XXVI CONVEGNO NAZIONALE
ASSOCIAZIONE ITALIANA DI SCIENZA E TECNOLOGIA DELLE MACROMOLECOLE
Torino, 6-9 Settembre 2026

Structural Control and Amyloid Reinforcement of Waste-Derived Protein Films

M. Colombo[1], E. Testa[1], E. Fasoli[1], D. Gentile[1], V. Barbera[1] , M. Galimberti[1]

[1] Department of Chemistry, Materials and Chemical Engineering “G. Natta”, Politecnico di Milano,
Via E. Bassini 6, 20131 Milano, Italy

Renewable carbon for materials production

Biomass generates approximately **40× more carbon** annually than crude oil production, yet only a small fraction is currently exploited for material applications.

Annual crude oil production:

~ 5 Gt/year[1]

x40

Annual biomass production:

~ 200 Gt dry biomass/year[2]

Replacing fossil carbon with **biogenic carbon** in plastics could **reduce GHG emissions**, according to a model published in Science in 2021[3].

Agricultural residues from biomass

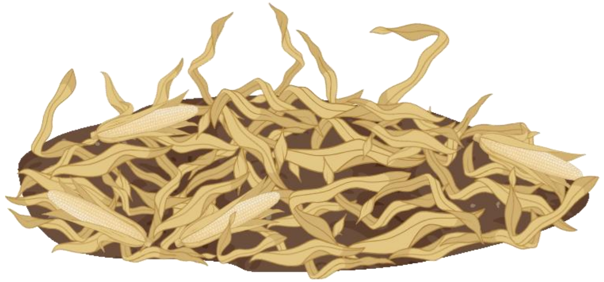
For example, a **significant fraction** of biomass generated as agricultural residues **remains underutilized**.

Annual dry biomass production:

~ 200 Gt/year

Annual agricultural dry biomass production:

~ 25 Gt/year[4]



Annual agricultural dry biomass residues unexploited:

~ 15-20 Gt/year[4]

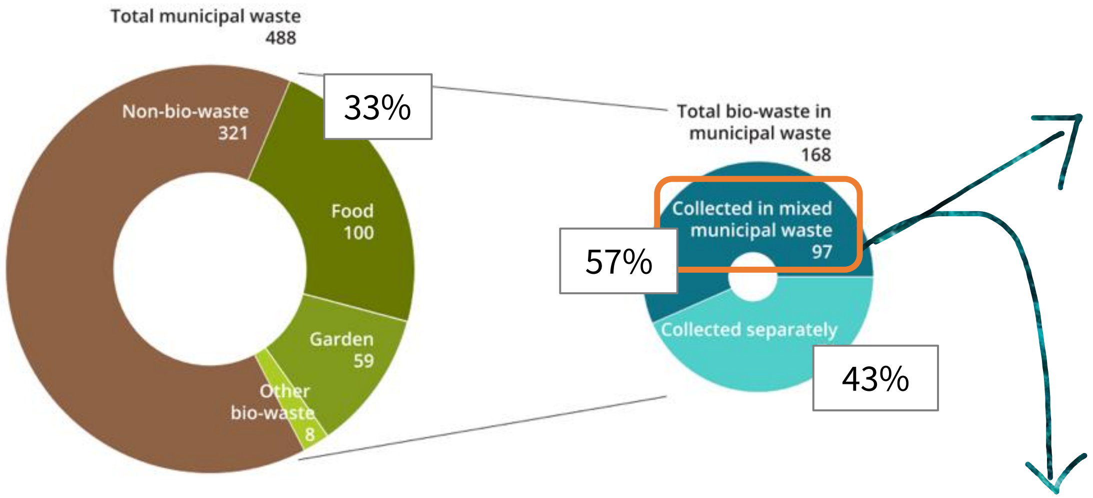
But **agricultural residues** represent **only one fraction** of the broader biomass stream.

Organic waste: an underutilized biomass resource

Organic Waste is defined as a "**waste containing carbon compounds**" and is typically assimilated to the concept of *biodegradable waste*, i.e. "any waste that is capable of undergoing anaerobic or aerobic decomposition, such as

food waste, garden waste, agricultural waste, animal waste, paper and paperboard"[6]

Organic waste at Municipal level (bio-waste, or OFMSW)[6], data expressed in Mt.

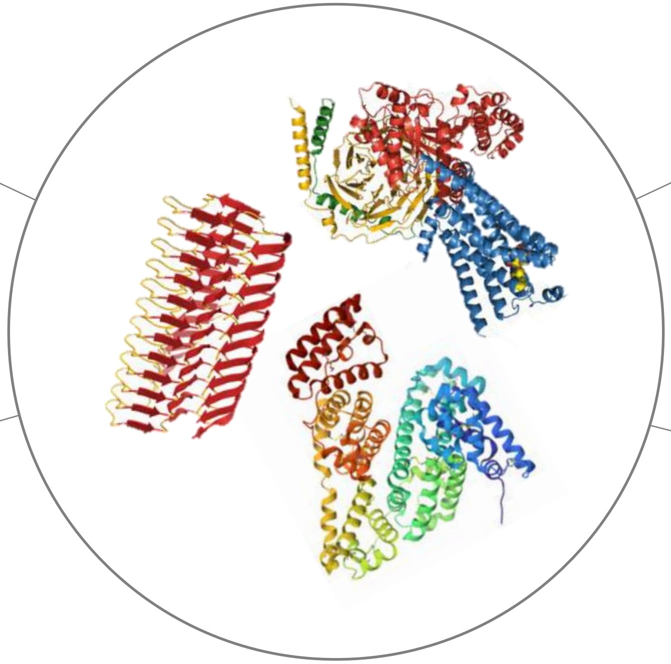


non-valorized

57% is incinerated or sent to landfill[6].
→ **GHG emissions**

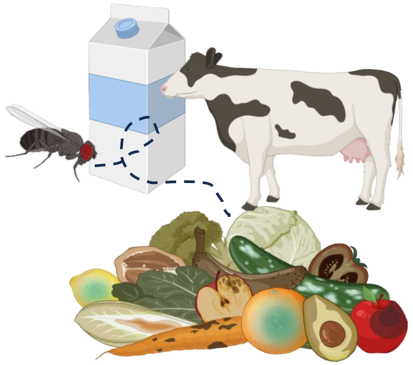
Biomass as a feedstock to recover biopolymers

PROTEINS



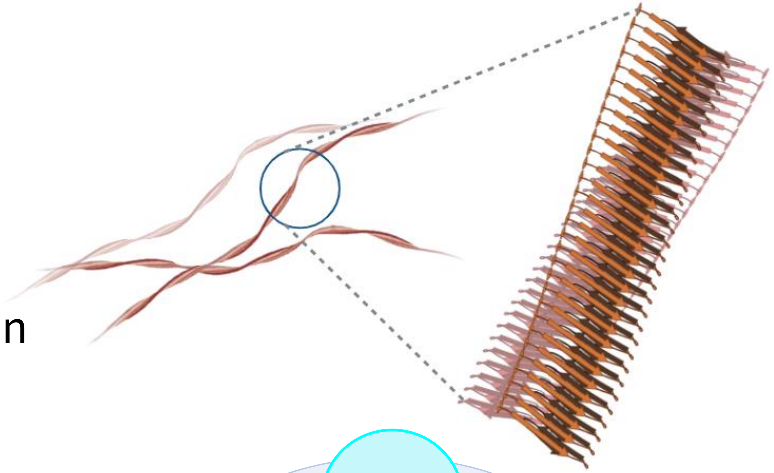
Proteins can be recovered from several of these biomass waste streams, since the production of **protein-rich waste** is reported to currently **reach** approximately **1 gigaton per year**[5].

From Proteins to Bioplastic Films: Experimental Workflow



1.
Protein source selection: BSA and BSF

2.
Protein recovery and characterization



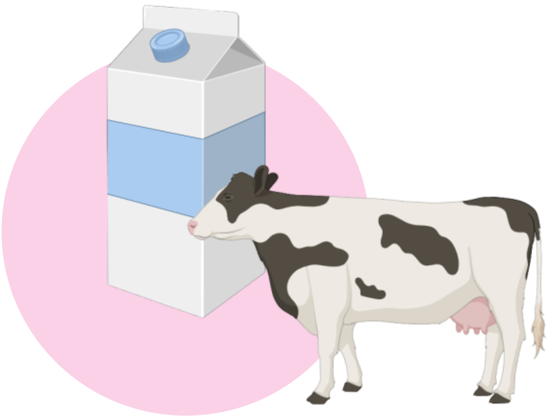
3.
Amyloid fibrils synthesis

4.
Bioplastic development

1. Protein source selection: BSA and BSF

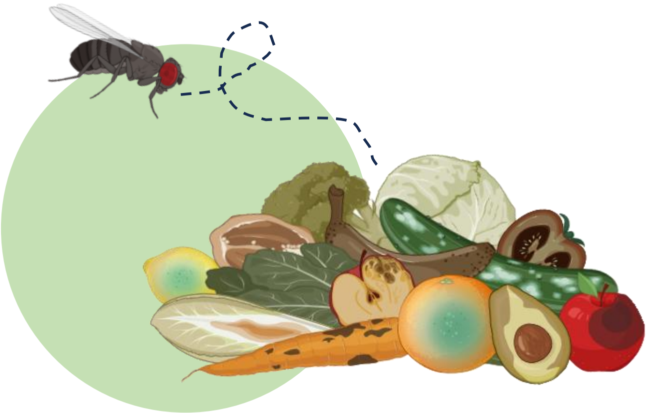
2. Protein recovery and characterization

Selected proteins employed in this work



Bovine serum albumin (BSA)

- Obtained from the **byproducts of the dairy** (milk whey) and **meat** (blood) processing **industries**;
- **globular protein** (MW = **66.5 kDa**) highly soluble in water, mainly arranged in **alpha-helices** secondary structures;
- production of **200.000 tons/year**[5].

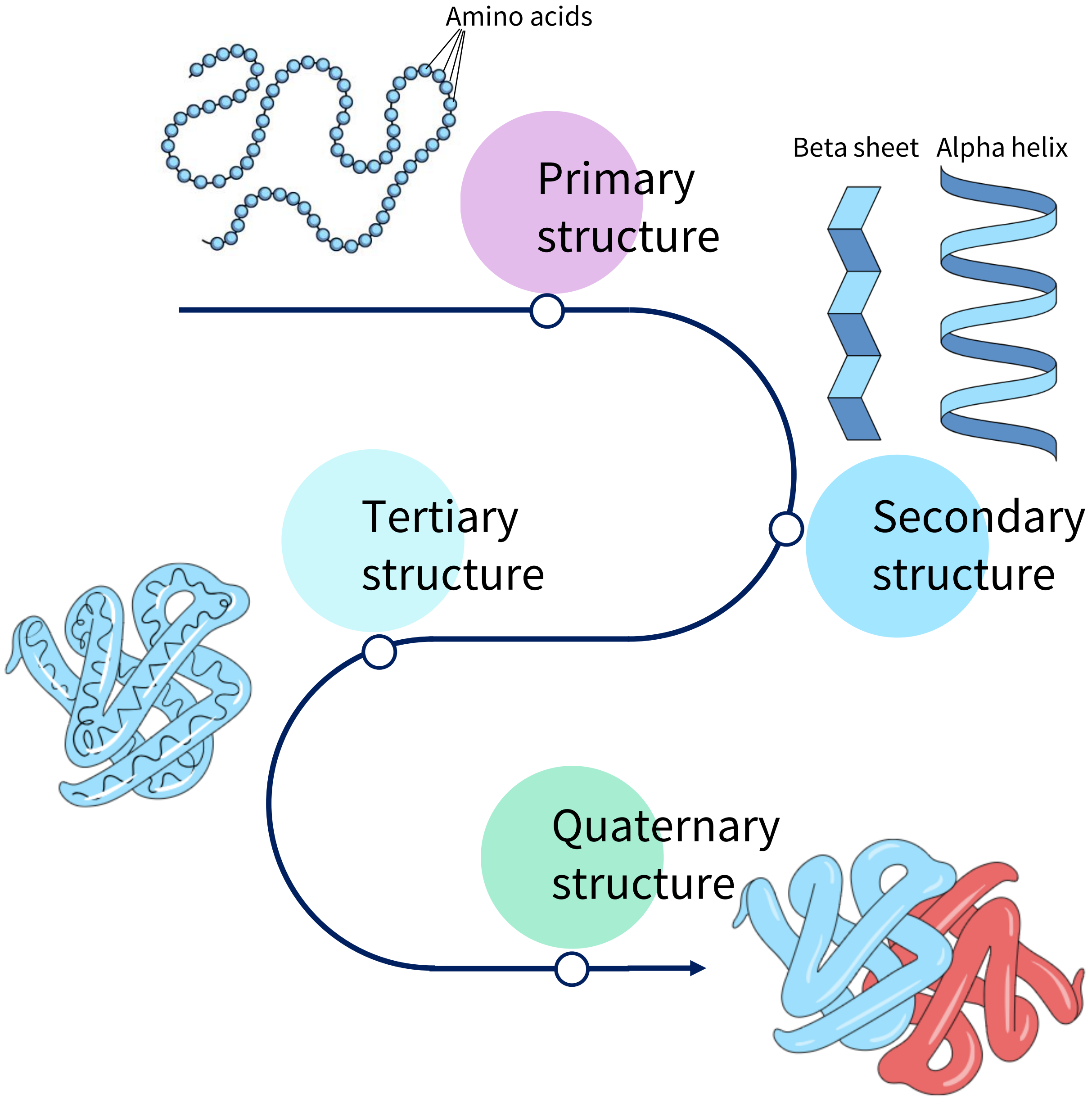


Black soldier fly (BSF) protein extract

- Obtained from the **freeze-dried biomass of BSF larvae or pupae** in an innovative circular bioeconomy chain for the **valorization of OFMSW (bioconversion)**;
- **protein content up to 35%** (wt%, on BSF dry mass)[8];
- different MW (**>250 kDa**, **75 kDa**, **55 kDa**, **20 kDa**, **10 kDa**) that our preliminary study correlated to muscular proteins namely **actin**, **myosin**, **tropomyosin** and **troponin**[7];

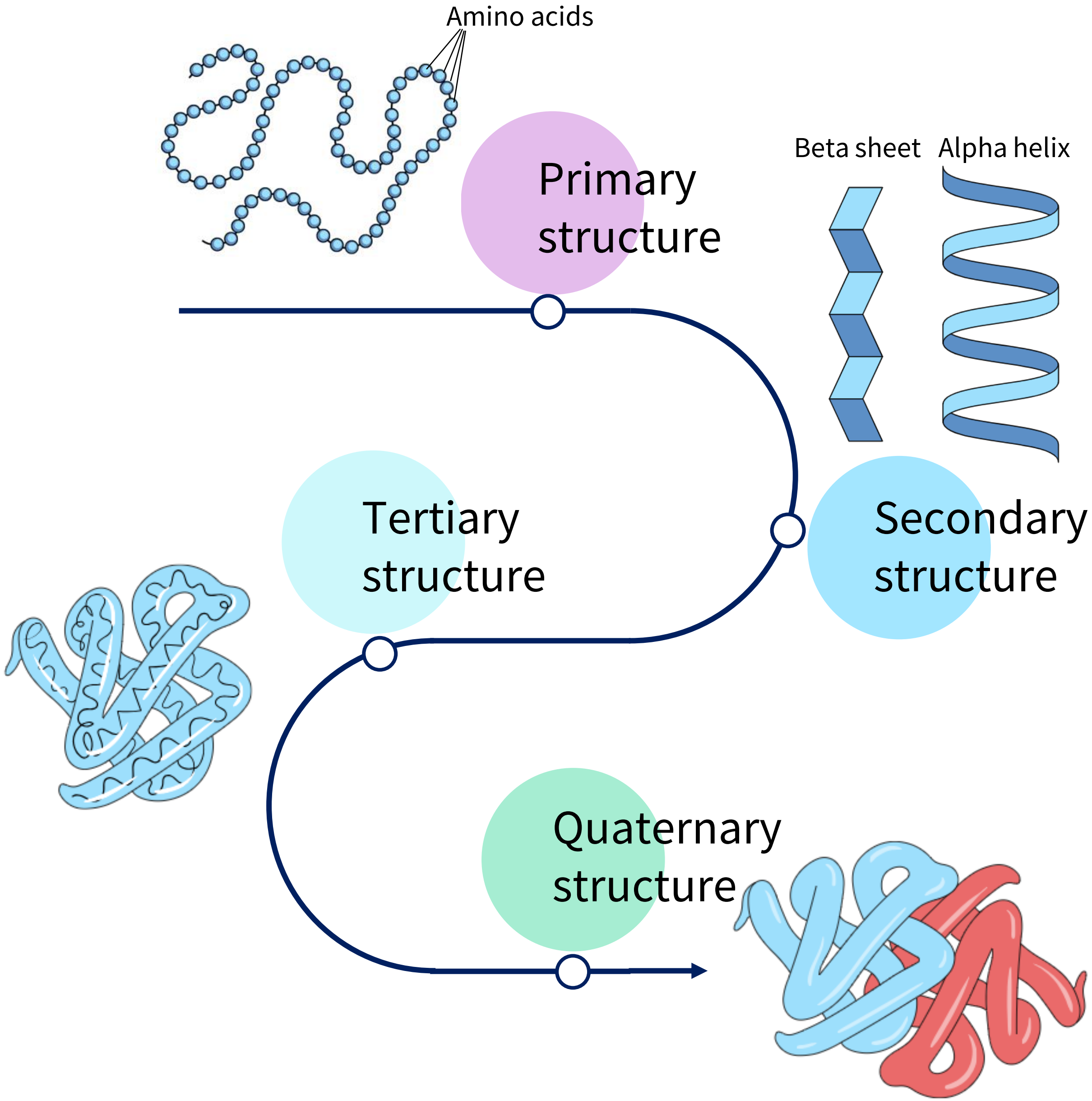
3.
Amyloid fibrils synthesis

Proteins fundamentals: hierarchical structure and peculiarities



- High differentiation and versatility
- Capacity to fulfill a broad range of functions
- Self-assemble through protein-protein interactions
- No-need for harmful solvents, catalysts or crosslinkers, as many other natural products
- Biodegrade/compost, upon triggers

Proteins fundamentals: hierarchical structure and drawbacks



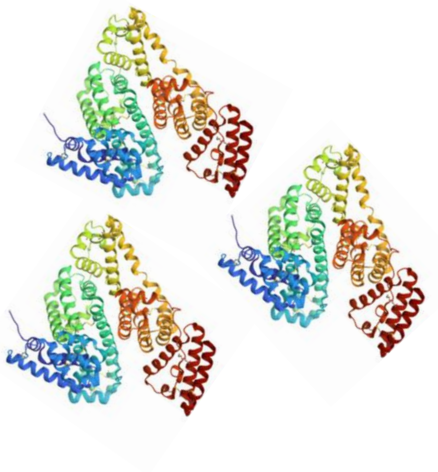
High moisture sensitivity

Low tensile strenght

Poor water-vapor barrier properties

Facing proteins-based bioplastics issues: amyloid fibrils introduction

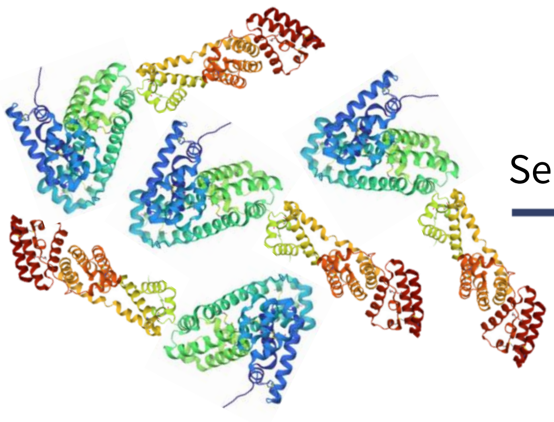
If the surrounding **environment conditions change**, protein molecules are more prone to undergo a **denaturation process:**



Folded protein molecules

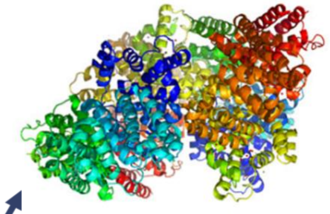
ΔT, ΔpH…

Denaturation

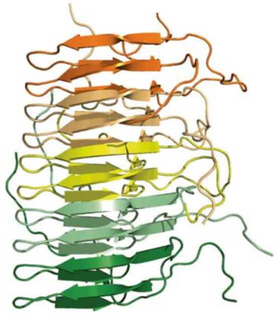


Misfolding of protein molecules and **exposure of hydrophobic core rich of β-sheets**

Self-assembling

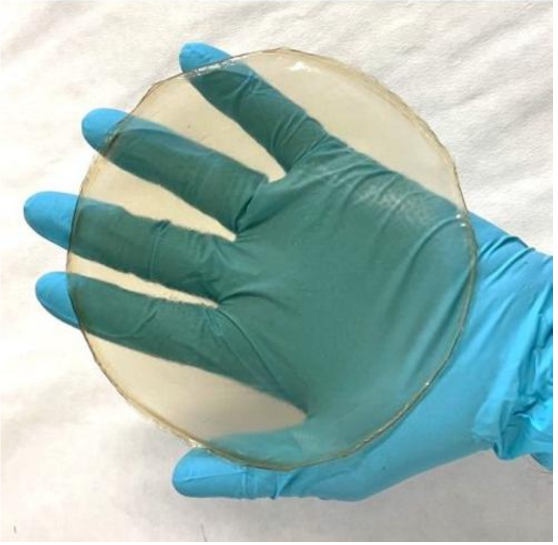


Amorphous aggregates



Ordered amyloid fibrils

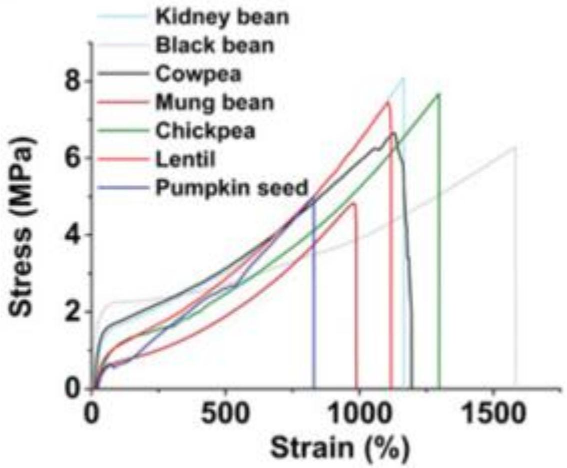
Amyloid fibrils as structural crystallites to tune protein film properties



E. Testa et al., ACS Sustainable Chem. Eng. 2025,13,2388−2400

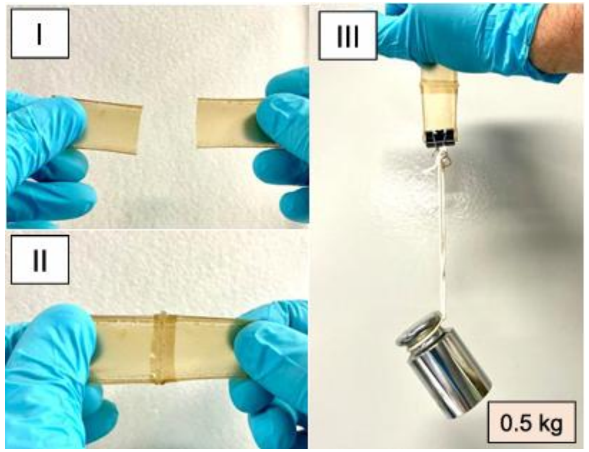
Amyloid fibril-based films

- Higher mechanical performances
- Higher homogeneity
- Higher transparency
- Higher hydrophobicity
- Lower permeability to gas and liquids



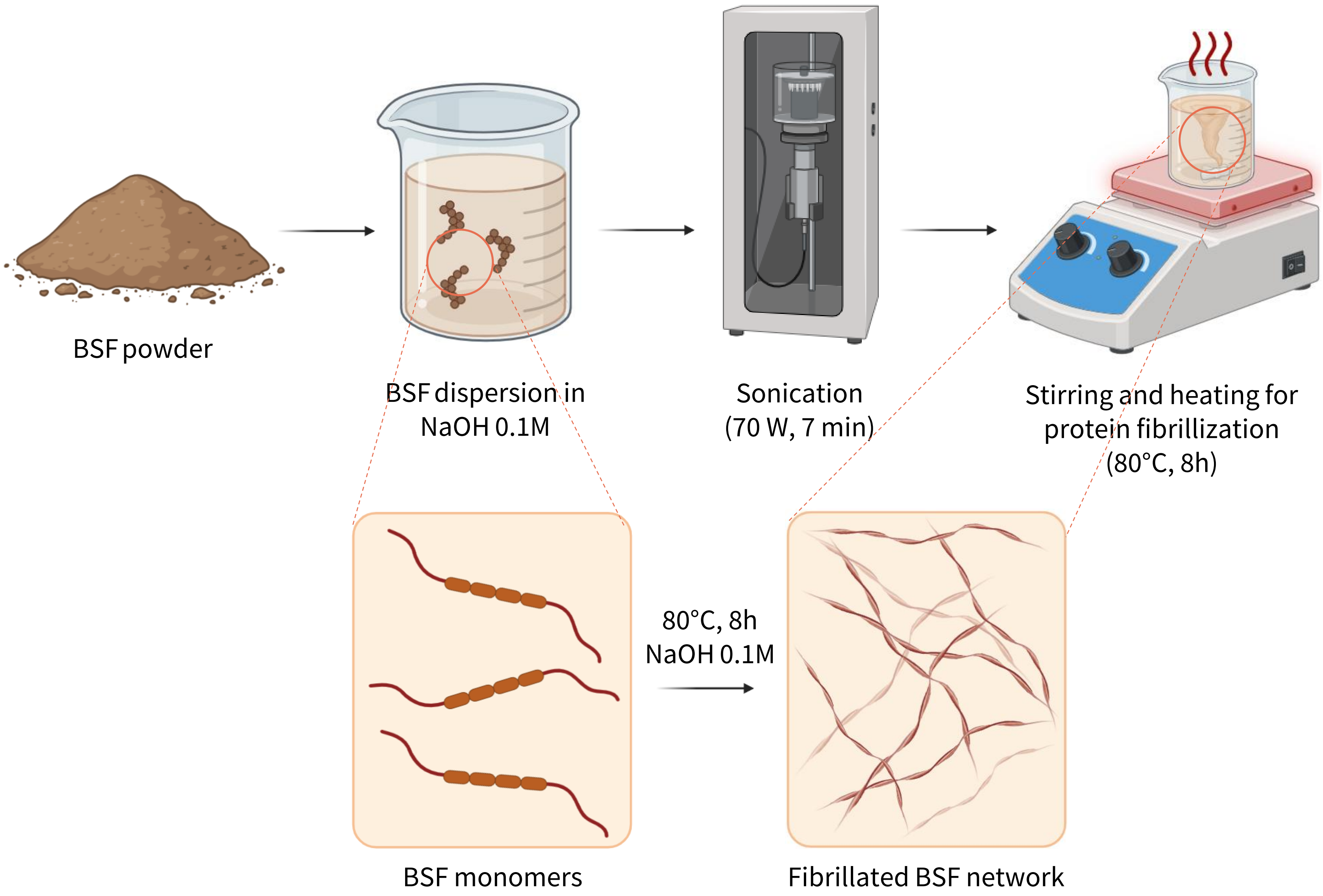
T. Li, J. Zhou, M. Peydayesh, Y. Yao, M. Bagnani, I. Kutzli, Z. Chen, L. Wang, R. Mezzenga, Plant Protein Amyloid Fibrils for Multifunctional Sustainable Materials. Adv. Sustainable Syst. 2023, 7, 2200414.

M. Peydayesh, M. Bagnani, and R. Mezzenga, Sustainable Bioplastics from Amyloid Fibril-Biodegradable Polymer Blends, ACS Sustain. Chem. Eng., vol. 9, no. 35, pp. 11916–11926, 2021, DOI: 10.1021/acssuschemeng.1c03937.

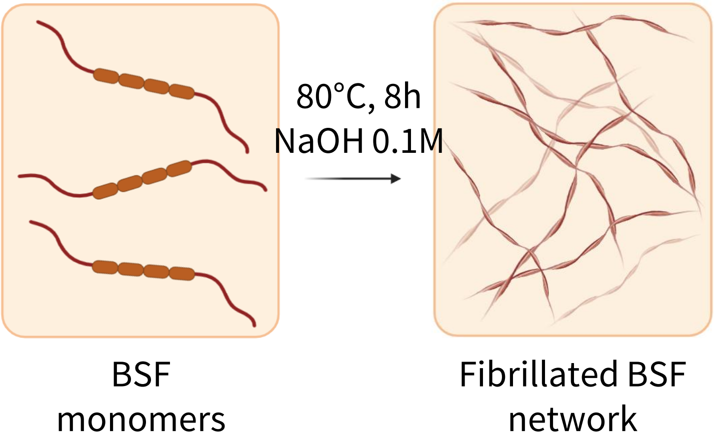


E. Testa et al., ACS Sustainable Chem. Eng. 2025,13,2388−2400

BSF fibrillization and amyloid fibrils formation evaluation

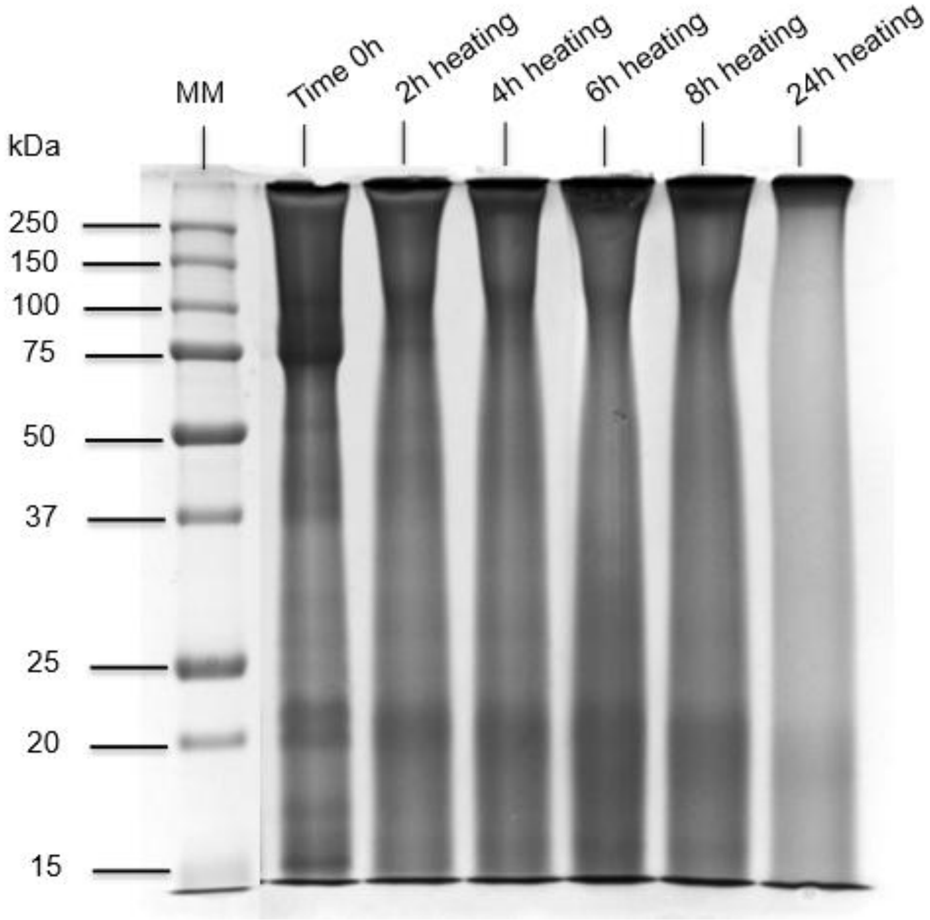


BSF fibrillization and amyloid fibrils formation evaluation



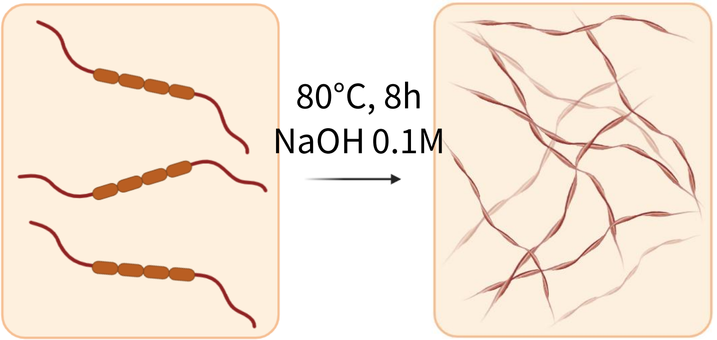
BSF fibrillization was evaluated through:

- SDS-PAGE (Mw variation)[9]



An overall **fading of the protein bands** as the heating time increased due to **hydrolysis**.
Besides, **bigger oligomers** (>250 kDa) **were formed** concurrently as the heating time increased.

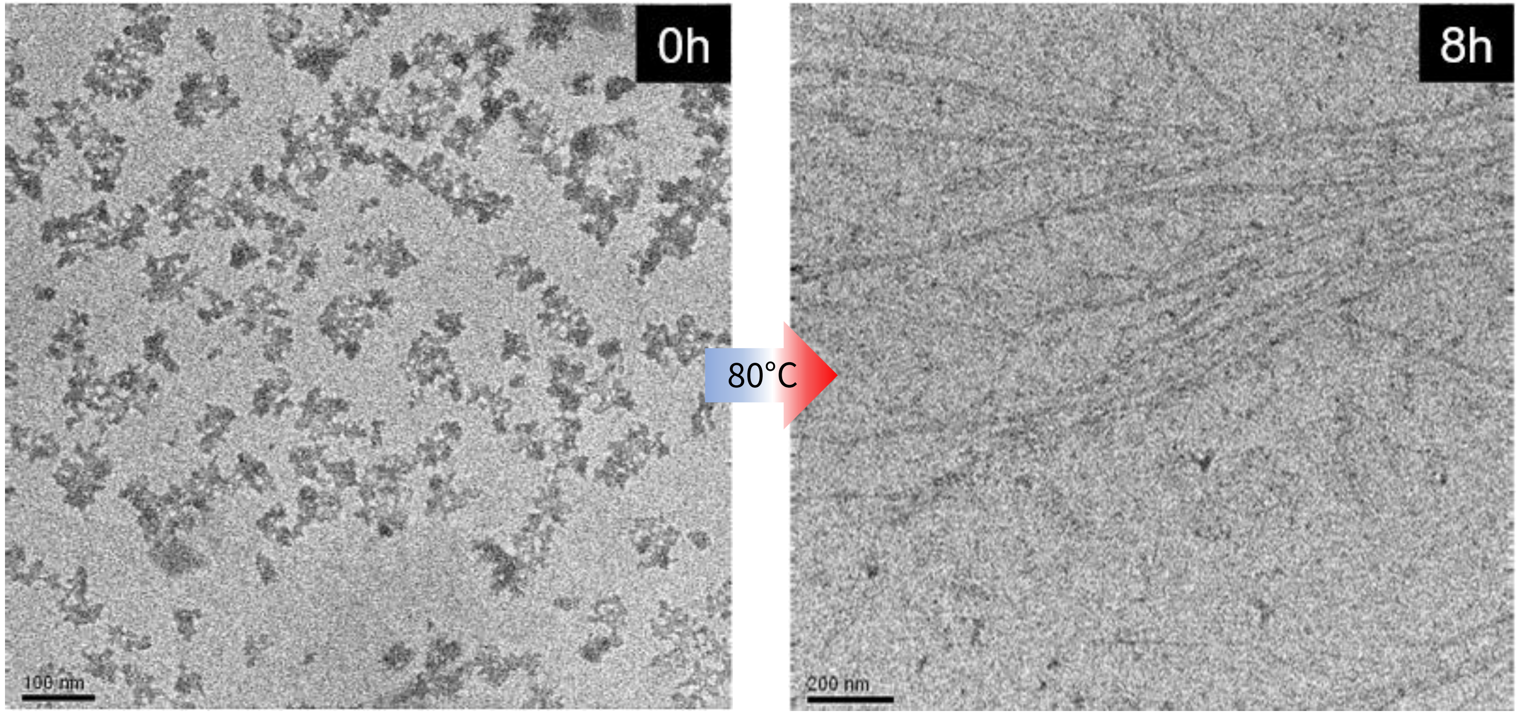
BSF fibrillization and amyloid fibrils formation evaluation



BSF fibrillization was evaluated through:

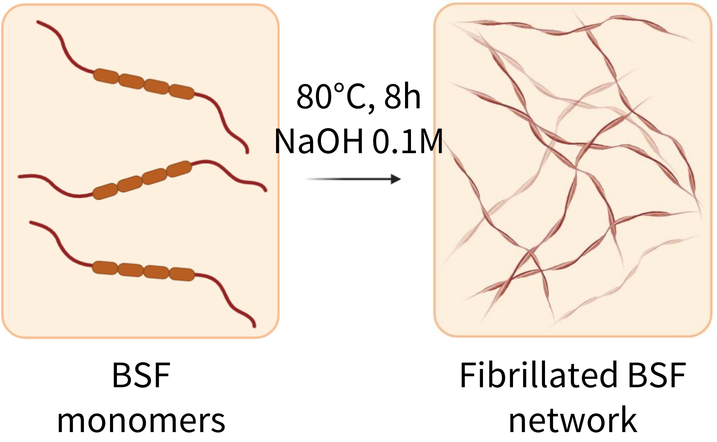
- SDS-PAGE (Mw variation)[9]

- TEM imaging[9]



Mature long fibrils (up to µm in length) were observed **after 8 hours of heating**

BSF fibrillization and amyloid fibrils formation evaluation

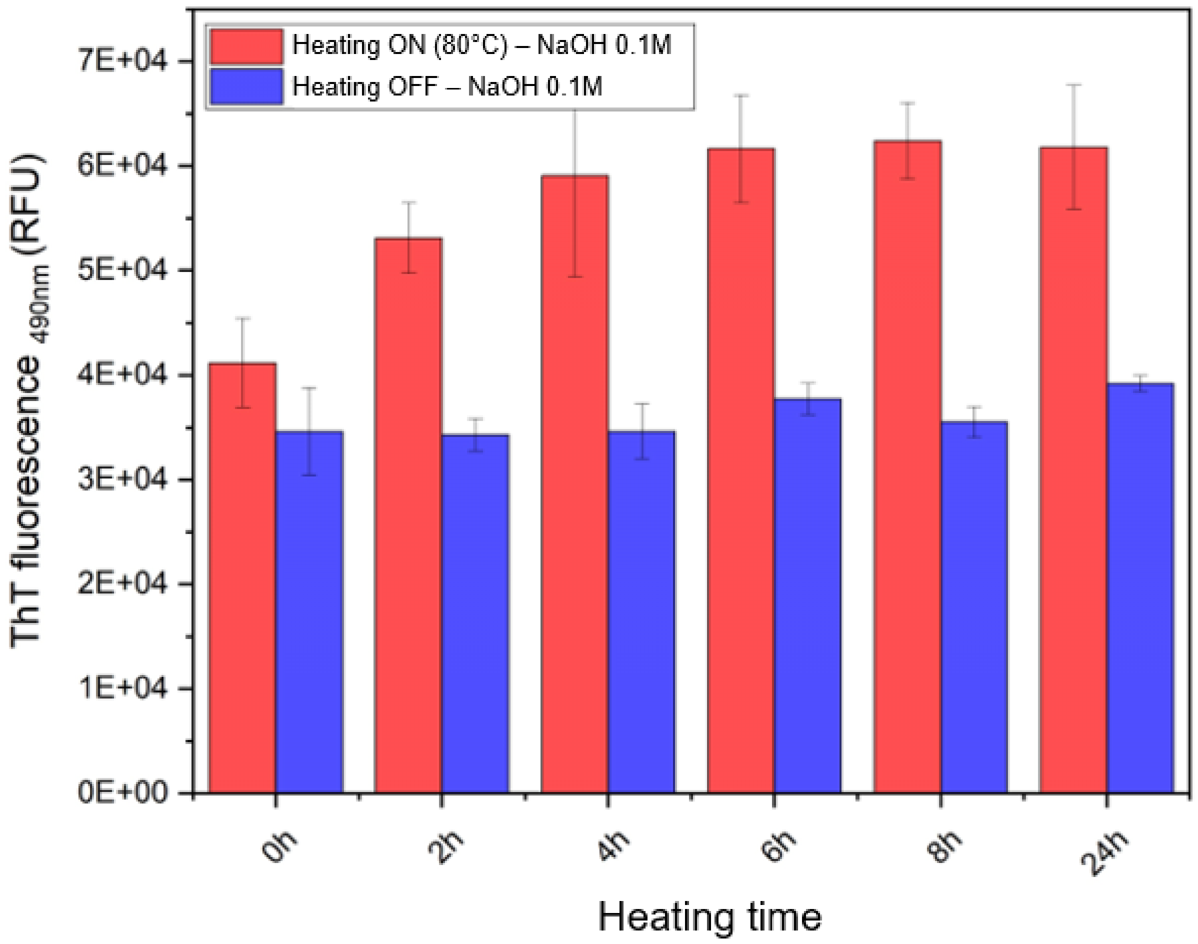


BSF fibrillization was evaluated through:

- SDS-PAGE (Mw variation)[9]

- TEM imaging[9]

- ThT fluorescence assay[9]

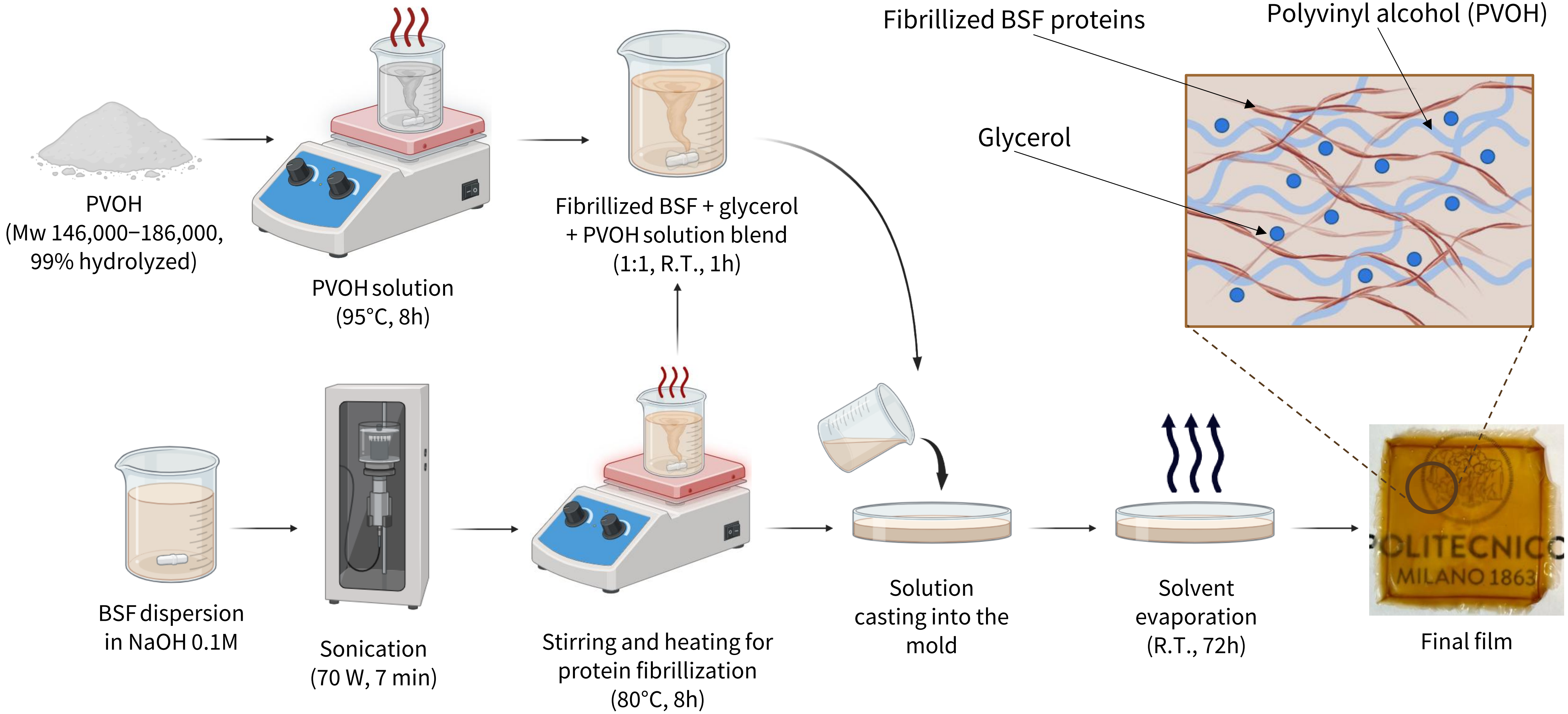


Increase in ThT fluorescence at 490 nm already after **4h of heating**

4.
Bioplastic development

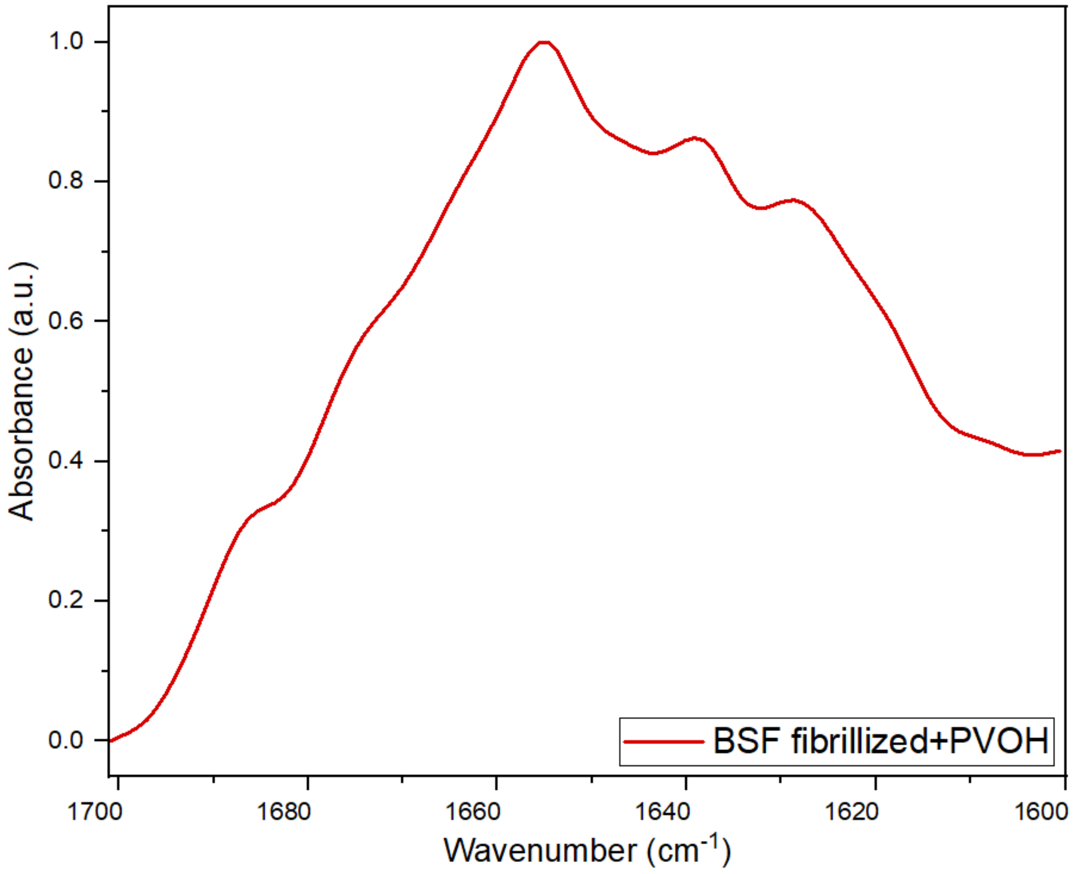
1st polymeric system: fibrillized BSF proteins and PVOH blend

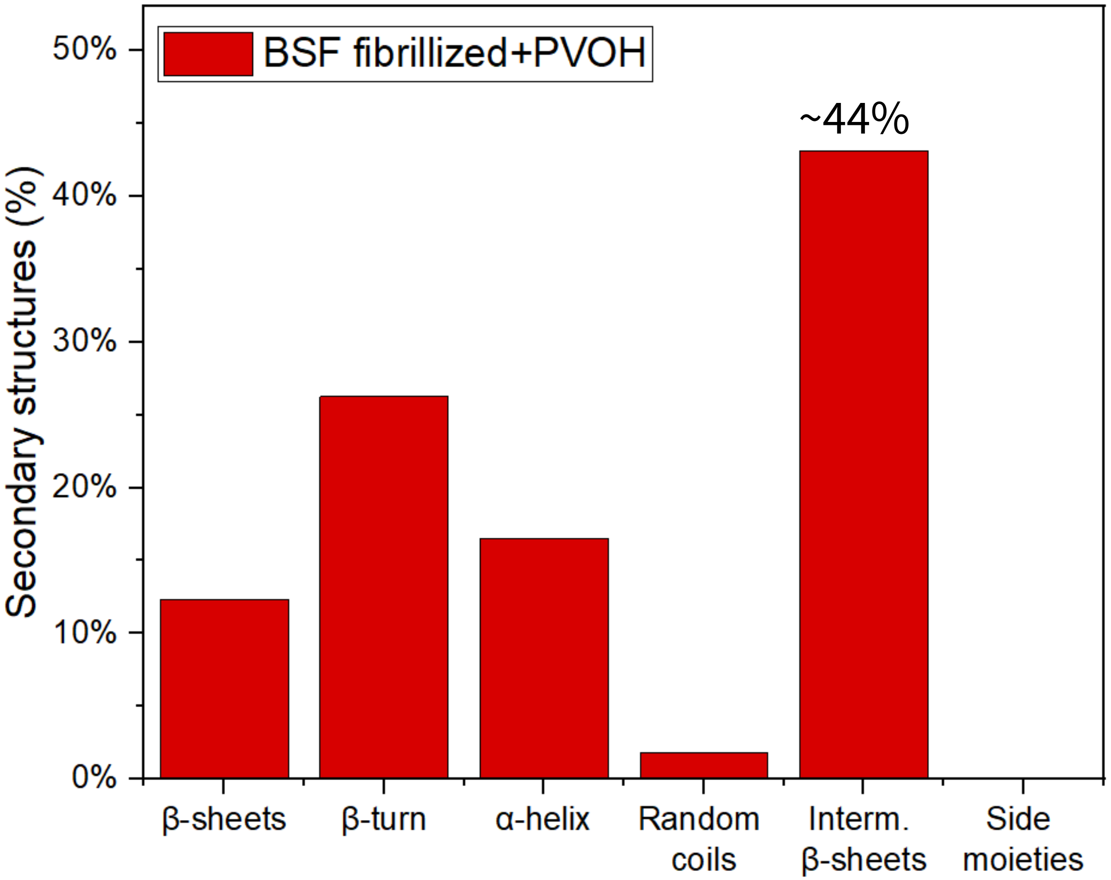
Fibrillized BSF + PVOH based film fabrication:



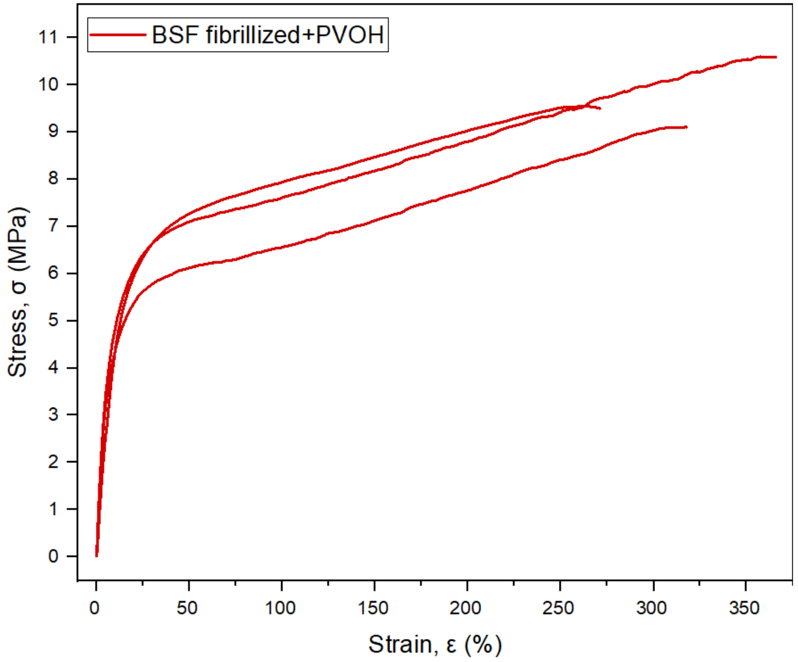
From protein organization to film properties: ATR-FTIR

ATR-FTIR spectroscopy was performed to assess the **protein organization** within the polymeric network. **Second-derivative deconvolution** of the amide I band (1600–1700 cm^{-1}) was used to quantify secondary structure contributions. The **marked presence of intermolecular β-sheet structures** (~1620 cm^{-1}) are a hallmark of **amyloid fibril formation and preservation** even after the PVOH incorporation and casting process.

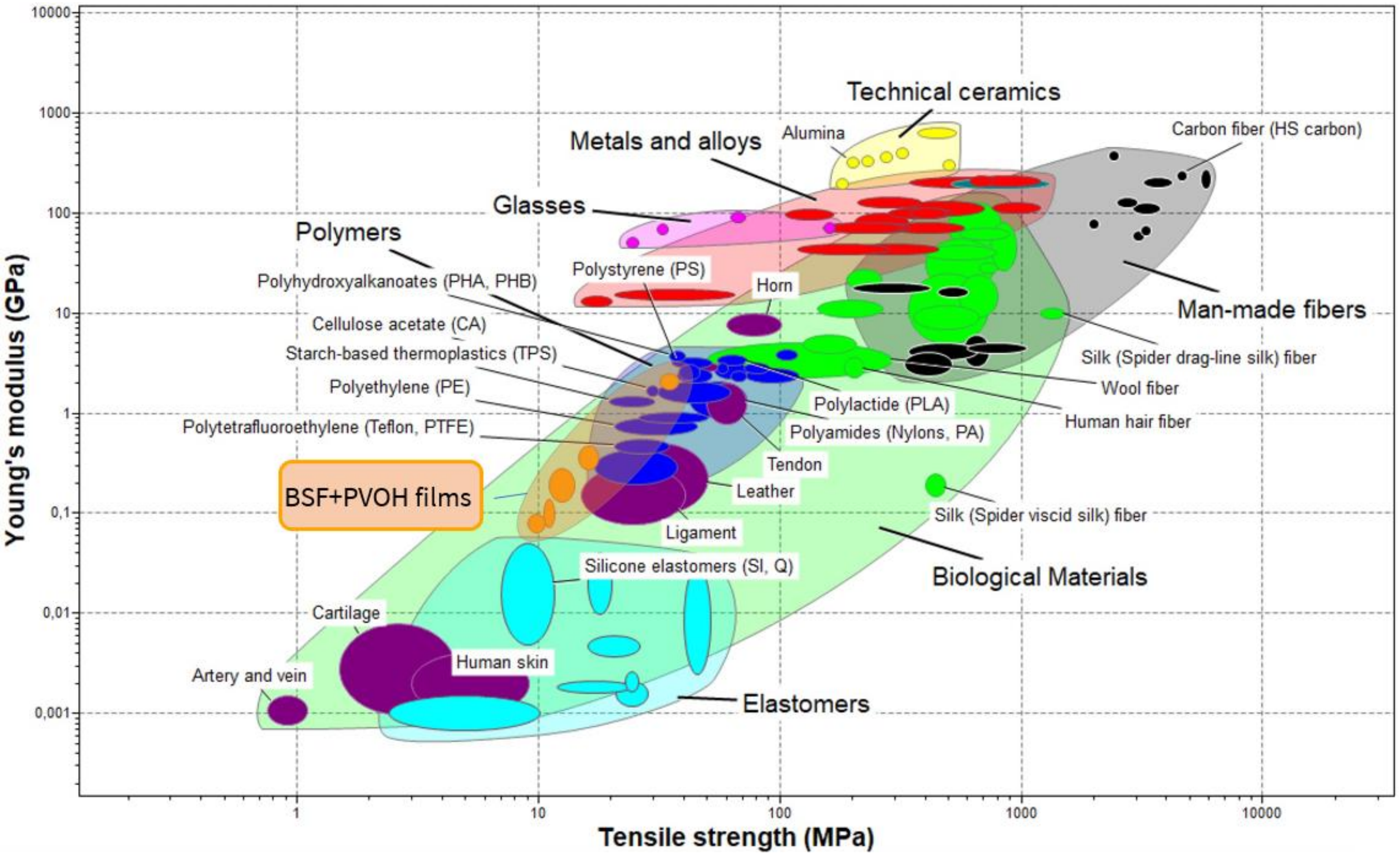




Quasi-static uniaxial tensile testing was carried out to evaluate the mechanical performance of these films. The **mechanical performance** of the films produced in this work **lies between** that of thermoplastic materials widely used in packaging, such as **PE** and **TPS**, and that of biological materials, such as **tendons** and **leather**[9].

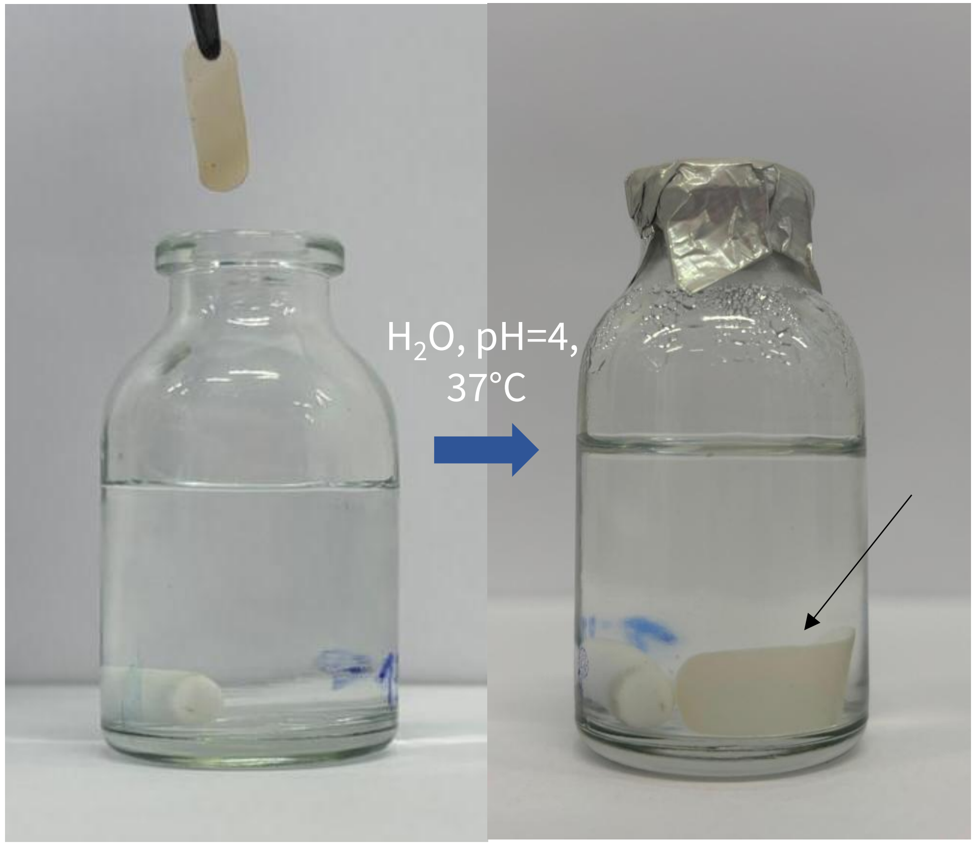


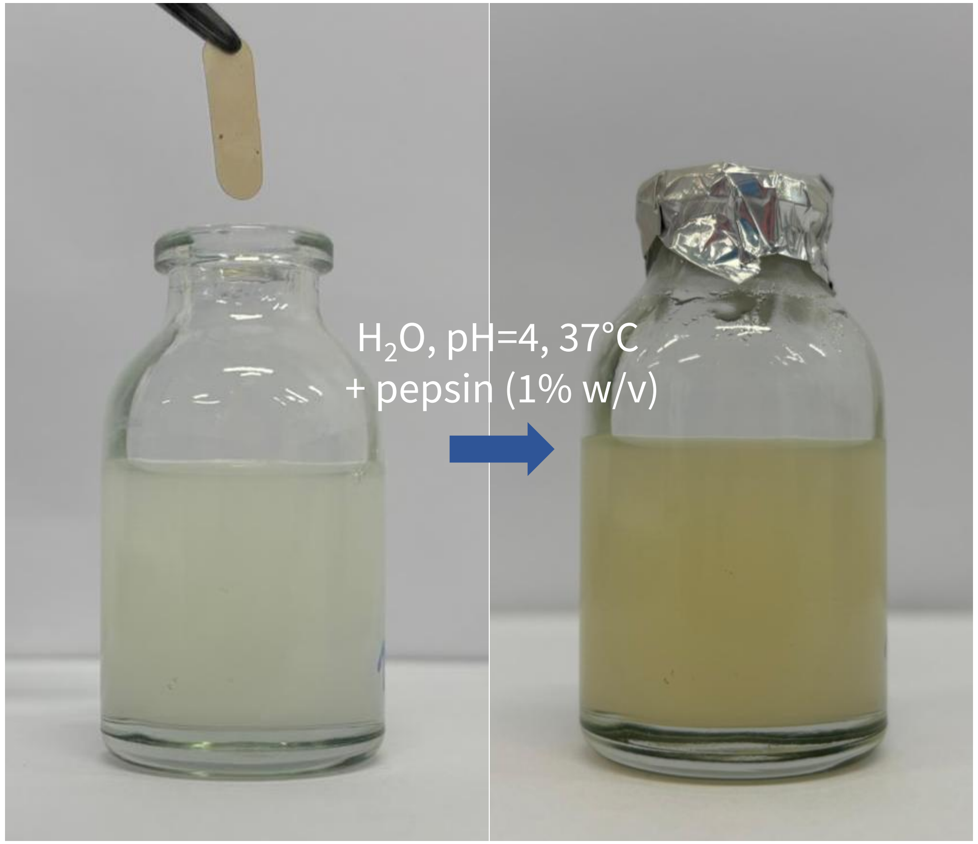
ε_b (%)	335 ± 73
σ_b(MPa)	10 ± 1
E (MPa)	87 ± 19



From protein organization to film properties: biodegradability

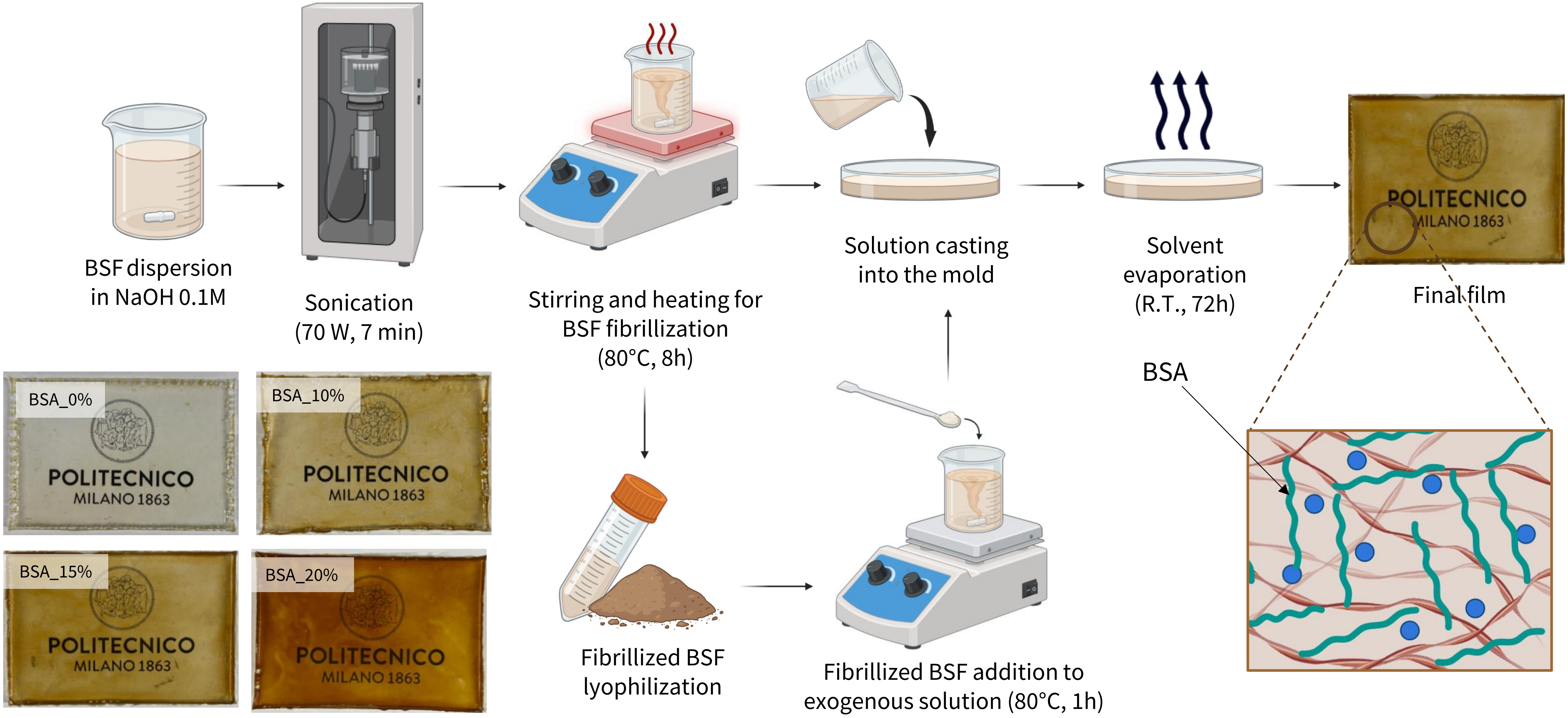
The **intermolecular hydrogen bonds** between the fibril β-sheet structures and the PVOH chains create sufficient cohesion to **resist dissolution**. However, the same material is **completely biodegradable within 5 days** when exposed to pepsin in acid solution (pH=4).





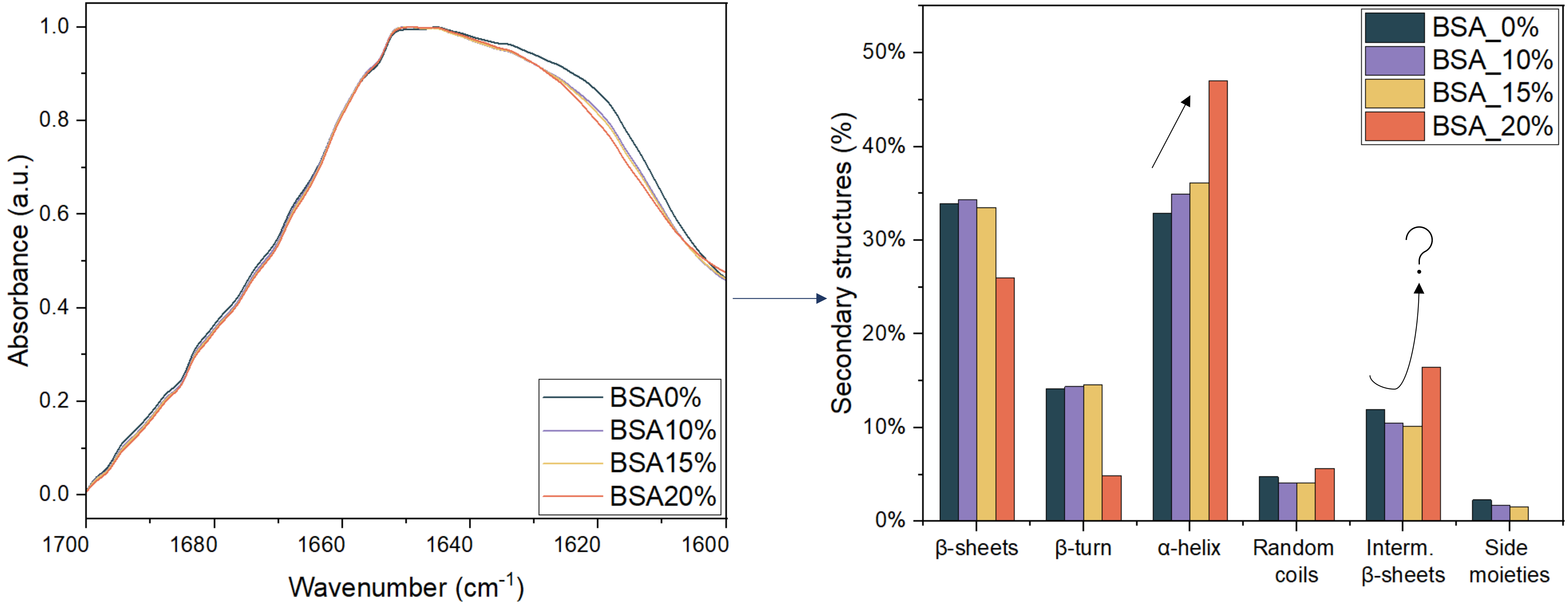
2nd polymeric system: fibrillized BSF proteins as reinforcing nanofillers

Fibrillized BSF + PVOH based film fabrication:

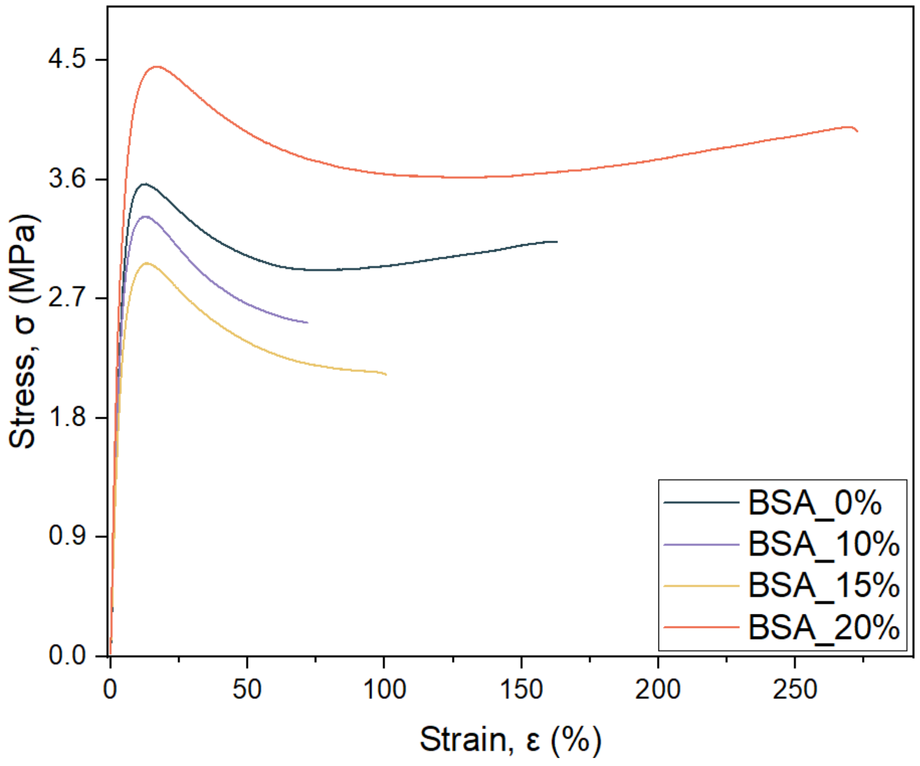


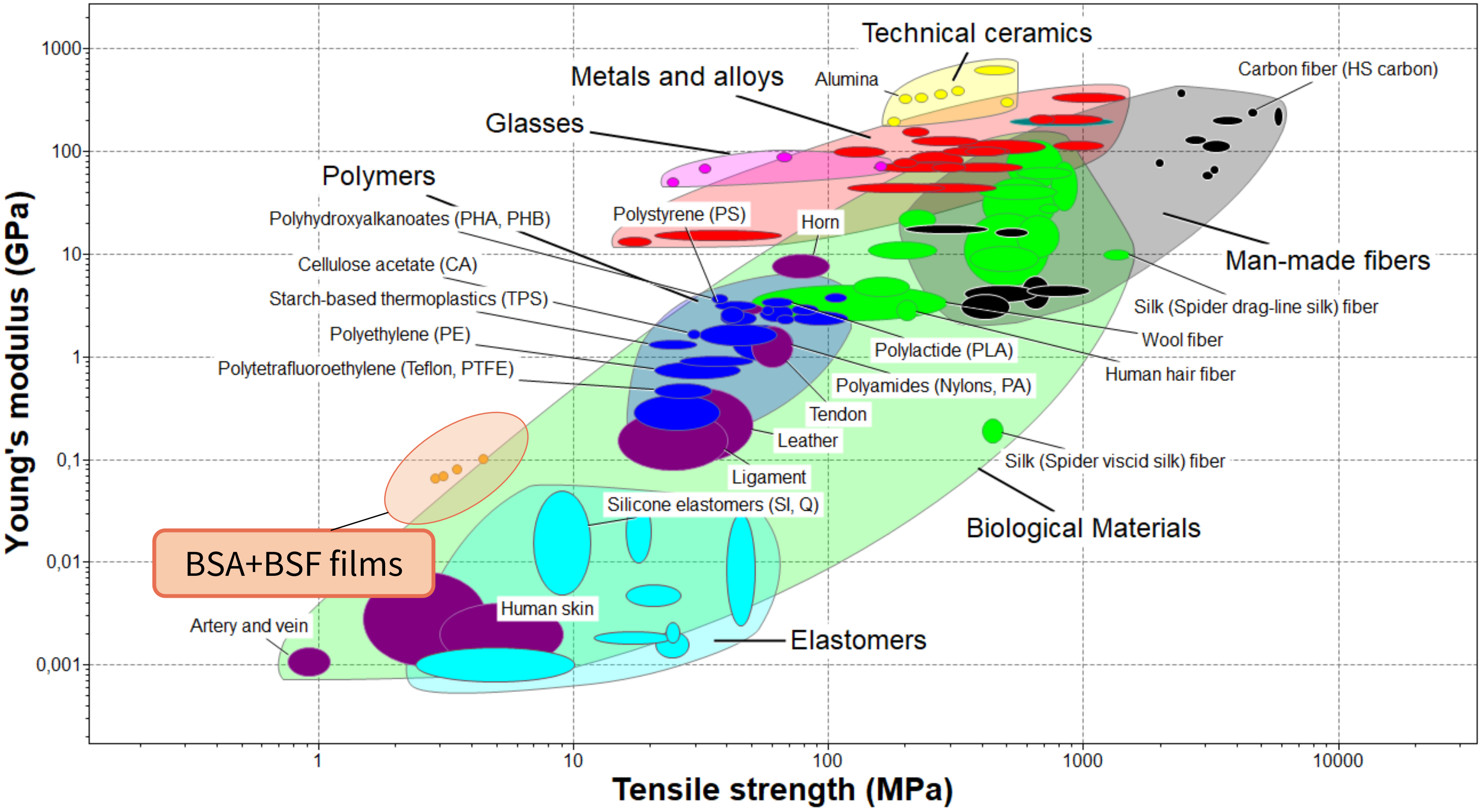
From protein organization to film properties: ATR-FTIR

At **10% and 15% of BSF** the **intermolecular β-sheets structures** remain relatively **stable**, while at **20% an increase** is observed. This pattern suggests that at high concentrations of BSF fibrils, a **more intense structural interaction** is established between the fibrils and the BSA matrix, in which amyloid fibrils **induce a partial conformational reorganization of the BSA chains**, creating a denser and more interconnected supramolecular network.



From protein organization to film properties: tensile test



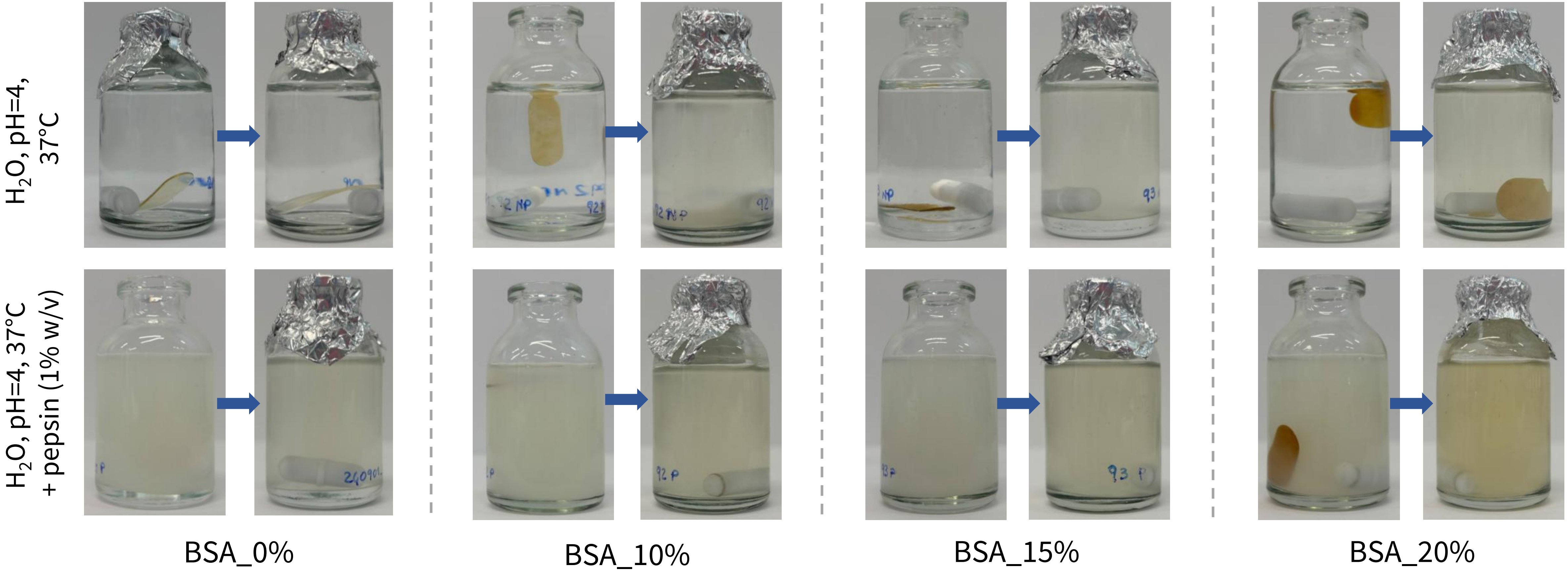


	BSA_0%	BSA_10%	BSA_15%	BSA_20%
ε_b (%)	177.25 ± 6.78	157.8 ± 4.5	119.55 ± 3.38	280.96 ± 3.12
σ_b(MPa)	2.53 ± 0.73	1.64 ± 0.91	0.67 ± 0.12	3.05 ± 1.03
E (MPa)	82.7 ± 2.76	66.9 ± 2.15	71.3 ± 1.48	104.8 ± 6.3

At **20% BSF**, a **β-sheet network forms enhancing tensile strength and Young's modulus**, indicating a threshold concentration for effective reinforcement.

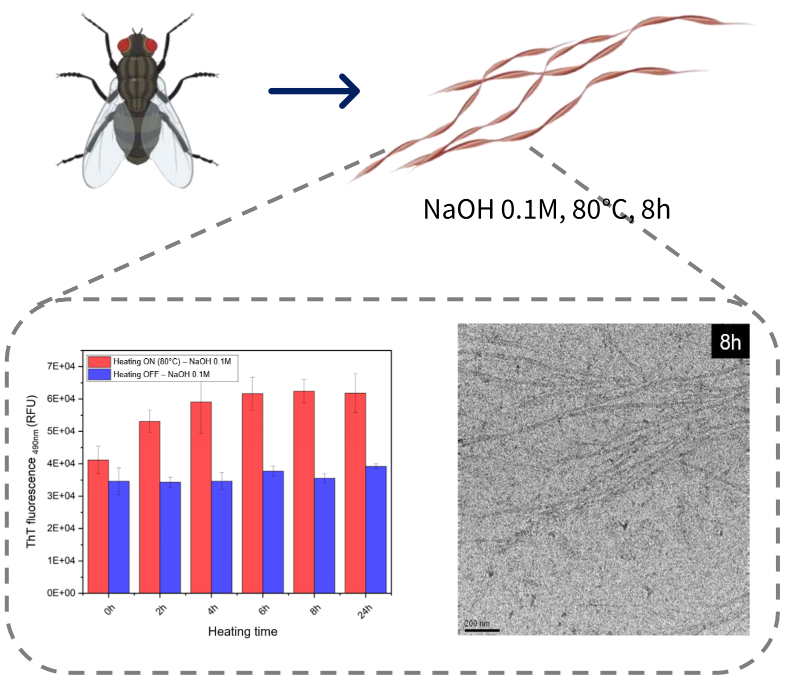
From protein organization to film properties: biodegradability

Water insolubility is observed with **0% and 20% BSF**, while intermediate concentrations remain soluble. At 20% BSF, the **denser intermolecular β-sheet network** provides sufficient structural cohesion to prevent dissolution. Despite different water stability, **all films remain enzymatically biodegradable within 24h**, highlighting the potential to combine water stability with biological degradability.

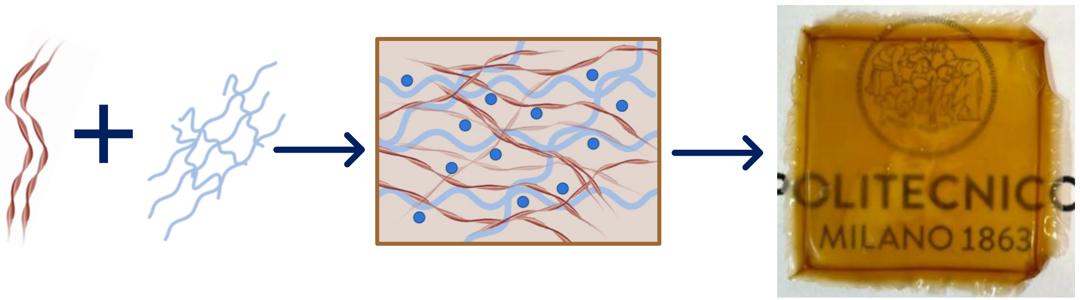


Conclusions

BSF proteins → Amyloid fibrillization

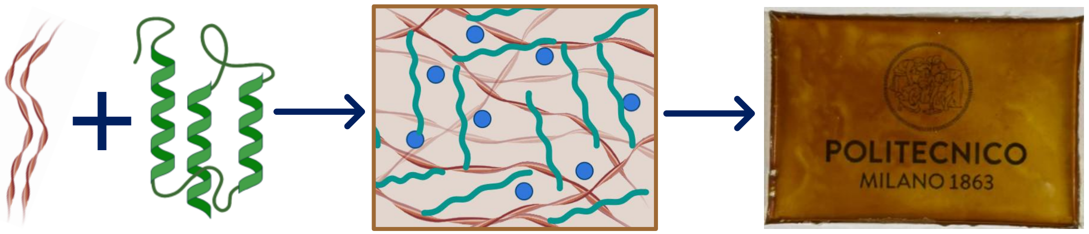


1st polymeric system: fibrillized BSF proteins and PVOH blend



- ✓ High ductility (ε = 335 ± 73%)
- ✓ High tensile strenght (σ = 10 ± 1 MPa)

2nd polymeric system: fibrillized BSF proteins as reinforcing nanofillers



- ✓ Increased σ (3.05 ± 1.03 MPa)
- ✓ Increased E (104.8 ± 6.3 MPa)

✓ BOTH SYSTEM ARE WATER STABLE YET FULLY **BIODEGRADABLE**

✓ **AMYLOID FIBRILLIZATION** IS A VERSATILE STRATEGY TO **TUNE THE STRUCTURAL AND MECHANICAL PROPERTIES** OF PROTEIN-BASED FILMS FROM WASTE-DERIVED PROTEIN

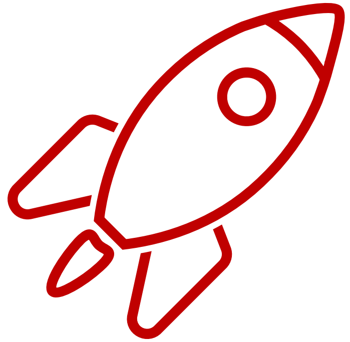
Future developments

- **Matrix substitution**: **replace BSA with sericin** to develop a fully waste-derived, all-protein bioplastic system aligned with circular bioeconomy principles

- Process optimization: apply Design of Experiments (DoE) to systematically **optimize fibril concentration,** plasticizer content and processing conditions

- Morphological characterization: **AFM of BSF fibrils in solution** (persistence length, contour length) **and SEM** of fractured film cross-sections to establish direct structure-property correlations

- **Barrier properties**: measure oxygen and water vapor permeability to benchmark both systems against conventional packaging polymers



Acknowledgements

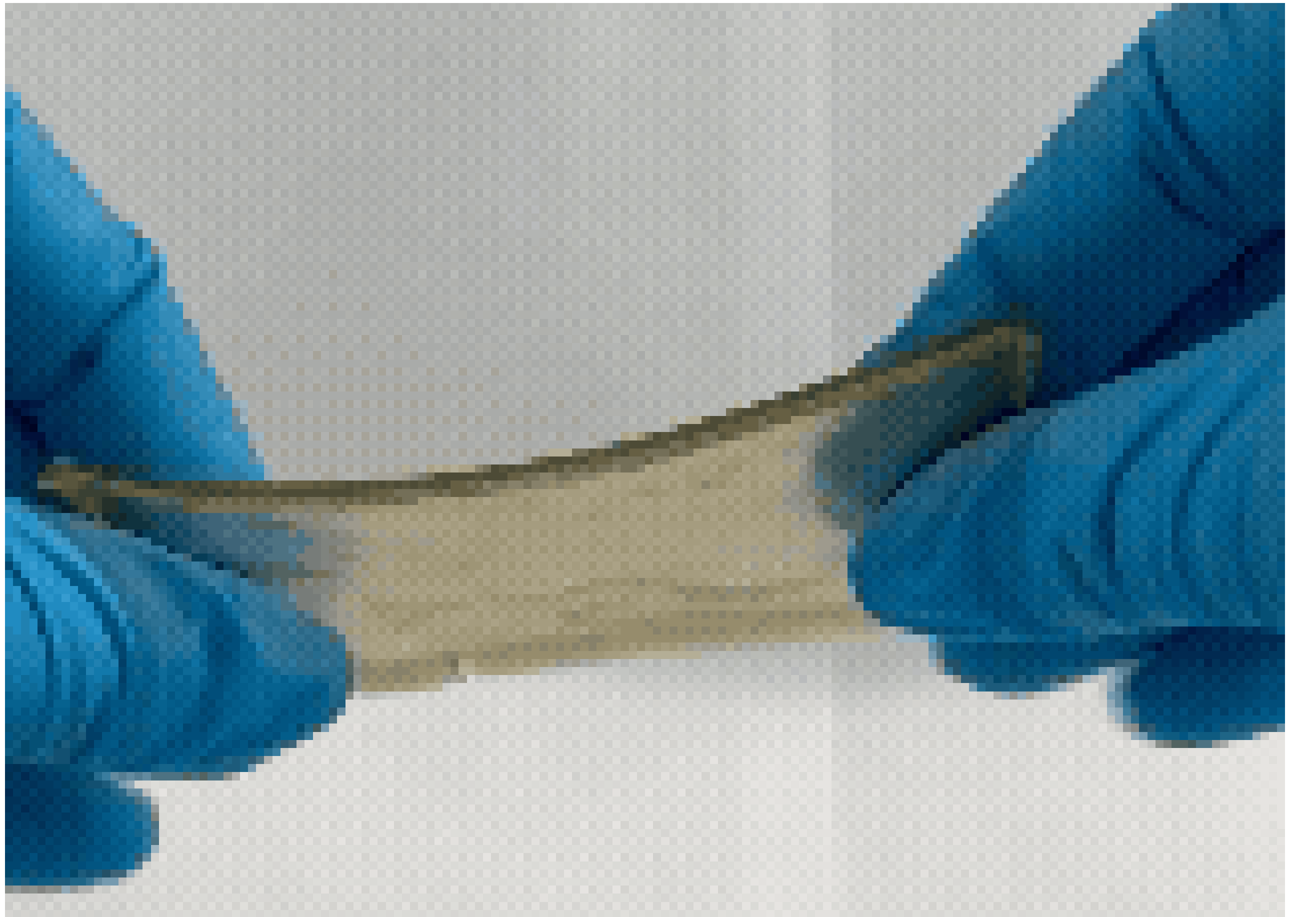
Progetto finanziato dall'Unione Europea – NextGenerationEU | PNRR – Piano Nazionale di Ripresa e Resilienza | MUR – Ministero dell'Università e della Ricerca

This PhD project is funded through ENI S.p.A. within the framework of the PNRR – NextGenerationEU program and was carried out at Politecnico di Milano – Department of Chemistry, Materials and Chemical Engineering 'Giulio Natta.

ISMaterials Group, Politecnico di Milano – Department of Chemistry, Materials and Chemical Engineering 'Giulio Natta.

Prof M. Galimberti, prof. V. Barbera, Dott. D. Gentile

Follow us on Instagram: @ismaterials.polimi



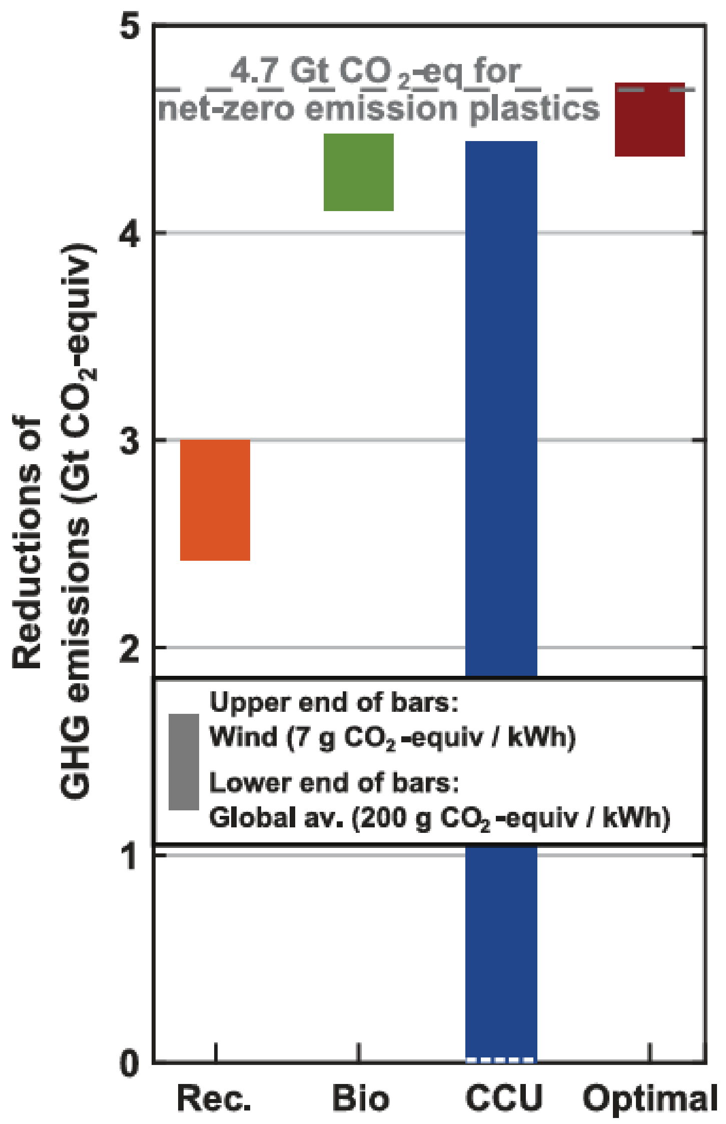
Thank you for your kind attention!

References

[1] https://iea.blob.core.windows.net/assets/209af090-8713-42b6-ac5b-650c6e68bccc/-12DEC2024_OilMarketReport.pdf
[2] “*Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components*”, Field et al., Science 281, 237-240 (1998)
[3] “*Achieving net-zero greenhouse gas emission plastics by a circular carbon economy*”, Meys et al., Science 374,71–76 (2021)
[4] "*What does sustainable mean in the context of plastics?*“, lecture of prof. Anthony (Tony) J. Ryan at Plastics & Circular Economy conference (2021)
[5] “*Turning Food Protein Waste into Sustainable Technologies*”, M. Peydayesh, M. Bagnani, W. L. Soon, R. Mezzenga (2022).
[6] https://circulareconomy.europa.eu/platform/sites/default/files/th-al-20-010-en-n_bio-waste_in_europe_-_18_06_20.pdf
[7] “*Electroconductive Bionanocomposites from Black Soldier Fly Proteins for Green Flexible Electronics*”, E. Testa et al., ACS Sustainable Chem. Eng. 2025,13,2388−2400
[8] “*Environmental impact scenarios of organic fraction municipal solid waste treatment with Black Soldier Fly larvae based on a life cycle assessment*”, N. Ferronato et al., Environmental Science and Pollution Research (2024) 31:17651–17669
[9] “*Amyloid-Like Self-Assembling of Black Soldier Fly Proteins and Development of Bioplastics*”, E. Testa et al., ACS Sustainable Chem. Eng. 2025,13,18713−18726

Supplementary information

Net-zero greenhouse gas emission plastics by a circular carbon economy



The graph shows **how much GHG emissions** (Y-axis, in Gt CO_2 equivalent) **are reduced compared to the fossil-based reference scenario**, for four different circular paths to net-zero plastics as of 2050.

The **recycling pathway** (Rec.), **reduces GHG emissions by 3.0 billion tons** (Gt) **of CO2 equivalent** (CO2-equiv) compared with the linear carbon pathway.

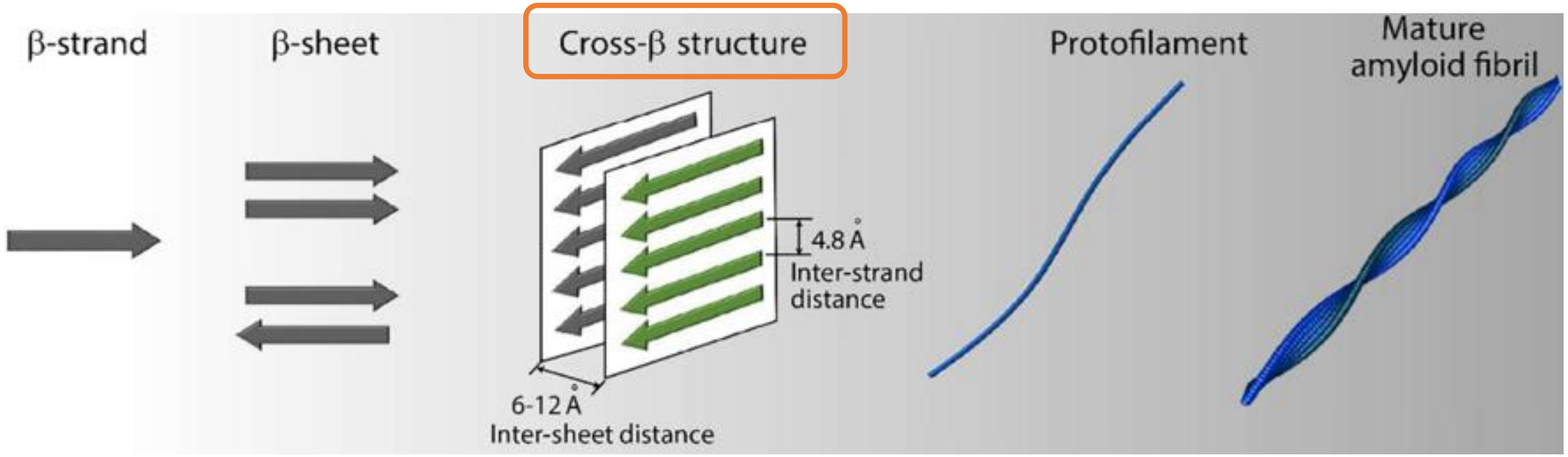
Up to **4.5 Gt of CO2-equiv** (95%) are reduced by a **biomass** (Bio) pathway.

Whereas plastic waste and biomass provide carbon and sufficient energy for conversion, **carbon capture and utilization (CCU) technologies** require electricity with a low carbon footprint to reduce GHG emissions, such as **wind power**, that enables CCU technologies to reduce up to **4.4 Gt of CO2-equiv**.

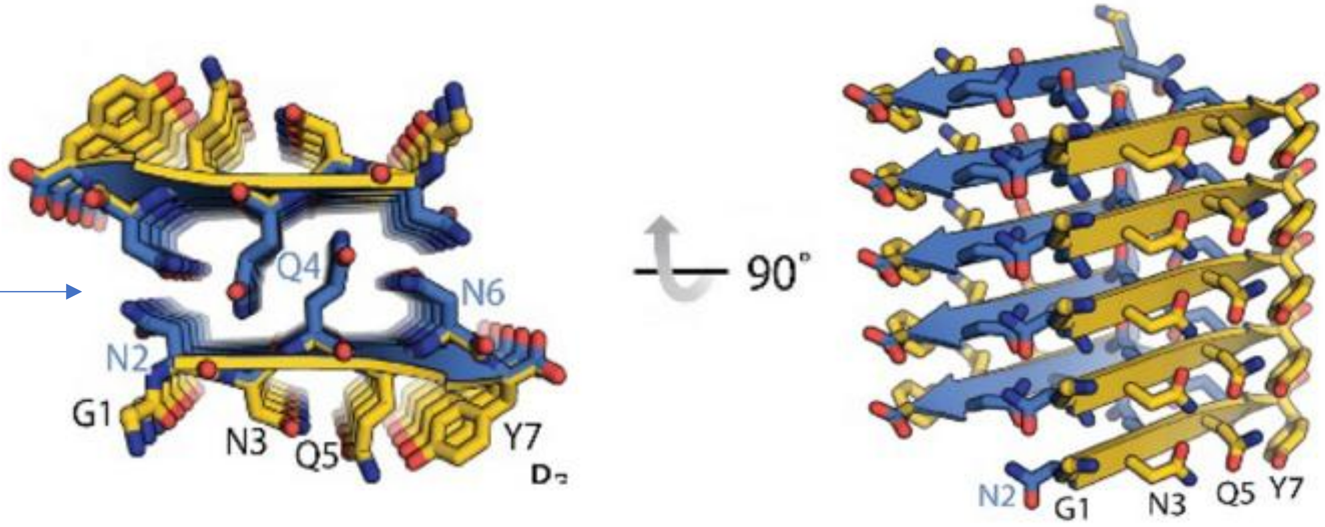
These single-technology pathways present residual GHG sources that prevent fully net-zero emission plastics. By contrast, the **circular carbon pathway that optimally combines recycling, biomass utilization, and CCU** reduces GHG emission of plastics by as much as **4.73 Gt of CO2-equiv**, assuming **wind-based electricity**. Thus, the plastics' life cycle would even be slightly net-negative.

Amyloid fibrils structure

Amyloid fibrils originate from the aggregation of β-sheets secondary structures



Steric zipper



This structure is super stable.

Cao, Y. & Mezzenga, R. Food protein amyloid fibrils: Origin, structure, formation, characterization, applications and health implications. *Adv Colloid Interface Sci* **269**, 334–356 (2019).

Preparation of films. Intermolecular aggregation between β-sheets