Controllable water-triggered degradation of PCL solution-blown nanofibrous webs made possible by lipase enzyme entrapment

Fnu Asaduzzaman and Sonja Salmon*

Textile Engineering, Chemistry and Science, Wilson College of Textiles, North Carolina State University, Box 8301, 1020 Main Campus Drive, Raleigh, NC-27695-8301.

*Corresponding Author

Email: sisalmon@ncsu.edu

SUPPLEMENTARY MATERIAL

Supplementary S1. Lipase assay calculation of free CALB and redissolved CALB-EFSBN-PCL

S1.1. Immobilized CALB stock solution in the buffer: 6.5 mg (exactly weighed) of 0.81 wt% CALB-EFSBN-PCL web was placed into a 1.5 ml microcentrifuge vial and incubated with 1.20 ml Tris HCl buffer (100 mM, pH 8.0) for 120 mins at 40 °C during which time EFSBN-PCL fibers were wholly dissolved to produce a stock solution.

S1.2. Free CALB stock solution (0.1 µl CALB/ml buffer): The free CALB liquid enzyme sample was first diluted (100 times) to 10 µl CALB/ml buffer in 100 mM Tris buffer (pH 8.0) and then further diluted (total dilution) to 0.1 µl CALB/ml buffer in the same buffer.

S1.3. p-nitrophenyl acetate substrate: The p-nitrophenyl acetate substrate was first dissolved in ethanol to prepare a 100 mM substrate stock solution and kept at -4 °C for further assay

measurements. Before each set of an assay, 100 mM p-NPAc was diluted with deionized water to a final concentration of 8 mM.

S1.4. Incubation: In the incubation step, 75 µl of EFSBN-PCL redissolved stock solution and 400 µl Tris HCl buffer (100 mM, pH 8.0) were mixed in a 1500 µl microcentrifuge tube and preheated for 5 mins at 45 °C. For free CALB activity, 475 µl of the dilution was added to a 1500 µl microcentrifuge tube and preheated for 5 mins at 45 °C. Then, 25 µl 8 mM p-NPAc substrate solution was added to the free CALB and immobilized CALB solutions and mixed thoroughly by gentle manual mixing. The final solution (total volume 500 µl) was incubated for 30 minutes at 45 °C. After incubation, the reaction was stopped by placing microcentrifuge tubes into ice water. Absorbance measurements of the assay solutions determine the concentration of p-nitrophenol (p-NP), the yellow-colored product of p-NPAc hydrolysis, with an absorbance maximum of 405 nm. Three replicates of each sample were measured. The blank control was made by maintaining the same procedure except adding an equivalent amount of SBN-PCL (neat PCL web) instead of EFSBN-PCL. Absorbance for One unit (U) of lipase activity is defined as the amount of enzyme that catalyzes the release of 1.0 µmole of p-NP per min from the p-NPAc substrate at 40 °C in the presence of 100 mM pH 8.0 Tris-HCl buffer. The micromoles of p-NP equivalents liberated were determined by using the standard curve.

S1.5. p-nitrophenol standard curve: A p-NP standard curve (Figure S1) was created by plotting absorbance versus known concentrations of p-NP (5-50 µg/ml), measured at 405 nm.

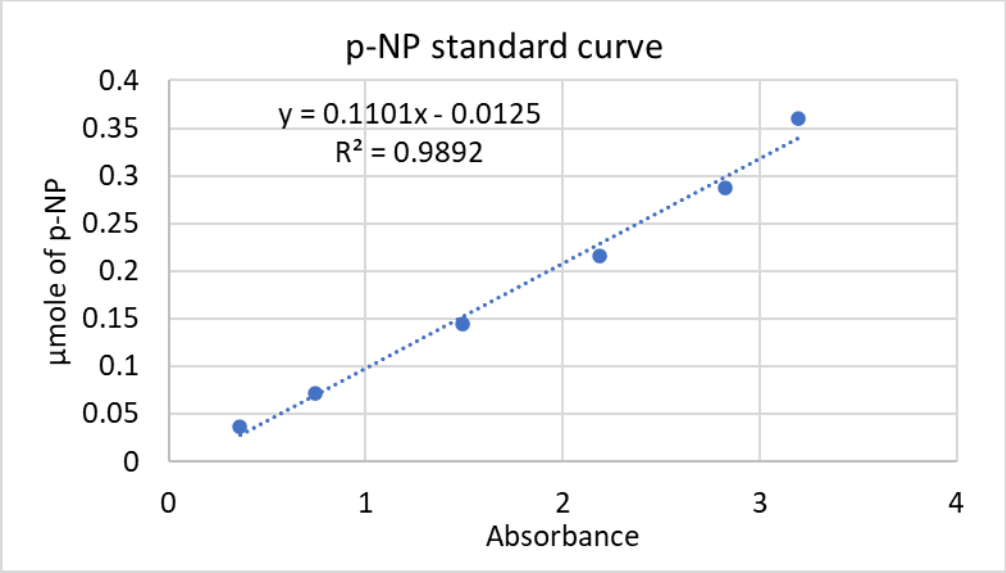

Figure S1. Calibration curve for p-nitrophenol (p-NP) concentration measured at 405 nm.

The lipase assay of the free liquid enzyme upon dilution was calculated using Eqns (i) and (ii):

$$\text{U/ml} = \frac{\mu\text{mole p-NP equivalent release} * \text{volume of total assay,ml}}{\text{Time}_{,\text{min}} * \text{volume of enzyme used,ml}} * \text{dilution} \quad \text{(i)}$$

$$\text{U/mg}_{\text{protein}} = \frac{\text{U/ml}}{\text{mg of} \frac{\text{protein}}{\text{ml}} \text{of liquid CALB}} \quad \text{(ii)}$$

The lipase assay of immobilized CALB per mg of the EFSBN-PCL webs protein was calculated using Eqn (iii):

$$\text{U/mg}_{\text{ EFSBNs protein}} = \frac{\mu\text{mole of p-NP equivalent release} * \text{volume of total assay,ml}}{\text{Time,min} * \text{EFSBN protein used in assay,mg} * \text{volume of enzyme solution,ml}} \quad \text{(iii)}$$

The relative activity of immobilized CALB was calculated as a percentage of the free lipase activity according to Eqn (iv):

$$\text{Relative activity (\%)} = \frac{\text{Activity of immobilized lipase}}{\text{Activity of free lipase}} * 100 \quad \text{(iv)}$$

S1.6. Example Calculations: A set of example calculations is presented in detail below.

Weight of 1.30 wt% CALB-EFSBN-PCL= 6.8 mg

The amount of protein in the webs = (6.8*1.30/100) = 0.0884 mg

The volume of tris buffer (100 mM, pH 8.0) used for web dissolution (stock solution 1) = 1200 µl

The volume (stock solution 1) used in lipase assay = 75 µl (make-up to 475 µl by adding buffer)

The amount of protein used in assay = (0.0884*75/1200) = 0.00552 mg = 5.52 µg

The conc. of free CALB in buffer (stock solution 2)= 0.1 µl CALB/ml buffer (10000 times dilution)

The volume of free CALB stock solution used in lipase assay = 475 µl

The substrate p-nitrophenyl acetate (8 mM) used in assay = 25 µl

So, the total volume of assay = 500 µl

Lipase activity of free CALB:

The absorbance of released p-NP by the free CALB = 1.964, 1.948, 1.818 = 1.91 (average)

The absorbance of the control samples = 1.270, 1.279, 1.320 = 1.290 (average)

The net absorbance of released p-NP by the free CALB = 1.91 – 1.290 = 0.62

Release of p-NP by the free CALB = (0.62*0.1101)-0.0125 = 0.0557 µmole

From equation (i), lipase activity of free CALB,

U/ml = U/ml = $\frac{0.0557\ \mu\text{mole} * 0.5\ \text{ml}}{30\ \text{min}\ * 0.475\ \text{ml}} * 10000$ = 19.56

And from equation (ii), lipase activity of free CALB,

$U/mg_{protein}$ = $\frac{27\ U/ml}{11.92\ mg/ml}$ = 1.64

Lipase activity of CALB-EFSBN-PCL:

The absorbance of released p-NP by the dissolute EFSBN-PCL CALB = 2.739, 2.657, 2.623 = 2.673

The absorbance of the control sample = 1.274, 1.283, 1.277 = 1.278 (average)

The net absorbance of released p-NP by the dissolute EFSBN-PCL CALB = (2.673-1.278) = 1.395

Release of p-NP by the dissolved EFSBN-PCL CALB = (1.395*0.1101)-0.0125 = 0.141 µmole

From equation (iii), the lipase activity,

$$\text{U/mg}_{\text{EFSBNs protein}} = \frac{0.141\ \mu\text{mole} * 0.5\ \text{ml}}{30\ min * 0.00552\ \text{mg} * 0.475\ \text{ml}} = 0.90$$

Relative activity:

Applying equation (iv),

Relative activity (%) = 0.90/1.64*100 =55%

Supplementary S2. Morphology of solution blown webs with and without enzyme

S2.1. Macroscopic appearance of solution blown webs: Photographs of SBN-PCL (A) and CALB-EFSBN-PCL (B) webs are shown in **Figure S2**.

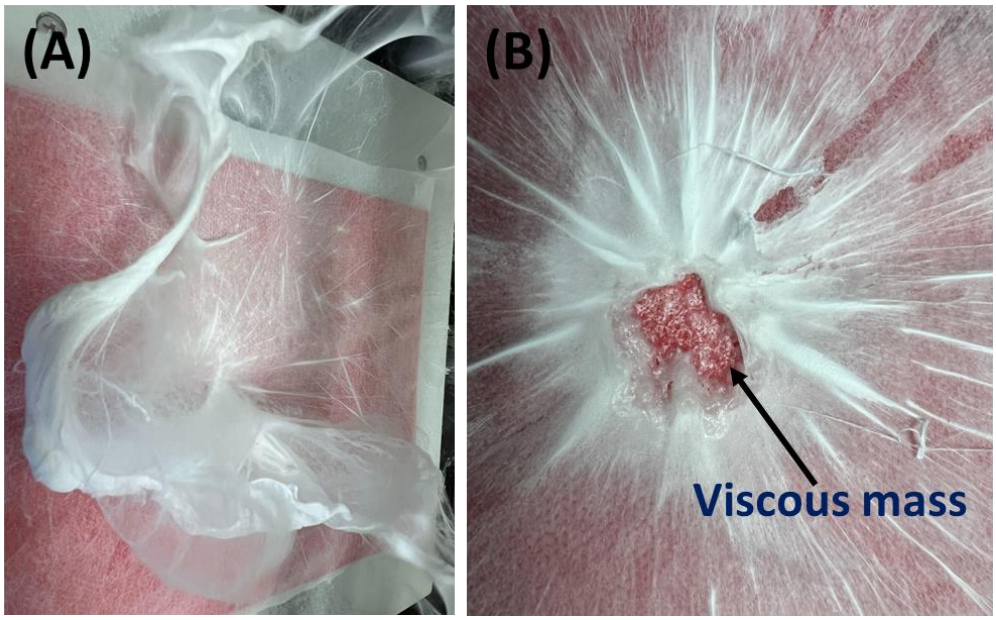

Figure S2: (A) SBN-PCL and (B) CALB-EFSBN-PCL webs produced using 11.2 v/v% CALB in polymer solution, shown on red collectors. Both webs were produced using AOT-chloroform solvent.

S2.2. Exemplary photographs of emulsion spinning solutions: Several exemplary images of the preparation and emulsion properties of PCL-chloroform-AOT solution blowing spinning solutions, with or without CALB, and with or without a water-soluble red dye, are shown, followed by ToF-SIMS ion abundance heat maps and SEM images of solution blown nanofiber webs.

To emphasize visualization of the emulsion, in one example, 0.1 milligram of dry powdered water-soluble dye (Solophenyl Red 3BL, Direct Red 80, C.I. 35780, Huntsman Textile Effects, High Point, NC 27265 USA) was combined with the appropriate amount of liquid lipase (CALB) needed to produce a nominal mixture of 0.7 v/v% CALB in 15 w/v% PCL in chloroform-AOT solvent (which

was the nominal spinning solution composition that corresponded to producing dry EFSBN-PCL webs containing ~0.5 wt% CALB). As shown in **Figure S3.A**, the red colored fraction was the aqueous phase, which includes CALB and the water-soluble red dye, while the colorless liquid was PCL dissolved in chloroform-AOT. Before mixing, these two liquid fractions were largely immiscible. After 90 seconds of vigorous vortex mixing (**Figure S3.B**), the liquid acquired a uniform red color throughout (**Figure S3.C**), indicating the formation of a water-in-oil emulsion. This emulsion was stable for at least 30 minutes (**Figure S3.D**), which was sufficient time to perform solution blow spinning.

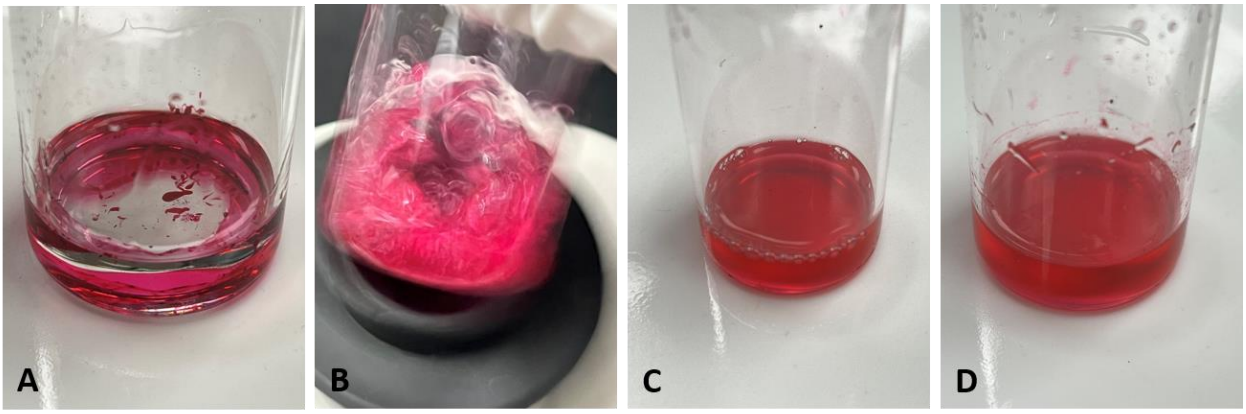

Figure S3: Exemplary preparation of a nominal 0.7 v/v% CALB in 15 w/v% PCL in chloroform-AOT solvent emulsion for solution blowing, where (A) shows the immiscibility of the red aqueous and colorless organic liquid phases, (B) shows homogenization of the red color during vortex mixing, (C) shows the resulting uniform red liquid appearance indicative of emulsion formation, and (D) shows the sample after standing for 30 minutes at ambient conditions. For scale, the vial diameter is 27 mm.

Optical microscopy images are shown below for the following: 15 w/v% PLC-chloroform-AOT solvent only, without (**Figure S4.A**) and with a cover glass (**Figure S4.B**); the emulsion liquid from **Figure S3.C** under a cover glass viewed at lower (**Figure S5.A**) and higher (**Figure S5.B**) magnification; the emulsion shown in **Figure S5** without (**Figure S6.a**) and with a cover glass

(**Figure S6.B**); a nominal 1.5 v/v% CALB in 15 w/v% PCL in chloroform-AOT solvent emulsion for solution blowing (without any added dye) viewed at lower (**Figure S7.A**) and higher (**Figure S7.B**) magnification under a cover glass, and viewed without a cover glass (**Figure S7.C**).

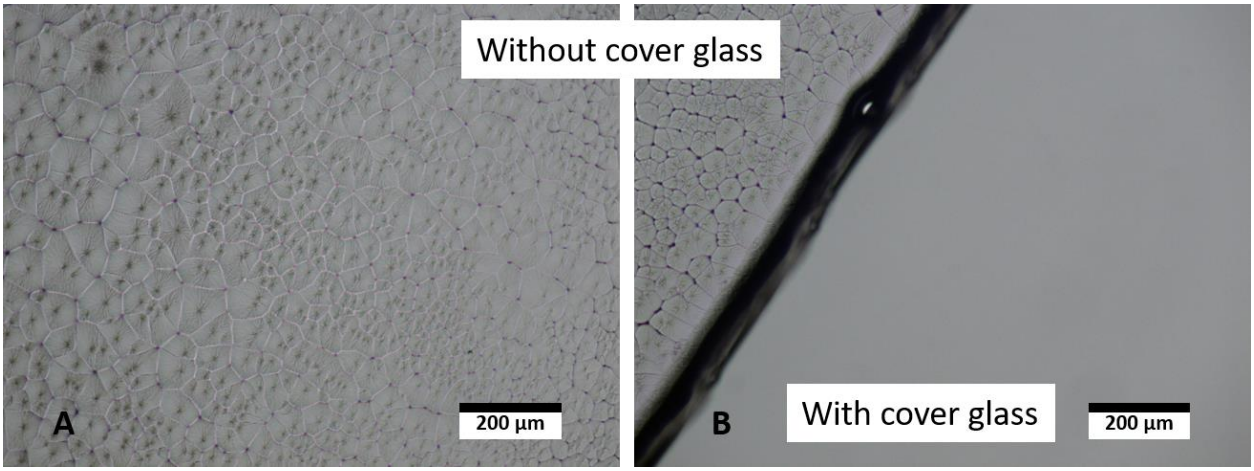

Figure S4: Optical microscopic images of 15 w/v% PCL in chloroform-AOT solvent applied to a glass slide (A) without a cover so that the solvent evaporated, leaving behind a spherulite PCL morphology, and (B) with a cover glass that prevented solvent evaporation (right side of image). The dark diagonal line in (B) shows the edge of the cover glass for visual reference, because a view of the transparent polymer solution alone with the cover glass is featureless.

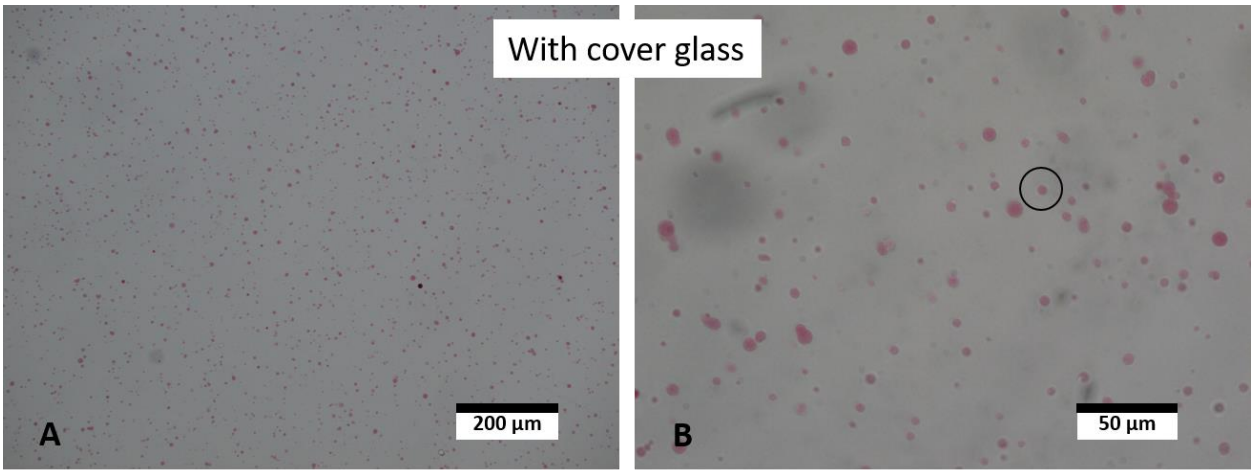

Figure S5: Optical microscopic images of a nominal 0.7 v/v% CALB in 15 w/v% PCL in chloroform-AOT solvent emulsion for solution blowing, with water-soluble dye included to enhance visualization of the micron-scale aqueous phase, containing CALB and red dye, dispersed within the surrounding colorless organic phase, containing PCL and chloroform-AOT solvent. AOT surfactant acts as an emulsion stabilizer. Images are shown at (A) lower magnification to illustrate emulsion uniformity and at (B) higher magnification to show aqueous dispersed phase droplet size. For comparative scale, a black circle is drawn around a droplet with ~5 µm diameter.

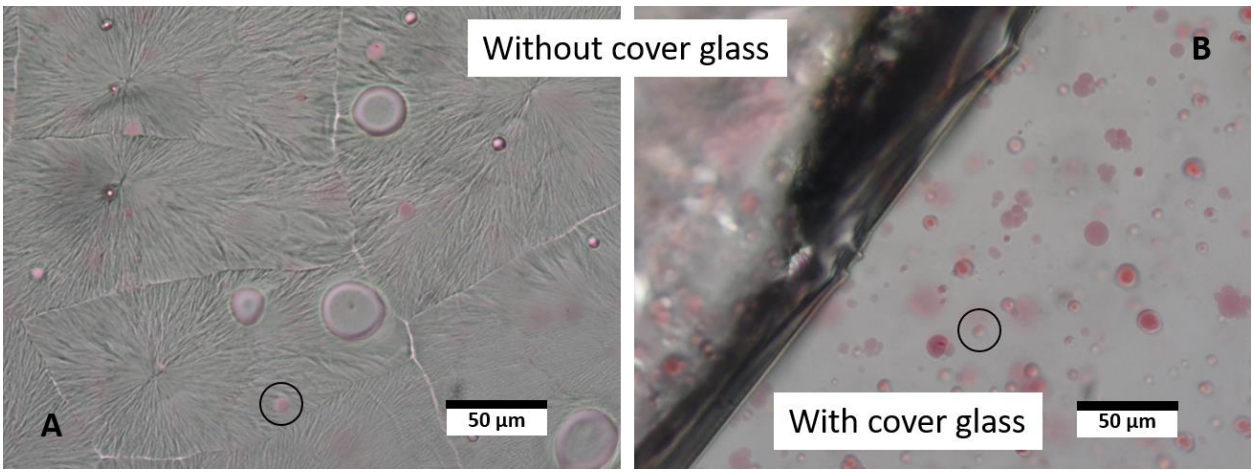

Figure S6: Optical microscopic images of the emulsion shown in Figure S5 (A) without a cover so that the solvent evaporated, leaving behind a spherulite PCL morphology with dispersed phase droplets embedded among the spherulites, and (B) with a cover glass that prevented solvent evaporation (right side of image). The dark diagonal line in (B) shows the edge of the cover glass for visual reference, where in this case, red dyed dispersed phase droplets are visible throughout the transparent organic phase. For comparative scale, black circles are drawn around droplets with ~5 µm diameter.

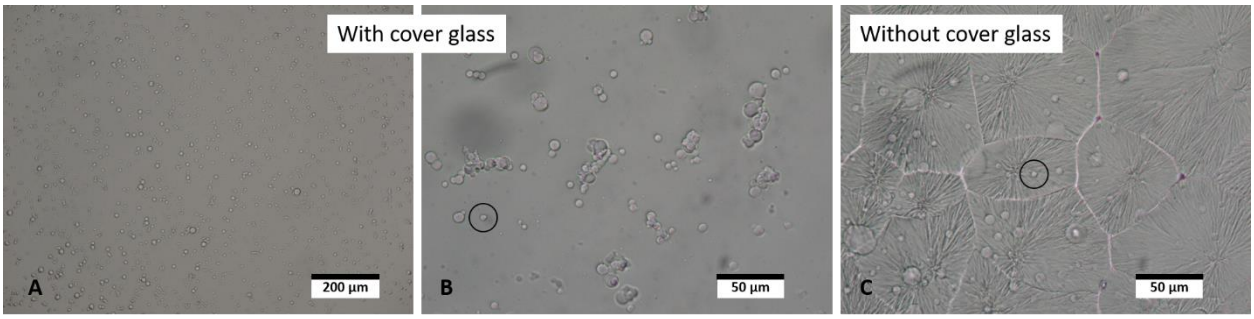

Figure S7: Optical microscopic images of a nominal 1.5 v/v% CALB in 15 w/v% PCL in chloroform-AOT solvent emulsion for solution blowing, with aqueous colorless CALB dispersed within the colorless organic phase, containing PCL and chloroform-AOT solvent. AOT surfactant acts as an emulsion stabilizer. Images are shown with cover glass at (A) lower magnification to illustrate emulsion uniformity, at (B) higher magnification to show aqueous dispersed phase droplet size, and (C) without cover glass at higher magnification to show the aqueous dispersed phase droplets embedded among PCL spherulites after evaporation of the chloroform solvent. For comparative scale, black circles are drawn around droplets with ~5 µm diameter.

S2.3. Surface chemistry of solution blown webs: ToF-SIMS heat maps of the abundance of specific ions detectable from the surface are shown in **Figure S8** for a SBN-PCL web, in **Figure S9** for a 0.53 wt% CALB-EFSBN-PCL web, and in **Figure S10** for a 1.30 wt% CALB-EFSBN-PCL web. **Figure S11** compares the total ion count of ions attributed to protein (CN+CNO) for SBN-PCL, 0.53 wt% CALB-EFSBN-PCL and 1.30 wt% CALB-EFSBN-PCL webs.

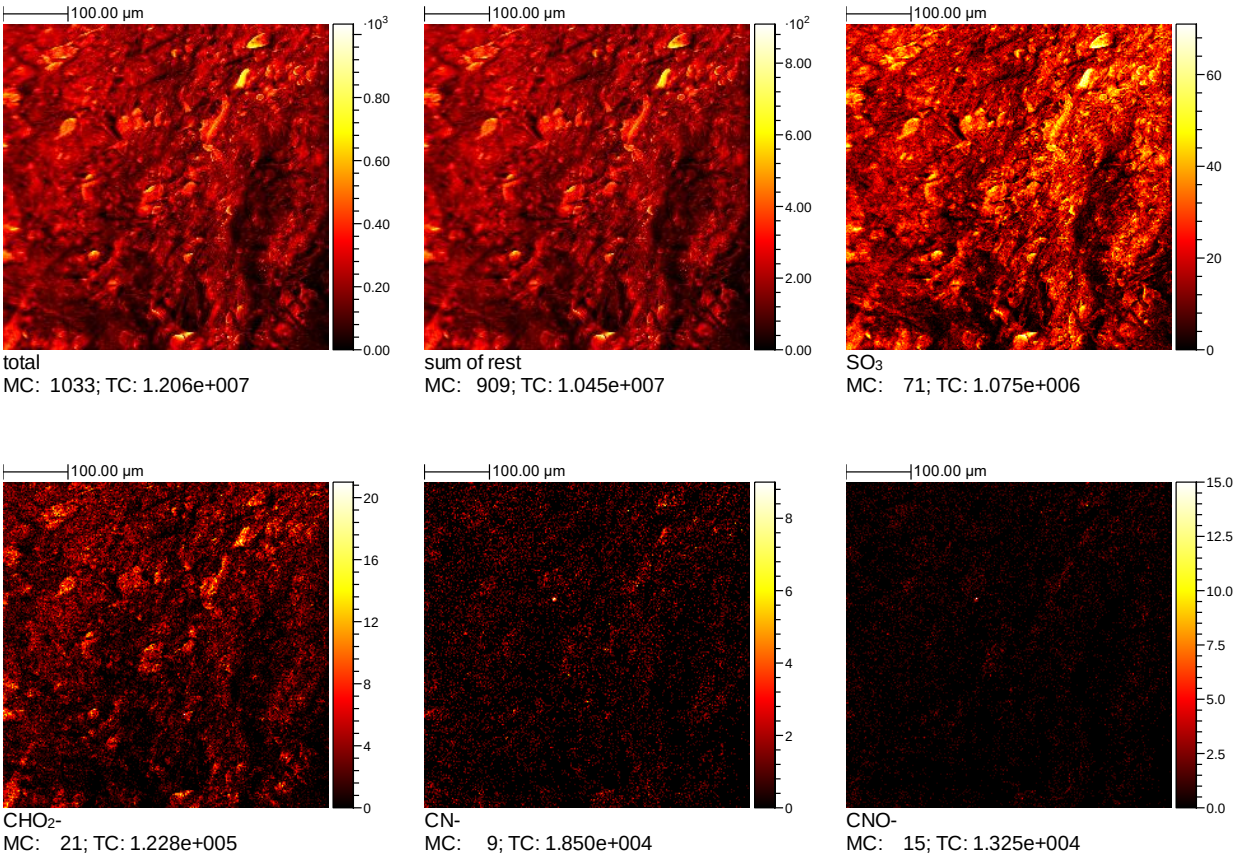

Figure S8: ToF-SIMS high lateral resolution mass spectral maps (analysis area 100x100 µm^2) of SBN-PCL webs.

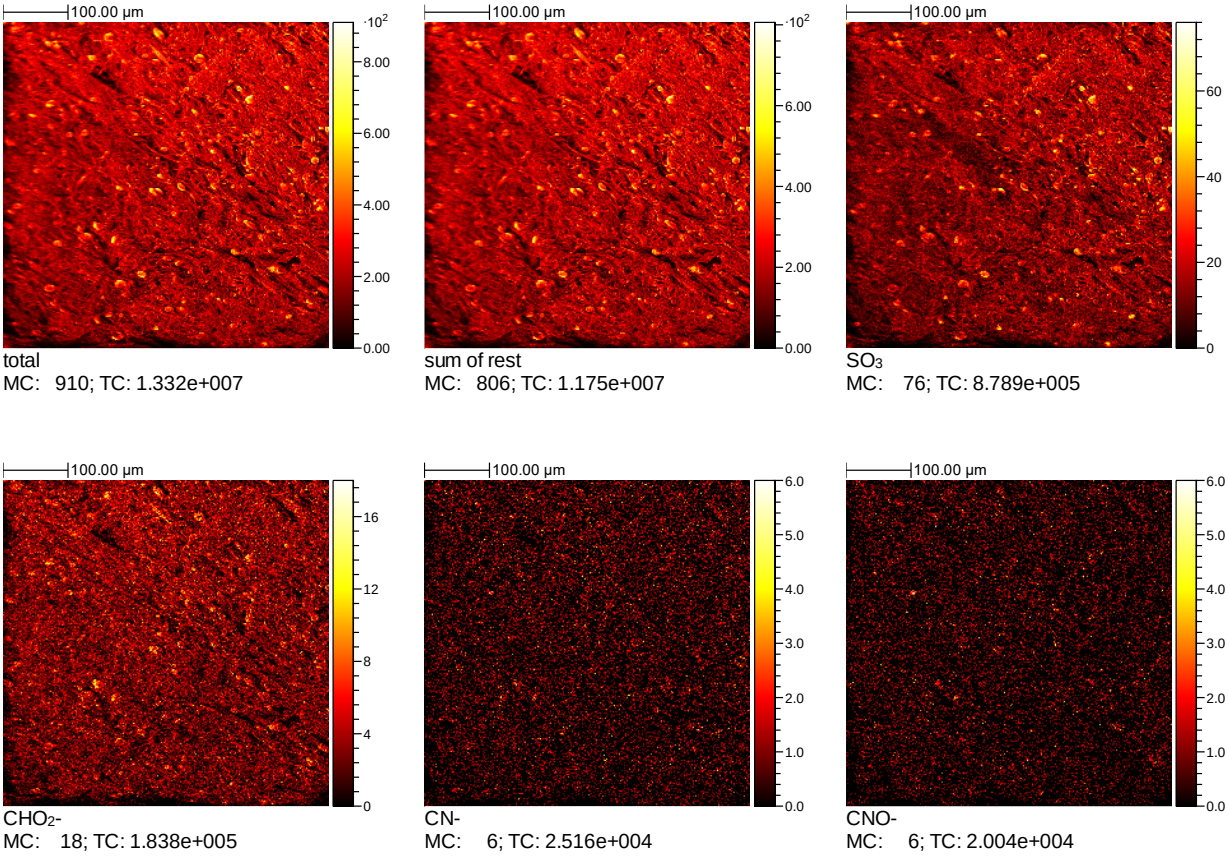

Figure S9: ToF-SIMS high lateral resolution mass spectral maps (analysis area 100x100 µm^2) of 0.53 wt% CALB-EFSBN-PCL webs.

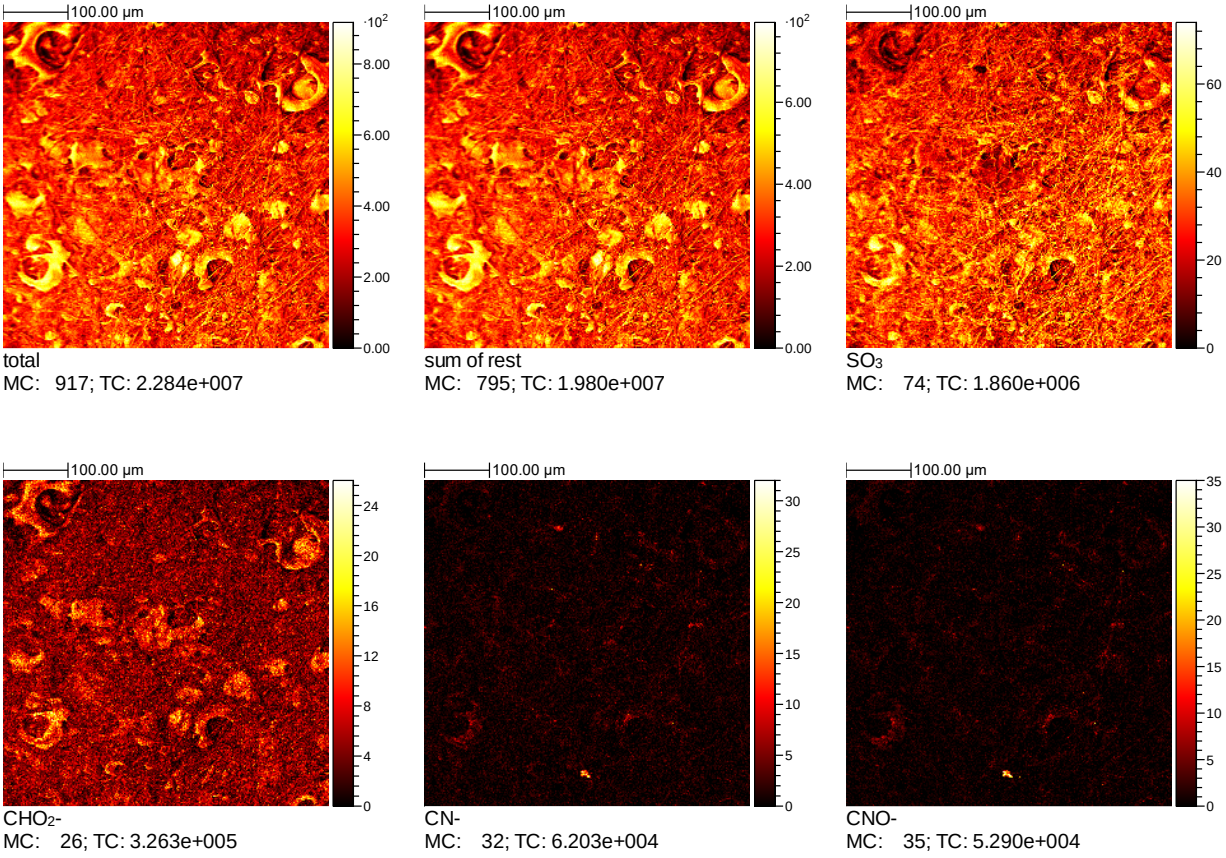

Figure S10: ToF-SIMS high lateral resolution mass spectral maps (analysis area 100x100 µm^2) of 1.30 wt% CALB-EFSBN-PCL webs.

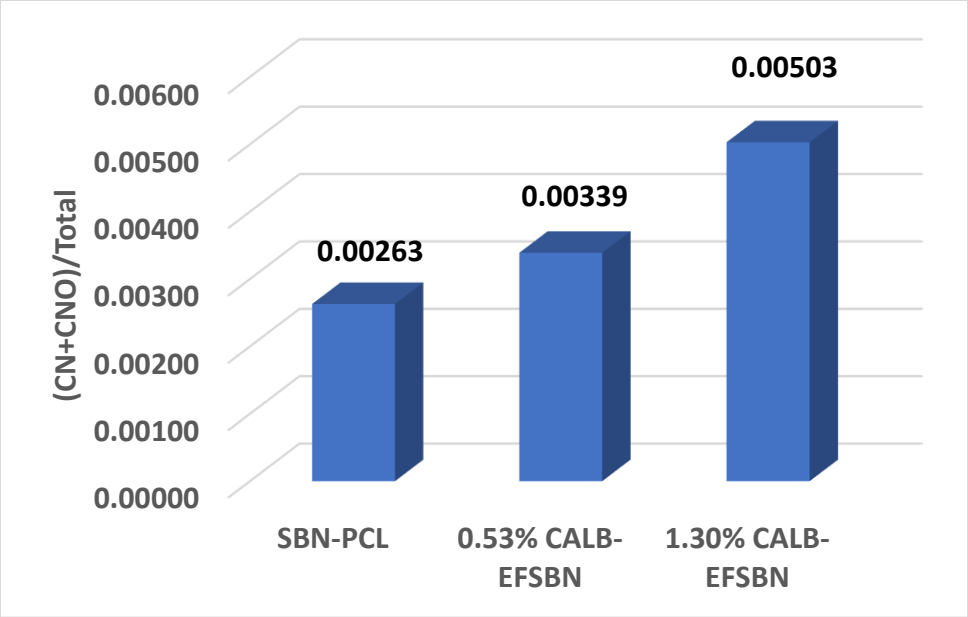

Figure S11: (CN+CNO)/total ion count graph of SBN-PCL, 0.53 wt% CALB-EFSBN-PCL and 1.30 wt% CALB-EFSBN-PCL webs.

S2.4. Microscopic appearance of solution blown webs: SEM micrographs of SBN-PCL webs and CALB-EFSBN-PCL webs with varying enzyme loading are shown in **Figure S12** and **Figure S13**.

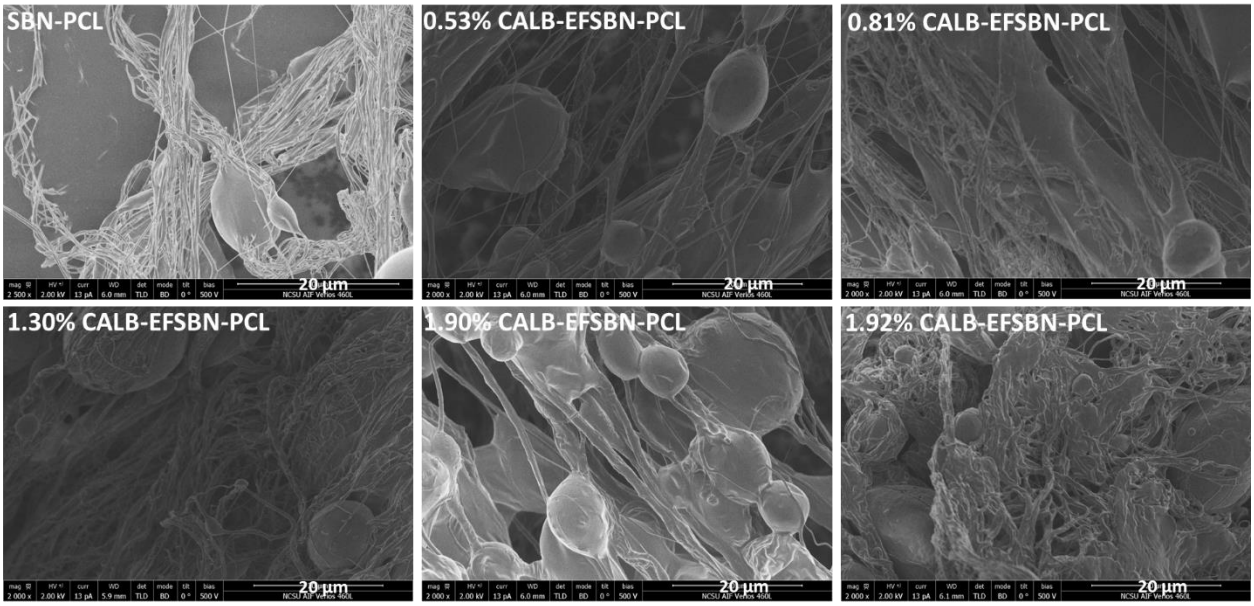

Figure S12: SEM micrograph (2000x magnification) of SBN-PCL webs and CALB-EFSBN-PCL webs with varying enzyme loading.

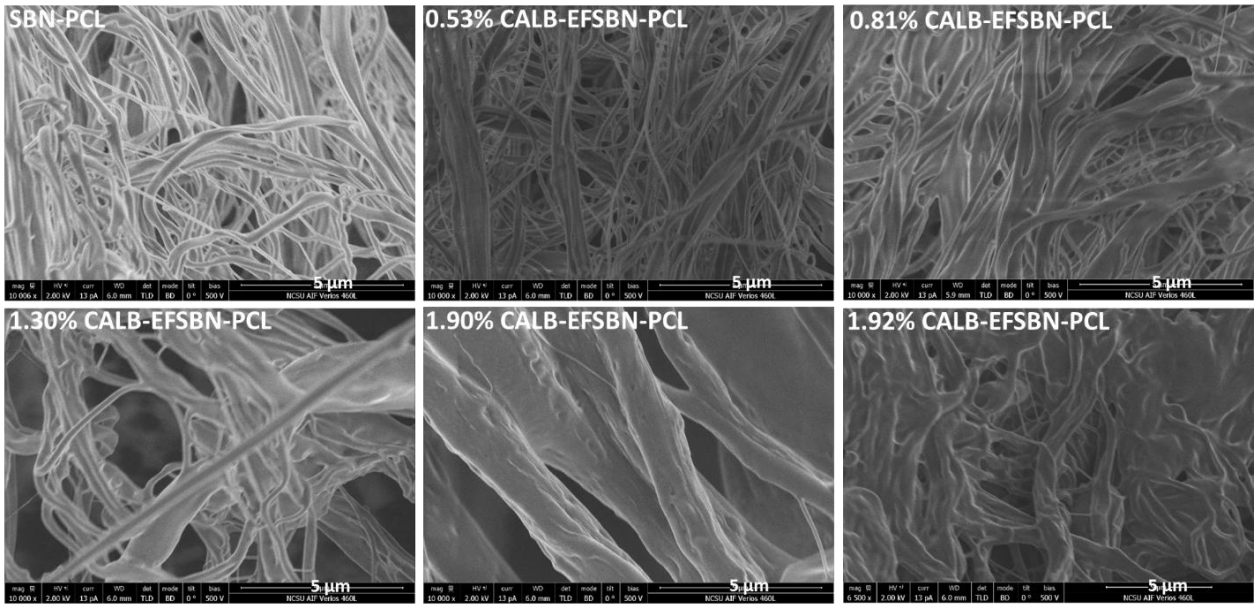

Figure S13: SEM micrograph (10000x magnification) of SBN-PCL webs and CALB-EFSBN-PCL webs with varying enzyme loading.

Supplementary S3. Relationship between degradation time and enzyme loading in EFSBN-PCL webs

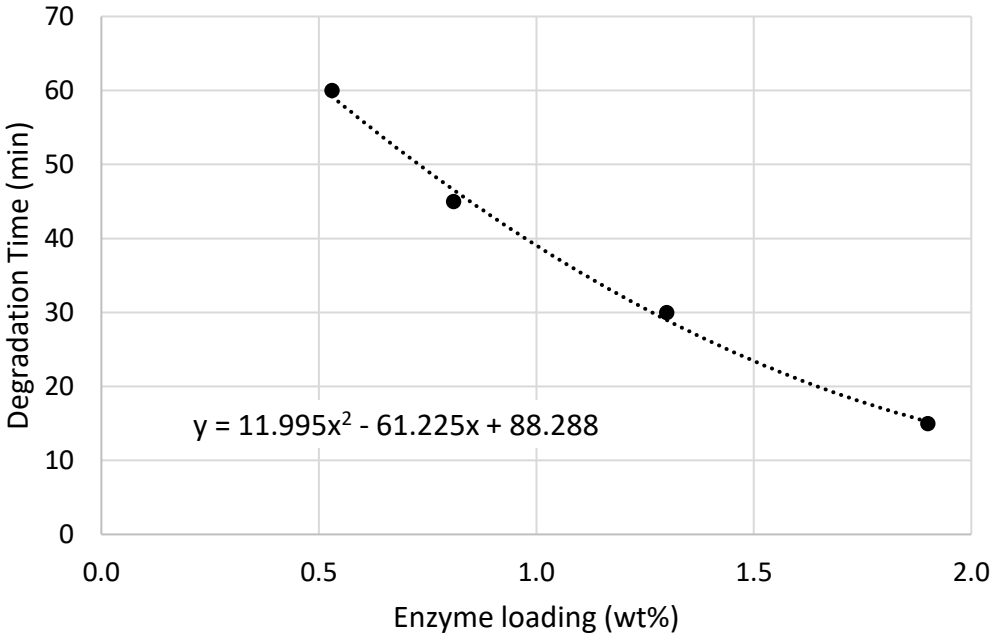

Figure S14: Relationship between approximate degradation time and level of CALB enzyme loading in EFSBN-PCL webs in pH 8 buffer at 40°C, where degradation time was the experimental time increment when solids were no longer visually observed in the incubated sample. Filled black circles are experimental data points; dotted line shows the polynomial trendline corresponding to the inserted equation.

Supplementary S4. Surface area (estimated) calculation of EFSBN-PCL and PCL bead

S4.1. Surface area of PCL bead

The volume of bead (consider PCL bead as a sphere), $V_1 = 4/3\pi r_1^3$ [r_1 = radius of sphere]

And density, $\rho = {}^{m_1}/_{V_1}$ [m_1 = mass of bead ~ 30 mg = 0.03 g]

- ⇨ $V_1 = {}^{m_1}/_{\rho}$
- ⇨ $4/3\pi r_1^3 = {}^{0.03\ g}/_{1.145\ \mathrm{g}/cm^3}$ [ρ = 1.145 g/cm^3]
- ⇨ r_1 = 0.184 cm

The surface area of bead, $A_1 = 4\pi r_1^2 = 4 * 3.1416 * 0.184^2 = 0.426$ cm^2

S4.2. Surface area of 1.30 % CALB-EFSBN-PCL fibers

Consider, the fibers are cylindrical shape.

The surface area of fibers, $A_2 = 2\pi r_2 h + 2\pi r_2^2$ [r_2 (radius) and h (length) of fibers]

$= 2\pi r_2 h$ [Average r_2 = 110 nm and $2\pi r_2 h >>> 2\pi r_2^2$; $2\pi r_2^2 \sim 0$]

The volume of fibers, $V_2 = \pi r_2^2 h$

And density, $\rho = {}^{m_2}/_{V_2}$ [m_2 = mass of fibers ~ 30 mg = 0.03 g]

- ⇨ $V_2 = {}^{m_2}/_{\rho}$
- ⇨ $\pi r_2^2 h = {}^{0.03\ g}/_{1.145\ \mathrm{g}/cm^3}$ [ρ ~ 1.145 g/cm^3]
- ⇨ $(A_2/2) * r_2 = {}^{0.03\ g}/_{1.145\ \mathrm{g}/cm^3} = 0.052$ cm^3
- ⇨ $A_2 = 0.052/r_2$
- ⇨ $A_2 = {}^{0.052}/_{(110 * 10^{-7})}$ = 4764 cm^2 [r_2 = 110 nm = 110 * 10^{-7} cm]

S4.3. Surface area ratio

The surface area ration, ${}^{A_2}/_{A_1} = 4764/0.426 = 11183$

- ⇨ $A_2 = 11183 * A_1$
- ⇨ Surface area of nanofibers = 11183 ~11000 times the surface area of beads