Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting Information for

Speeding it Up: Dual Effects of Biostimulants and Iron on the Biodegradation of Poly(lactic acid) at Mesophilic Conditions

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S1. Compost physicochemical characteristics

Some compost was collected and sent to the Soil and Plant Nutrient Laboratory at Michigan State University (East Lansing, MI, USA) to evaluate its physicochemical parameters (dry solids, volatile solids, and C/N ratio) as previously described elsewhere [1]. The physicochemical parameters are reported below in **Table S1**.

Table S1. Physicochemical parameters and total nutrient analysis of compost used in the biodegradation test.

Parameter	**Compost**
Dry solids, %	42.5
Volatile solids, %	41.7
pH	8.0
C/N ratio	10.1
Carbon, %	24.2
Nitrogen, %	2.42
Phosphorus, %	1.21
Potassium, %	3.15
Calcium, %	5.07
Magnesium, %	2.82
Sodium, %	0.58
Sulfur, %	0.58
Iron, ppm	9878
Zinc, ppm	480
Manganese, ppm	413

Copper, ppm	107
Boron, ppm	41
Aluminum, ppm	6751

S2. CO_2 evolution and mineralization of PLA in the presence of skim milk, gelatin, and ethyl lactate

Figure S1 a and b shows the CO_2 evolution and % mineralization of cellulose, skim milk, PLA, cellulose + skim milk, and PLA + skim milk. Cellulose reached mineralization of 87.7% whereas skim milk attained over 100 % of its carbon conversion over a period of 180 days. Since skim milk and cellulose are readily biodegradable and can be easily utilized as a carbon source by the microorganisms present in the compost, no lag phase was observed. Skim milk was added to the compost with the goal of inducing the protease activity of the microbes present. PLA shows similar CO_2 evolution when compared to blank (compost only). This indicates that PLA is still undergoing chemical hydrolysis and is yet to breakdown to M_n of 10k Da, where it can be assimilated by microorganisms.

Due to the compost amendment with skim milk, PLA (PLA + skm1) shows a mineralization of approximately 60%. In order to account for the effect of skim milk, a separate mineralization plot (PLA + skm2) is derived. Around 35% mineralization is observed for the same depicting that the enzymatic degradation of PLA is enhanced due to the presence of skim milk. This is corroborated by the molecular weight analysis (PLA $k = 0.0045 \pm 0.0001$ and PLA-skim milk $k = 0.0053 \pm 0.0003$).

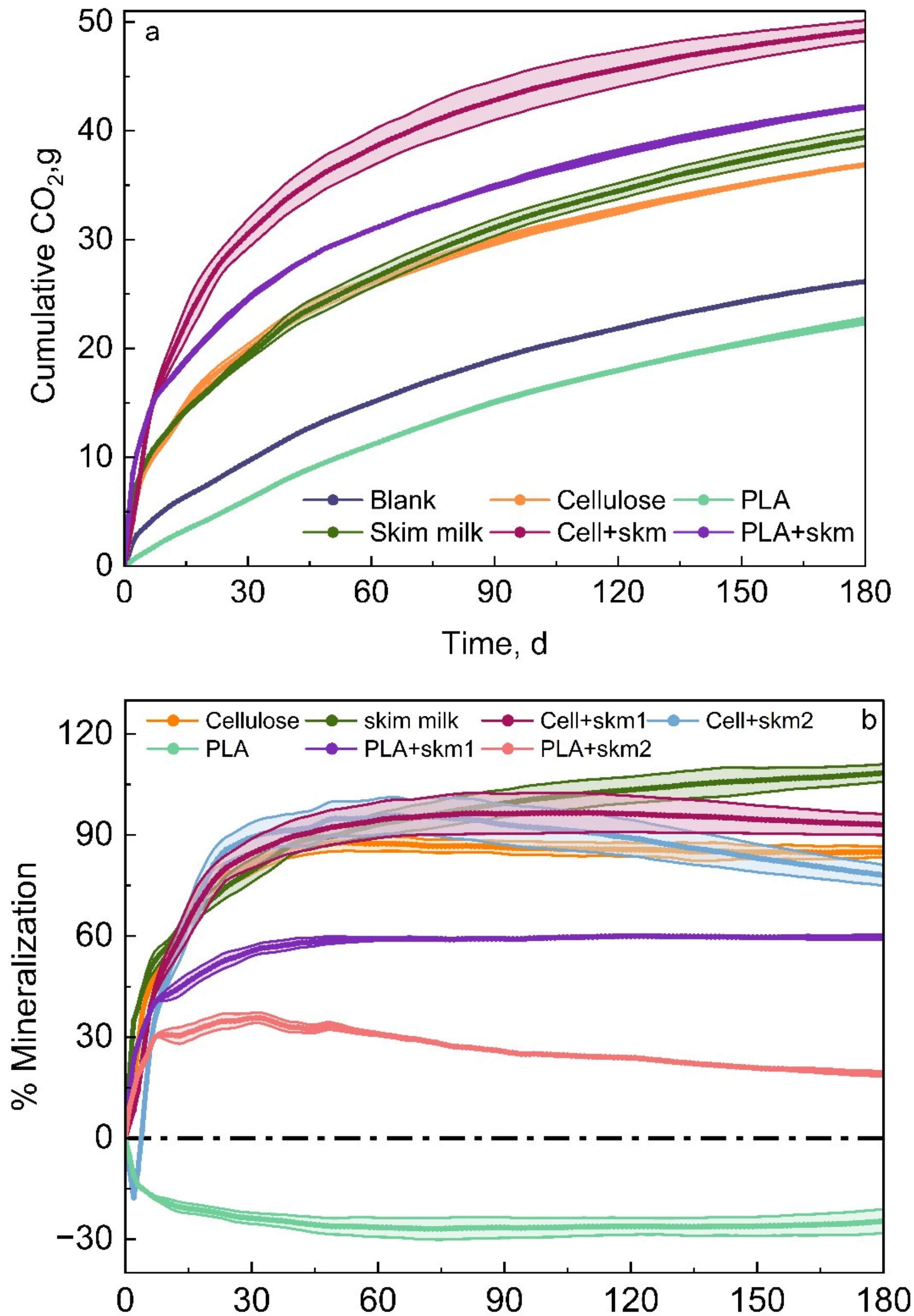

Figure S1. Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, skim milk, cellulose + skim milk (Cell+skm), PLA + skim milk (PLA+skm) in compost at 37°C.

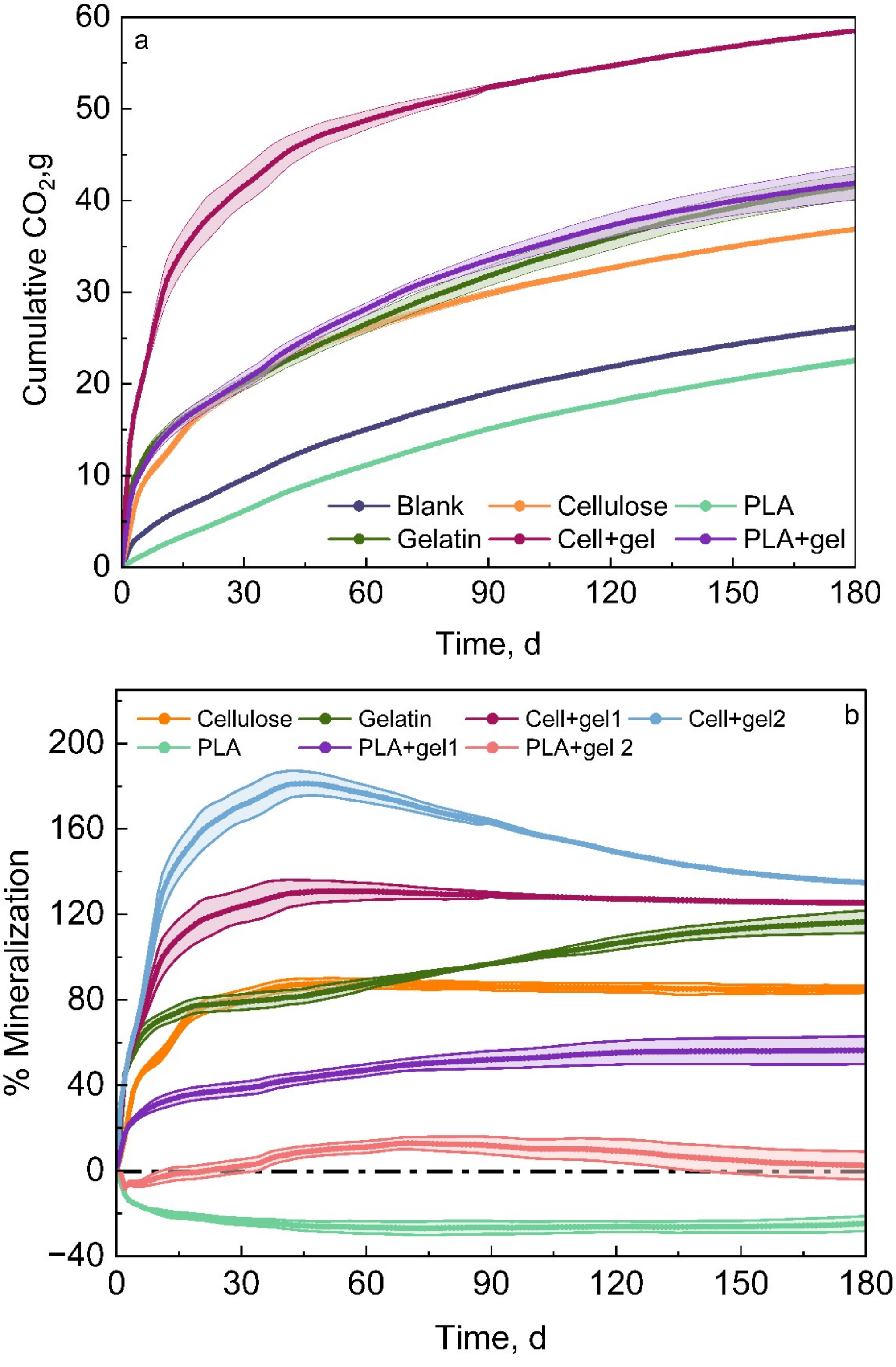

Figure S2. Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, gelatin, cellulose + gelatin (Cell+gel), PLA + gelatin (PLA+gel) in compost at 37°C.

Figure S2 a and b shows the CO_2 evolution and % mineralization of cellulose, gelatin, PLA, cellulose + gelatin, and PLA + gelatin. Cellulose reached mineralization of 87.7% whereas gelatin attained over 100 % of its carbon conversion over a period of 180 days. Since gelatin and cellulose are readily biodegradable and can be easily utilized as a carbon source by the microorganisms present in the compost, no lag phase was observed.

Gelatin is composed of protein which the microorganisms in compost use for their biochemical process. The microorganisms secrete protease enzyme to digest gelatin which is the same mechanism when PLA is introduced in gelatin amended compost. Due to the compost amendment with skim milk, PLA (PLA + gel1) shows a mineralization of approximately 60%. In order to account for the effect of gelatin, a separate mineralization plot (PLA + gel2) is derived. Though there seems to be negative mineralization, the molecular weight analysis [2] shows that gelatin helps in the enzymatic degradation of PLA. This coupled with the chemical hydrolysis of PLA [3], produces a significant difference with respect to the kinetic rate of degradation (PLA $k = 0.0045 \pm 0.0001$ and PLA-gelatin $k = 0.0060 \pm 0.0002$) [2].

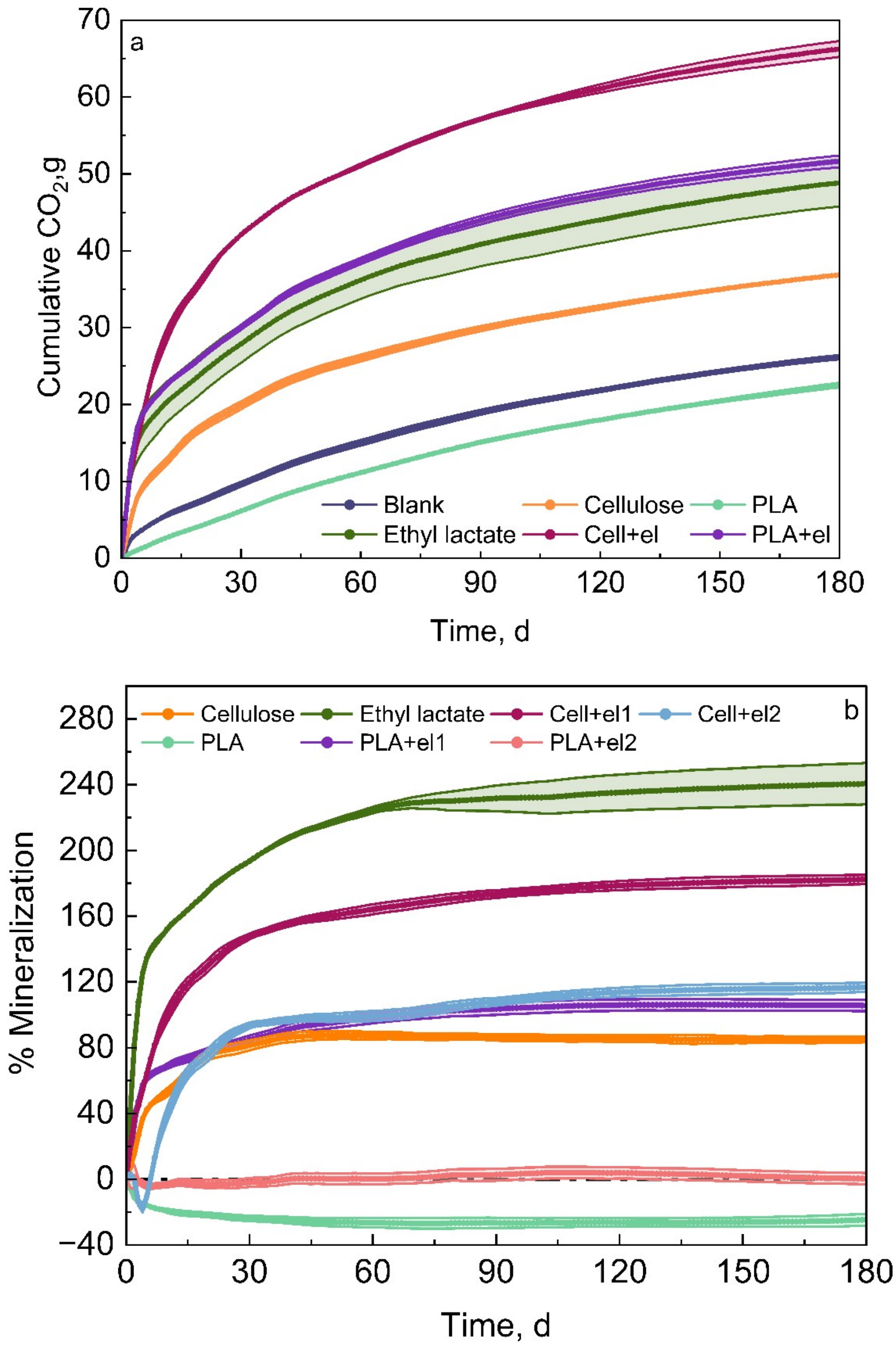

Figure S3. Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, ethyl lactate, cellulose + ethyl lactate (Cell+el), PLA + ethyl lactate (PLA+el) in compost at 37°C.

Figure S3 a and b shows the CO_2 evolution and % mineralization of cellulose, ethyl lactate, PLA, cellulose + ethyl lactate, and PLA + ethyl lactate. Cellulose reached mineralization of 87.7% whereas ethyl lactate attained over 100 % of its carbon conversion over a period of 180 days. Since ethyl lactate and cellulose are readily biodegradable and can be easily utilized as a carbon source by the microorganisms present in the compost, no lag phase was observed. Ethyl lactate was used to stimulate the lactate utilizing microbial community present in the compost.

Ethyl lactate evolves around 51.5 g of CO_2, and the corresponding mineralization reaches around 270%. The CO2 evolution of the amended compost containing both cellulose and ethyl lactate (Cell-el 1) was only around 66.2 g and there was a corresponding mineralization of 182.4%. PLA in the presence of ethyl lactate (PLA-el 1) evolved around 51.6 g of CO2, followed a similar trend as ethyl lactate over 180 days, and showed maximum mineralization of 105%. A positive mineralization behavior is seen for PLA when the effect of ethyl lactate is accounted for (PLA-el 2), indicating the lactate-stimulating activity of ethyl lactate. This is further confirmed by kinetic rates for PLA alone and PLA in compost amended with ethyl lactate (PLA $k = 0.0045 \pm 0.0001$ and PLA-ethyl lactate $k = 0.0058 \pm 0.0002$) [2].

S3. CO_2 evolution and mineralization of PLA in the presence of Fe_3O_4 nanopowder, skim milk + Fe, and ethyl lactate + Fe

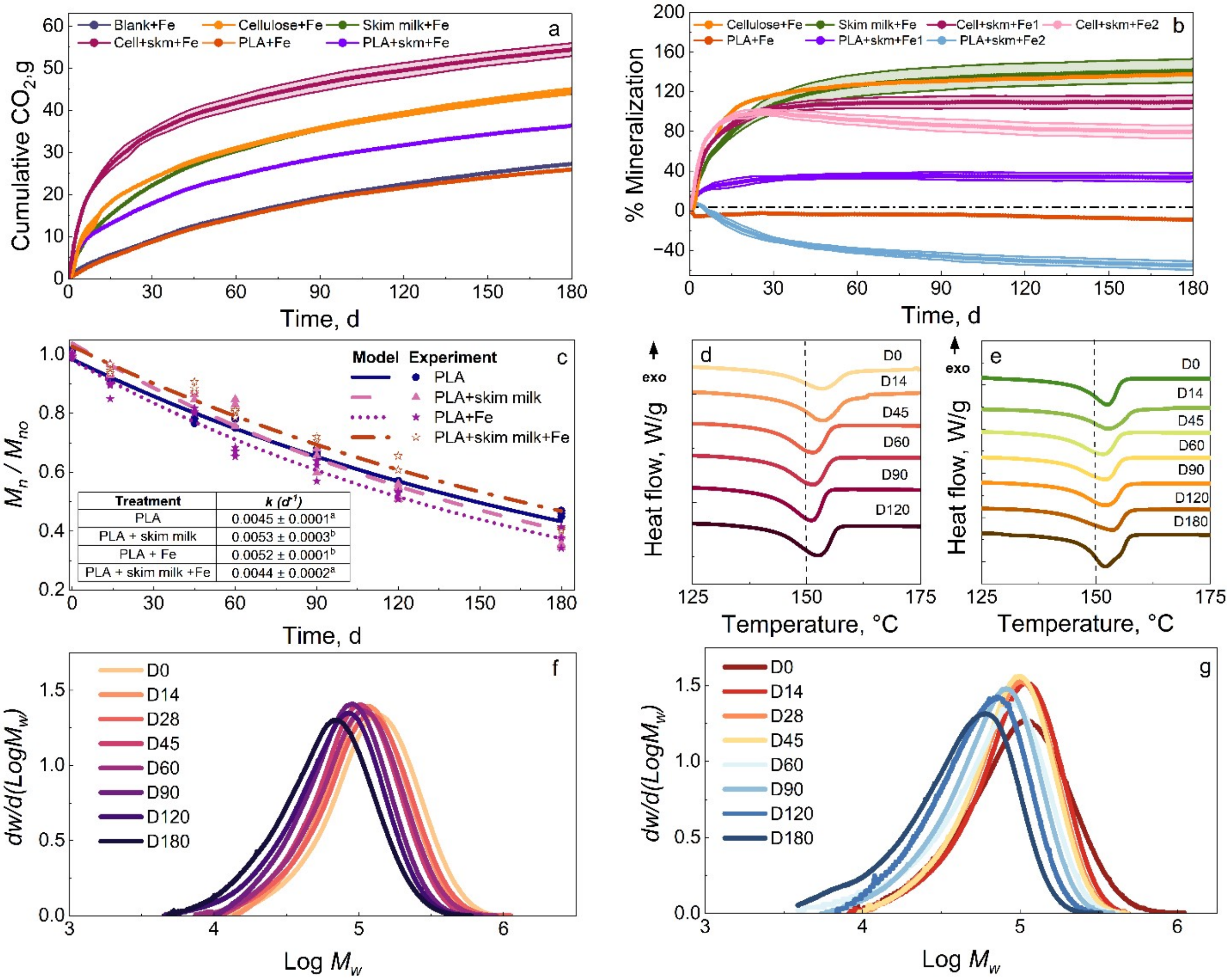

Figure S4. Cumulative CO_2 evolution (a) and Mineralization (b) of blank + Fe, cellulose + Fe, PLA + Fe, skim milk + Fe , cellulose + skim milk + Fe (Cell+skm+Fe), PLA + skim milk + Fe (PLA+skm+Fe) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated by gelatin, and gelatin + Fe. 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 in the column with different lowercase letters are statistically different (α = 0.05 Tukey-Kramer Test). (d) and (e) depict DSC thermograms for PLA + Fe and PLA + Fe in compost biostimulated by skim milk. (f) and (g) shows the MWD of PLA + Fe in compost and compost biostimulated with skim milk.

Figure S4 a and b shows the CO_2 evolution and mineralization of cellulose + Fe, skim milk + Fe, PLA + Fe, cell + skm + Fe, and PLA + skm + Fe in compost at 37°C. Skm + Fe shows around 60.1 g of CO_2 evolution and mineralization of 247.8 % in 180 days. Skim milk was combined with Fe to target chemical hydrolysis and enzymatic degradation steps. The CO_2 evolution in this case (Cell+skm+Fe) sees a higher production of 54.5 g, which is as expected and higher compared to the individual values for cellulose + Fe (44.5 g). Cell+skm+Fe 1 shows a mineralization of 121.6% whereas Cell+skm+Fe 2 after accounting for skm+Fe shows a mineralization of 99.4% indicating that the presence of skim milk in no way affects the degradation of cellulose.

To understand the influence of skim milk + Fe on PLA degradation, PLA was introduced in the compost amended with skim milk and Fe. The bioreactor containing both PLA and skim milk + Fe (PLA+skm+Fe 1) shows CO_2 evolution of around 36.4 g and maximum mineralization of 35.3 % by the end of the test. Improved mineralization is observed as opposed to no CO_2 evolution for PLA alone without any biostimulation of compost. The effect of skim milk + Fe on PLA degradation is calculated by plotting the mineralization of PLA+skm+Fe 2 (subtracting skim milk + Fe). The negative mineralization does not necessarily indicate the absence of skim milk's protease activity in PLA's enzymatic degradation. The significant difference in the evolution of X_c from 28.3% to 31.4% for PLA, and from 28.3% to 37.9% for PLA samples biostimulated with skim milk + Fe, as seen in Figure S4 d and e**,** respectively further shows the improvement in the enzymatic degradation of PLA due to the presence of skim milk + Fe. Skim milk acts as a precursor for protease activity and the broadening and change in the intensity of peaks as seen in Figure S4 f and g enforces that the addition of skim milk and Fe does enhance PLA degradation.

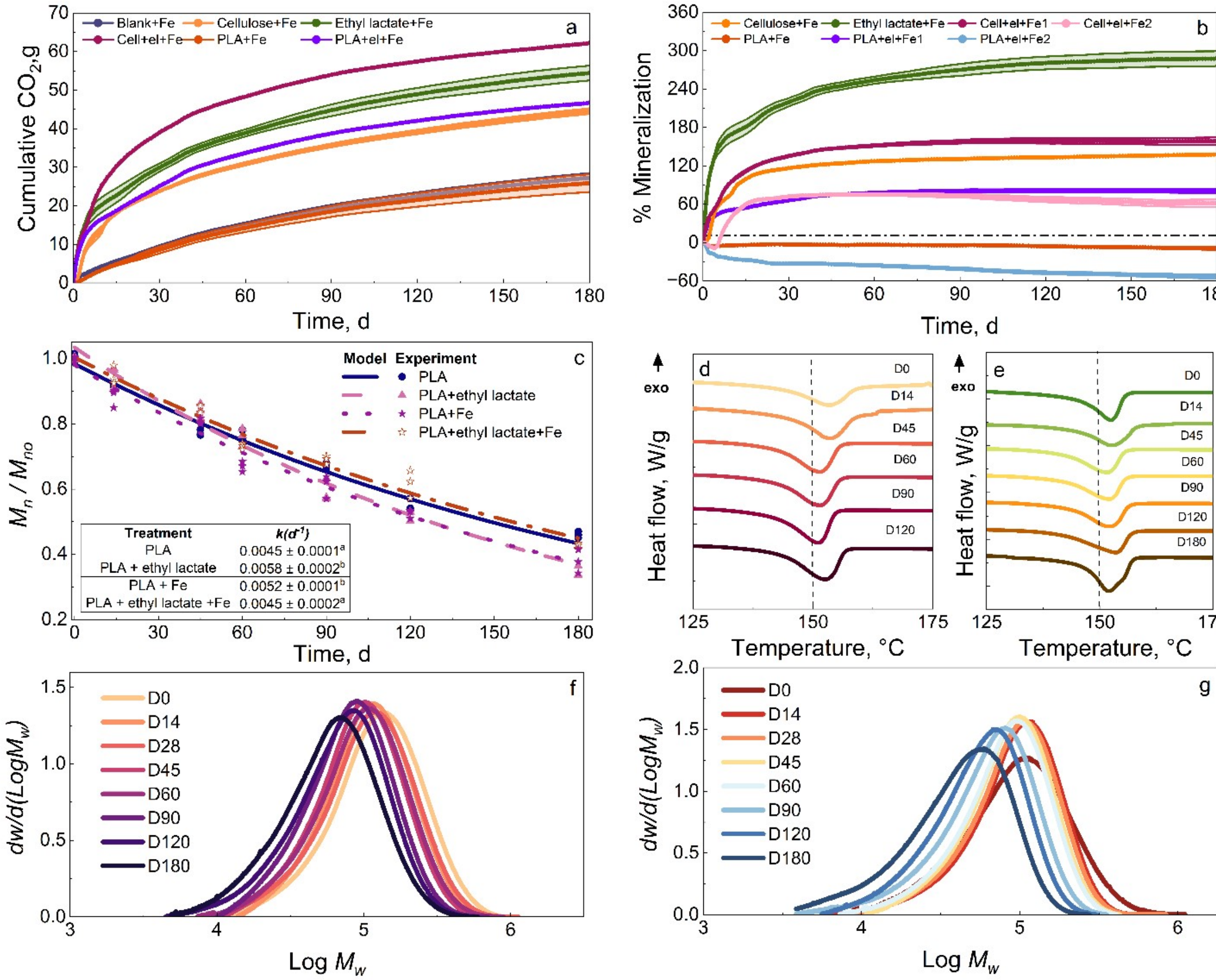

Figure S5. Cumulative CO_2 evolution (a) and Mineralization (b) of blank + Fe, cellulose + Fe, PLA + Fe, ethyl lactate + Fe , cellulose + ethyl lactate + Fe (Cell+el+Fe), PLA + ethyl lactate + Fe (PLA+el+Fe) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated by gelatin, and gelatin + Fe. 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 in the column with different lowercase letters are statistically different (α = 0.05 Tukey-Kramer Test). (d) and (e) depict DSC thermograms for PLA + Fe and PLA + Fe in compost biostimulated by ethyl lactate. (f) and (g) shows the MWD of PLA + Fe in compost and compost biostimulated with ethyl lactate.

Figure S5 a and b shows the CO_2 evolution and mineralization of cellulose + Fe, ethyl lactate + Fe, PLA + Fe, cell + el + Fe, and PLA + el + Fe in compost at 37°C. El + Fe shows around 54.5 g of CO_2 evolution and mineralization of 288.1 % in 180 days. Ethyl lactate was combined with Fe to target chemical hydrolysis and lactate utilizing microbes in compost. The CO_2 evolution in this case (Cell+el+Fe) sees a higher production of 62.2 g, which is as expected and higher compared to the individual values for cellulose + Fe (44.5 g). Cell+el+Fe 1 shows a mineralization of 156.7% whereas Cell+el+Fe 2 after accounting for el+Fe shows a mineralization of 75.1% indicating that the presence of ethyl lactate does not affect the degradation of cellulose.

To understand the influence of ethyl lactate + Fe on PLA degradation, PLA was introduced in the compost amended with ethyl lactate and Fe. The bioreactor containing both PLA and ethyl lactate + Fe (PLA+el+Fe 1) shows CO_2 evolution of around 46.5 g and maximum mineralization of 88.8 % by the end of the test. Enhanced mineralization is observed as opposed to no CO_2 evolution for PLA alone without any biostimulation of compost. The effect of ethyl lactate + Fe on PLA degradation is calculated by plotting the mineralization of PLA+el+Fe 2 (subtracting ethyl lactate + Fe). The negative mineralization does not necessarily indicate the absence of ethyl lactate’s lactate stimulating microbial activity in PLA's enzymatic degradation. The significant difference in the evolution of X_c from 28.3% to 31.4% for PLA, and from 28.3% to 39.9% for PLA samples biostimulated with ethyl lactate + Fe, as seen in Figure S5 d and e**,** respectively further shows the improvement in the enzymatic degradation of PLA due to the presence of ethyl lactate + Fe. As seen in Figure S5 f and g ethyl lactate stimulates the lactate utilizing microbial community in the compost which aids in the degradation of PLA.

References

[1] E. Castro-Aguirre, R. Auras, S. Selke, M. Rubino, and T. Marsh, “Insights on the aerobic biodegradation of polymers by analysis of evolved carbon dioxide in simulated composting conditions,” *Polym. Degrad. Stab.*, vol. 137, pp. 251–271, Mar. 2017.

[2] P. C. Mayekar and R. Auras, “Accelerating Biodegradation : Enhancing Poly(lactic acid) Breakdown at Mesophilic Environmental Conditions with Biostimulants,” *Macromol. Rapid Commun.*, vol. 2300641, pp. 1–10, 2023.

[3] P. C. Mayekar, W. Limsukon, A. Bher, and R. Auras, “Breaking It Down: How Thermoplastic Starch Enhances Poly(lactic acid) Biodegradation in Compost─A Comparative Analysis of Reactive Blends,” *ACS Sustain. Chem. Eng.*, vol. 11, no. 26, pp. 9729–9737, 2023.