination of



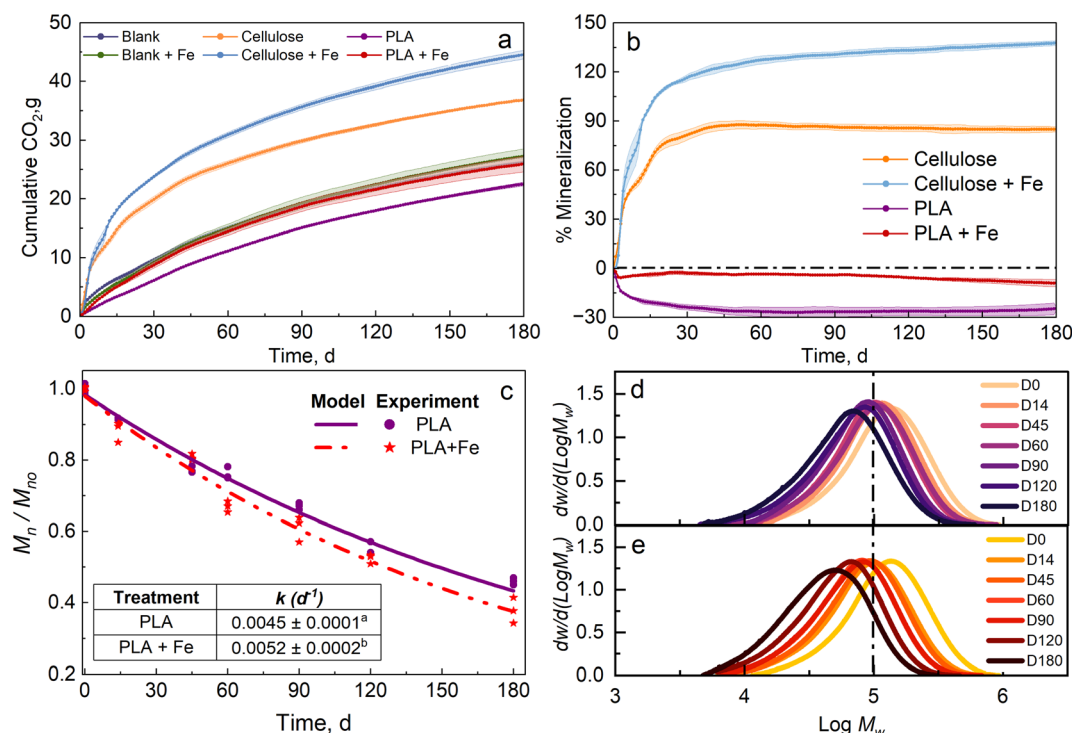
gelatin, skim milk, and ethyl lactate without and with  $\text{Fe}_3\text{O}_4$  nanopowder was tracked over a test duration of 180 days at mesophilic conditions (37 °C). Samples were retrieved at specific times to evaluate the  $M_n$  and  $X_c$  evolution and determine the kinetic degradation rate.

### 3.1. Effect of $\text{Fe}_3\text{O}_4$ on cellulose and PLA degradation

Fig. 1a and b show the  $\text{CO}_2$  evolution and mineralization, respectively, of cellulose, cellulose in compost biostimulated with  $\text{Fe}_3\text{O}_4$  nanopowder (hereafter referred to as cellulose + Fe), PLA, and PLA in compost biostimulated with  $\text{Fe}_3\text{O}_4$  nanopowder (hereafter referred to as PLA + Fe) at 37 °C. Control compost (blank) evolved around 26.1 g of  $\text{CO}_2$ , and compost biostimulated with  $\text{Fe}_3\text{O}_4$  nanopowder (hereafter referred to as blank + Fe) evolved around 27.2 g of  $\text{CO}_2$ . The minor difference can be attributed to the difference in weight of the compost introduced in the bioreactor, and the levels are not significantly different ( $p > 0.05$ ). Cellulose in compost evolved around 36.8 g of  $\text{CO}_2$ , and reached a mineralization of 87.7%, whereas cellulose + Fe evolved around 44.6 g of  $\text{CO}_2$ , depicting a mineralization of 137.7%. The primary reason for the priming effect (>100% mineralization) observed in the case of cellulose + Fe may be attributed to the over-deterioration of the endemic carbon present in the compost.<sup>4</sup> PLA in compost showed around 22.5 g of  $\text{CO}_2$  evolution, whereas blank produced around 26.1 g of  $\text{CO}_2$ , implying that no carbon from PLA was degraded. The negative mineralization values indicate more  $\text{CO}_2$  production in

the blank bioreactors than in the PLA bioreactors. PLA offers a physical hydrophobic barrier to water, making it difficult for the microorganisms to utilize it as a carbon source at the beginning of the test and until day 180 due to the low contribution of chemical hydrolysis at mesophilic temperatures. Overall, we did not see any mineralization in PLA due to the low temperature of 37 °C, which is insufficient to activate the biotic stage. These values are very low compared to the degradation of PLA at thermophilic temperatures<sup>4,44</sup> but are similar to earlier reported values.<sup>5,46</sup>

In the case of PLA and PLA + Fe, we observed the difference in the  $\text{CO}_2$  evolution right from the start of the test. PLA + Fe evolved around 25.9 g of  $\text{CO}_2$  compared with 22.5 g of  $\text{CO}_2$  evolution for PLA. Similar to the case of cellulose + Fe, the reason for the difference in the  $\text{CO}_2$  evolution for PLA can be attributed to the presence of  $\text{Fe}_3\text{O}_4$ .  $\text{Fe}_3\text{O}_4$  promotes microbial activity in the soil and enhances the nitrification potential.<sup>47</sup>  $\text{Fe}_3\text{O}_4$  is also known to induce changes by enhancing enzymatic activity and microbial growth.<sup>32</sup> This characteristic can be corroborated by the kinetic degradation rate ( $k$ ), as seen in Fig. 1c inset (PLA  $k = 0.0045 \pm 0.0001 \text{ d}^{-1}$  and PLA + Fe  $k = 0.0052 \pm 0.0002 \text{ d}^{-1}$ ). The significant difference can be credited to the presence of  $\text{Fe}_3\text{O}_4$ . Fig. 1d and e present the molecular weight distribution (MWD) for PLA in compost and compost biostimulated with  $\text{Fe}_3\text{O}_4$ . The peak amplitude for PLA in Fig. 1d remained approximately the same throughout the test duration. No broadening of the peak and negligible shift



**Fig. 1** Cumulative  $\text{CO}_2$  evolution (a) and mineralization (b) of blank, cellulose, PLA, blank +  $\text{Fe}_3\text{O}_4$  (blank + Fe), cellulose +  $\text{Fe}_3\text{O}_4$  (cellulose + Fe), PLA +  $\text{Fe}_3\text{O}_4$  (PLA + Fe) in compost at 37 °C. (c) Represents the normalized  $M_n$  reduction as a function of time for PLA in control compost and compost biostimulated with  $\text{Fe}_3\text{O}_4$ . The experimental data was fitted using a first-order reaction of the form  $M_n/M_{n0} = e^{-kt}$ , where  $M_{n0}$  is the initial  $M_n$ ,  $k$  is the rate constant, and  $t$  is the time. The inset shows the  $k$ -fitted values; values with different lowercase letters are statistically different ( $\alpha = 0.05$ , Tukey–Kramer test). (d) and (e) show the MWD of PLA in compost and compost biostimulated with  $\text{Fe}_3\text{O}_4$ , respectively.





indicates that the chemical hydrolysis proceeded slowly at a mesophilic temperature of 37 °C. In contrast, for PLA + Fe, a significant shift to low  $M_n$  and broadening of the peak are observed in Fig. 1e, depicting the reduction in  $M_n$  as shown in Fig. 1c.

### 3.2. $M_n$ reduction for PLA with biostimulants in compost

PLA samples were retrieved separately from the control compost and compost biostimulated with skim milk, gelatin, and ethyl lactate. Fig. 2 shows the reduction in  $M_n$  of PLA and biostimulated PLA tracked until the end of the test (180 days). A significant difference was observed in the kinetic reduction rates of PLA with biostimulant treatment compared with no biostimulation treatment. The  $\text{CO}_2$  evolution values for PLA, PLA + skim milk, PLA + gelatin, and PLA + ethyl lactate are provided in Section S1 of the ESI.†

Skim milk was added to the compost to induce protease activity by the microbes.<sup>48–50</sup> Serine protease (3.4.21.112) belongs to peptidases and is the class of extracellular enzymes able to hydrolyze the peptide bonds linked to amino acids in the protein structure. Skim milk is composed of different proteins, such as lactose, casein, and whey protein, making it a good precursor for enzymatic activity, as mentioned earlier. Other researchers have previously used skim milk to demonstrate extracellular protease synthesis. The microorganisms present in compost secrete protease to hydrolyze the milk protein. This protease is used by microorganisms capable of PLA degradation to depolymerize PLA.<sup>51</sup> This increase in  $k$  can be deduced as a final reduction of around 75 days on the biodegradation time when PLA in compost biostimulated with skim milk reaches  $M_n \leq 10$  kDa at 420 days (Fig. 2). Microorganisms assimilate the

PLA  $n$ -mers at this stage, accelerating the biodegradation stage.<sup>44</sup> On the other hand, PLA needs at least 494 days to reach the same  $M_n$  – an effective 15% reduction of time.

Gelatin is composed of protein and amino acids, and is a precursor for protease activity.<sup>19,52–54</sup> The addition of gelatin to compost produced an acceleration of PLA with an enhancement of  $k$  translated to a final reduction of around 124 days when PLA is biostimulated with gelatin, reaching an  $M_n \leq 10$  kDa at 371 days compared with at least 494 days for PLA alone – an effective 25% reduction of time (Fig. 2).

Ethyl lactate, on the other hand, was used to stimulate the lactate utilizing microbes in the compost. Ethyl lactate undergoes hydrolysis to produce ethanol and lactate, where both can act as a constant long-term supply of hydrogen sources as electron donor compounds for reductive degradation and microbial redox process. Lactate has been shown to act as an electron donor compound in the case of anaerobic degradation.<sup>55,56</sup> Lactate has previously been used for anaerobic degradation, trichloroethane dichlorination, and sulfate reduction.<sup>57,58</sup> PLA + ethyl lactate resulted in a change in  $k$ , which can be translated to around 111 days when PLA is biostimulated with ethyl lactate, reaching an  $M_n \leq 10$  kDa at 384 days compared to PLA alone – an effective 22% reduction of time (Fig. 2).

A detailed discussion of the effect of gelatin, skim milk, and ethyl lactate on the biodegradation of PLA is provided in our previous work.<sup>20</sup>

### 3.3. Effect of $\text{Fe}_3\text{O}_4$ on cellulose and PLA degradation with gelatin as a biostimulant

Since gelatin resulted in the most significant  $M_n$  reduction for PLA, it was selected to discuss the effect of a combination of biostimulants (*i.e.*,  $\text{Fe}_3\text{O}_4$  nanopowder and gelatin). The data for  $\text{Fe}_3\text{O}_4$  nanopowder, skim milk, and ethyl lactate are provided in Section S3 of the ESI.† When  $\text{Fe}_3\text{O}_4$  nanopowder was introduced in compost amended with gelatin, the gelatin acted as a precursor for the protease enzyme secretion by the microbes present in the compost and the  $\text{Fe}_3\text{O}_4$  nanopowder provided the metal to catalyze the hydrolysis.

Fig. 3a and b show the  $\text{CO}_2$  evolution and mineralization of cellulose + Fe, gelatin + Fe, PLA + Fe, cellulose + gelatin + Fe (hereafter referred to as cell + gel + Fe), and PLA + gel + Fe in compost at 37 °C. Gelatin + Fe resulted in around 60.1 g of  $\text{CO}_2$  evolution and mineralization of 247.8% in 180 days. Gelatin was combined with Fe to target the chemical hydrolysis and enzymatic degradation steps. The  $\text{CO}_2$  evolution in this case (Cell + gel + Fe 1) was 64.6 g, which is as expected and higher compared with the individual values for cellulose + Fe and gelatin + Fe and a mineralization of 144.9%. To better understand the interaction and to account for the degradation behavior of cellulose in the presence of gelatin (Cell + gel + Fe 2), the mineralization value was estimated at 97.7% by subtracting the background signal from the bioreactor containing gelatin + Fe. This higher value indicates that cellulose degradation was not affected by gelatin, and both are used up by the microorganisms as carbon sources.

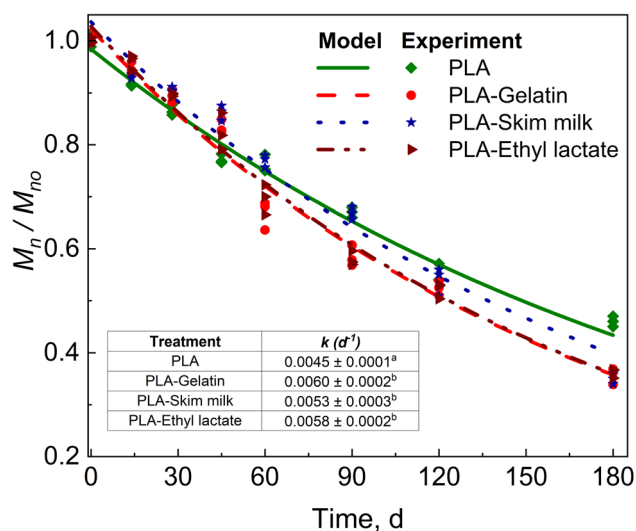
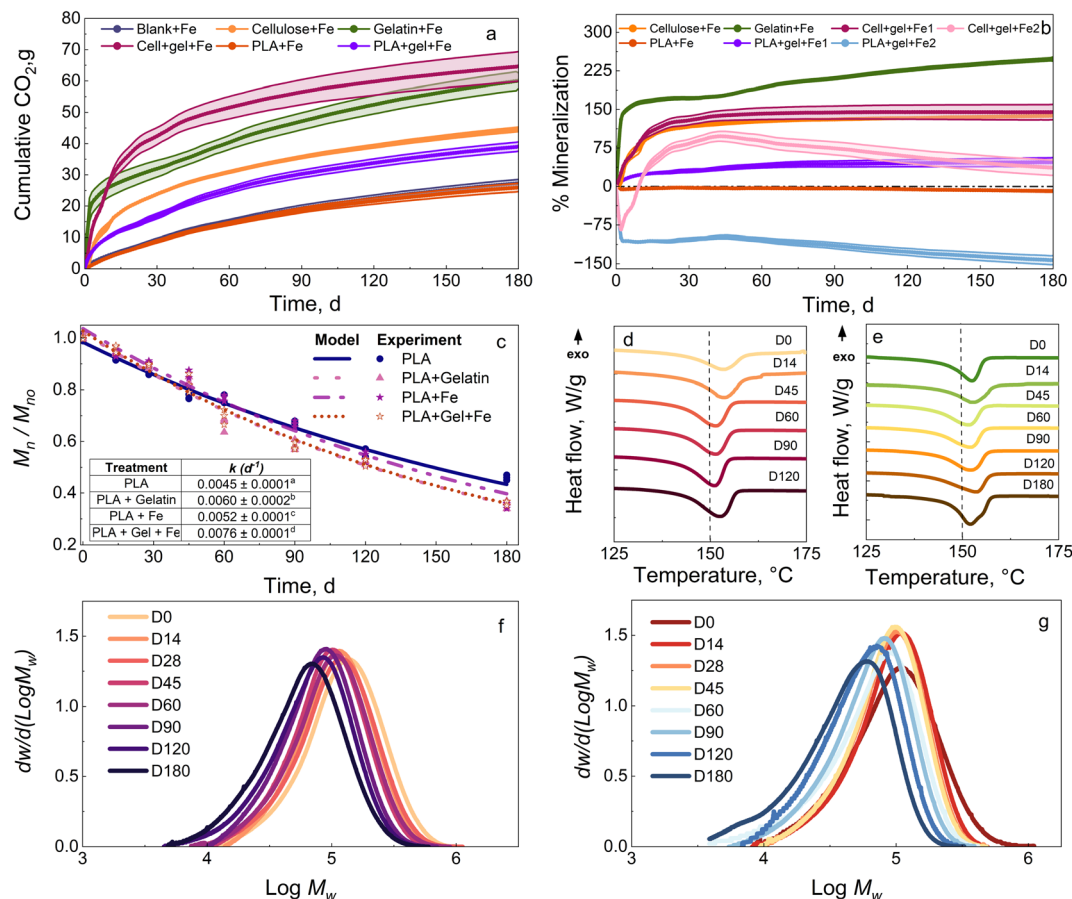


Fig. 2 Normalized  $M_n$  reduction as a function of time for PLA in control compost and compost biostimulated with skim milk, gelatin, and ethyl lactate. The experimental data was fitted using a first-order reaction of the form  $M_n/M_{no} = e^{(-kt)}$ , where  $M_{no}$  is the initial  $M_n$ ,  $k$  is the rate constant, and  $t$  is the time. The inset shows the  $k$ -fitted values; values with different lowercase letters are statistically different ( $\alpha = 0.05$ , Tukey–Kramer test).





**Fig. 3** Cumulative  $\text{CO}_2$  evolution (a) and mineralization (b) of blank + Fe, cellulose + Fe, PLA + Fe, gelatin + Fe, cellulose + gelatin + Fe (Cell + gel + Fe), PLA + gelatin + Fe (PLA + gel + Fe) in compost at  $37^{\circ}\text{C}$ . (c) Represents the normalized  $M_n$  reduction as a function of time for PLA in control compost and compost biostimulated with gelatin, and gelatin + Fe. The experimental data was fitted using a first-order reaction of the form  $M_n/M_{n0} = e^{(-kt)}$ , where  $M_{n0}$  is the initial  $M_n$ ,  $k$  is the rate constant, and  $t$  is the time. The inset shows the  $k$ -fitted values; values with different lowercase letters are statistically different ( $\alpha = 0.05$ , Tukey–Kramer test). (d) and (e) depict DSC thermograms for PLA + Fe and PLA + Fe in compost biostimulated with gelatin. (f) and (g) show the MWD of PLA + Fe in compost and compost biostimulated with gelatin.

To understand the influence of gelatin + Fe on PLA degradation, PLA was introduced in the compost amended with gelatin + Fe. The bioreactor containing both PLA and gelatin + Fe (PLA + gel + Fe 1) generated  $\text{CO}_2$  evolution of around 40.5 g and maximum mineralization of 56.5% by the end of the test. Improved mineralization was observed as opposed to no  $\text{CO}_2$  evolution for PLA alone without any biostimulation of the compost. The effect of gelatin + Fe on PLA degradation is calculated by plotting the mineralization of PLA + gel + Fe 2 (subtracting gelatin + Fe), as mentioned earlier. Negative mineralization does not necessarily indicate the absence of gelatin's protease activity in PLA's enzymatic degradation. This finding is validated by the significant difference observed in the kinetic rate of degradation for PLA in compost, with and without any biostimulation with gelatin, as seen in Fig. 3c inset (PLA + Fe  $k = 0.0052 \pm 0.0001$  and PLA + Gel + Fe  $k = 0.0076 \pm 0.0002$ ). The significant difference in the evolution of  $X_c$  from 28.3% to 31.4% for PLA, and from 28.3% to 40.9% for PLA biostimulated with gelatin + Fe, as seen in Fig. 3d and e, respectively, further shows the improvement in the enzymatic degradation of PLA due to the presence of gelatin + Fe. Gelatin

acts as a precursor for the microbes to release protease enzyme, aiding in the enzymatic degradation of PLA. The broadening and change in the amplitude of the MWD peaks through the test duration of 180 days, as seen in Fig. 3g for PLA in compost biostimulated with gelatin + Fe compared to PLA + Fe alone in Fig. 3f, show compelling evidence for enhanced degradation for PLA in the presence of gelatin and Fe. Iron is an essential micronutrient, necessary for life-sustaining processes, and plays a critical role in cell growth of microbes.<sup>59</sup> Iron also functions as a cofactor, promoting and increasing enzymatic activity. In addition, Iron plays an important role in various biological processes such as respiration, oxido-reduction mechanism, nitrogen fixation, tricarboxylic acid cycle, and electron transport.<sup>33</sup> S. He *et al.* demonstrated that a soil matrix amended by Fe nanoparticles shifted the microbial community composition and stimulated the metabolic activity of the bacterial community present by enhancing their growth rate.<sup>33</sup> The soil nitrification potential of the Fe-amended soil was improved from 10% to 19% compared to control soil, indicating that adding Fe aided in the increase of biomass capacity and eventually enhanced and boosted carbon cycling.



Zhang *et al.* further showed that the addition of Fe nanoparticles promoted the degradation of organic matter and amplified the dehydrogenase and urease activities, significantly improving the overall microbial activity and nitrogen mineralization.<sup>47</sup> Thus, adding Fe<sub>3</sub>O<sub>4</sub> nanopowder improves microbial metabolic activity, nitrification potential, and microbial population. When supplemented with the enzymatic activity associated with gelatin, these changes improve the degradation of PLA in compost compared with that of control PLA with no biostimulants present. Y. He *et al.* showed enhanced enzymatic and nitrification activity for organic matter degradation in a food-waste composting system due to compost amendment with Fe-carbon particles.<sup>60</sup> In addition, the bacterial and fungal communities exhibited significant improvement in the composting process due to the presence of iron, which can explain the improved PLA degradation found in the presence of Fe<sub>3</sub>O<sub>4</sub> nanopowder.

Overall, the changes obtained in *k* indicate a final reduction of around 148 days when PLA stimulated with gelatin reaches an *M<sub>n</sub>* ≤ 10 kDa at 346 days compared to at least 494 days for PLA – an effective 25% reduction of time. Similarly, time reductions of 17% or 30% were observed when PLA + Fe<sub>3</sub>O<sub>4</sub> or gelatin and Fe<sub>3</sub>O<sub>4</sub> nanopowder, respectively, when included in the compost.

As organic waste disposal is becoming more stringent worldwide and landfill disposal bans are increasing, several food industries are being impacted and need to find alternative end-of-life scenarios. The gelatin industry produces a large amount of sludge, resulting in a tremendous amount of waste generated, which includes collagen fibers, bone residues, and other inorganic materials. This gelatin sludge usually ends up in landfill or waste management treatment without any pretreatment and creates several problems such as water contamination, greenhouse gas emission, and health risks for local habitats.<sup>61</sup> So, this waste could be diverted from landfills and used to produce mature compost along with the organic fraction of municipal solid waste. Gelatin sludge is high in nitrogen and organic matter content and can act as a valuable plant nutrient.<sup>61</sup> The nutritional value of the compost generated from treating gelatin waste and Fe<sub>3</sub>O<sub>4</sub> nanopowder, considering the benefits mentioned earlier, complement each other to improve soil fertility once the compost is applied to agricultural land. The selection and combination of specific compounds can open a new route to accelerate the degradation of compostable polymers in industrial and home composting operations.

## 4. Conclusion

We investigated the role of different compounds—skim milk, gelatin, and ethyl lactate in combination with Fe<sub>3</sub>O<sub>4</sub> nanopowder—on PLA degradation at 37 °C by biostimulating the compost media. The different compounds were selected to target different stages of the biodegradation process. Without any biostimulant compounds, PLA continued to undergo a long abiotic lag phase affirming a slow hydrolysis phase, which was seen as a negative mineralization for the test duration of 180 days, whereas a boost in CO<sub>2</sub> evolution for PLA was observed in compost amended by Fe<sub>3</sub>O<sub>4</sub> nanopowder in combination with

gelatin, skim milk, or ethyl lactate. This observation was verified by the molecular weight change and crystallinity evolution. PLA biodegradation was accelerated by 30% to reach the biotic phase of *M<sub>n</sub>* ≤ 10 kDa by the addition of gelatin and Fe<sub>3</sub>O<sub>4</sub> nanopowder. The addition of biostimulants opens new avenues to improve PLA biodegradation in home composting conditions.

## Author contributions

P. M. and R. A. conceived and designed the experiments; P. M. performed the experiments, analyzed the data, and drafted the manuscript. The authors thank Anibal Bher for helping with the test, data acquisition, and discussion about biodegradation. R. A. provided mentoring and financial support. All authors have read, reviewed, and agreed to the published version of the manuscript.

## Conflicts of interest

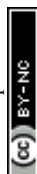
The authors declare no conflict of interest.

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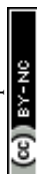
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