Bridging the gap from raw microalgae to bioplastic: conversion of *Chlorella vulgaris* into thermoplastic starch

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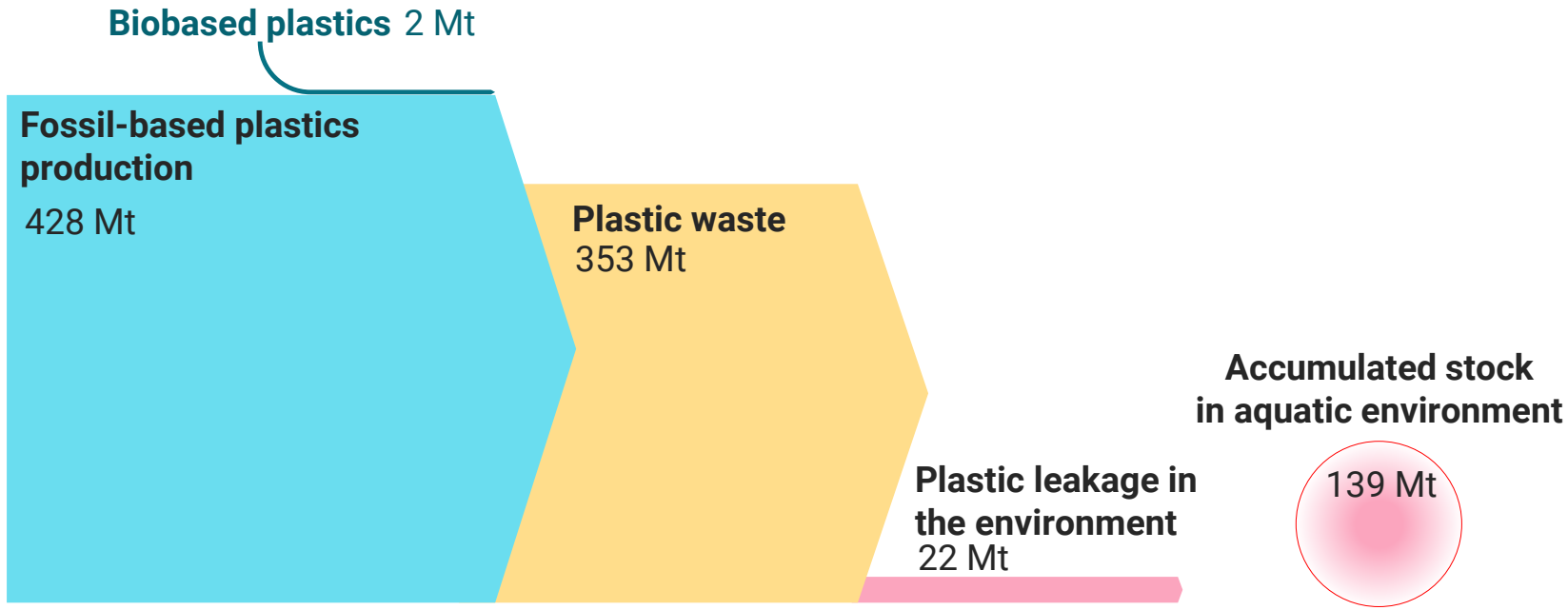
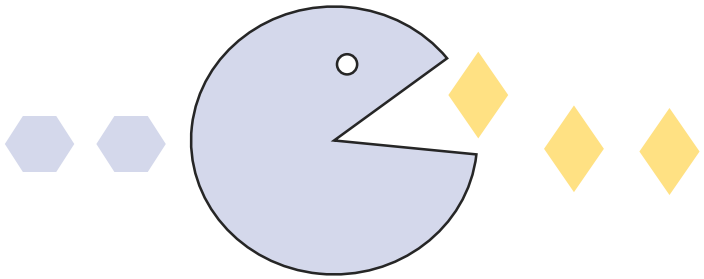


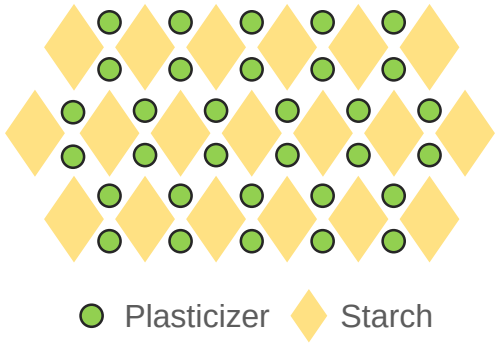
Plastic life-cycle in 2019 (figure on scale). These numbers are expected to triple by 2060.
Source: OECD, Global Plastics Outlook, 2023.

Starch is a main feedstock for more than 80 % of biobased-biodegradable plastics.



Fermentation

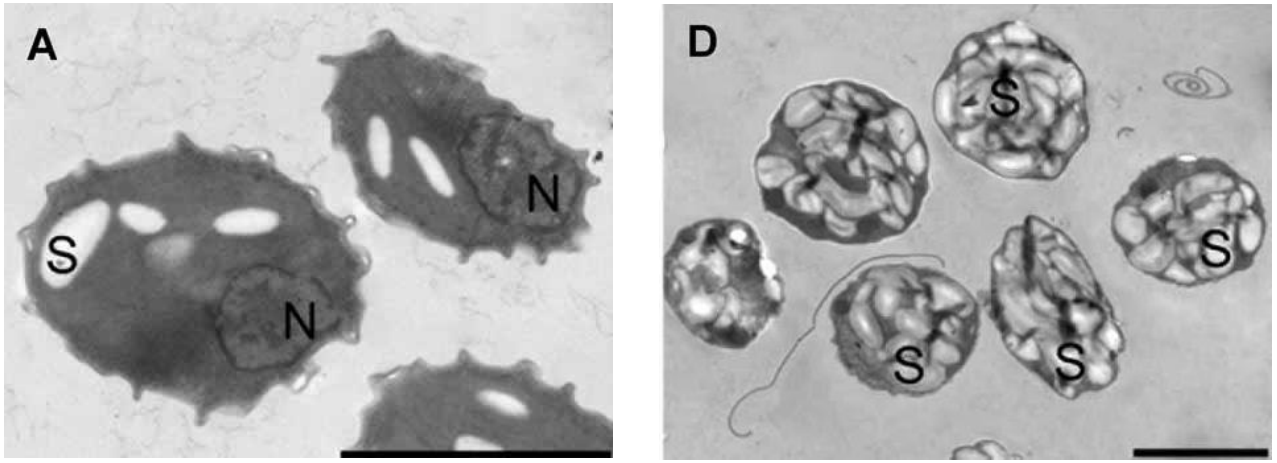
Polyhydroxyalkanoate PHA
PLA, PBAT, PBS



Direct plasticization

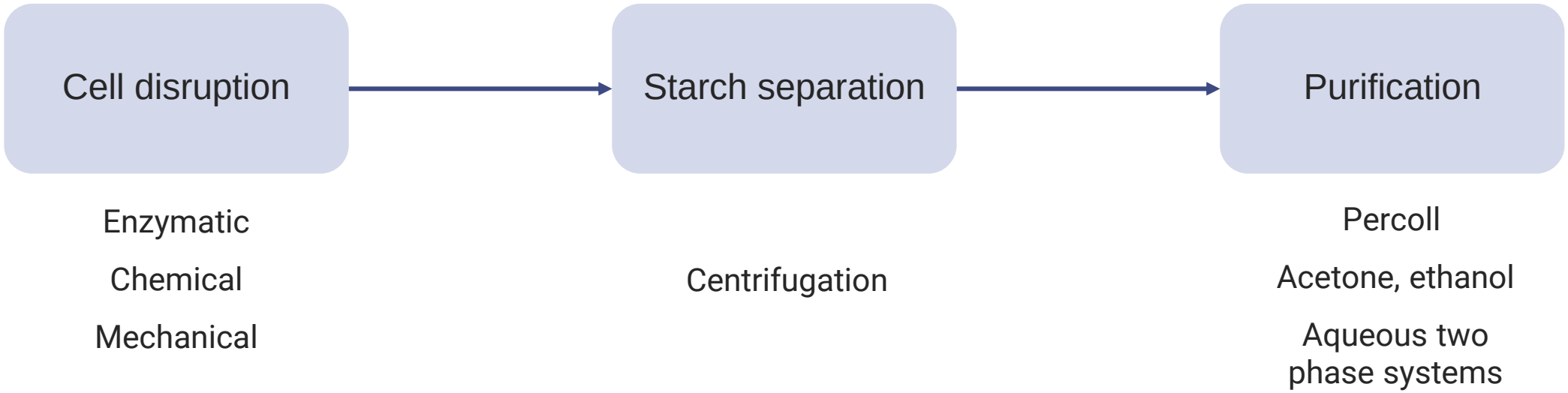
Thermoplastic starch TPS

Microalgae are a promising source of starch.



Brányiková et al. 2011:
Chlorella vulgaris CCALA924 produced 7 t_{starch}/ha/year.
(Data extrapolated from one pilot-scale experiment.)

Starch extraction from microalgae with thick cell wall is a challenging task.



Delrue et al., 1992; Di Caprio et al., 2023; Gifuni, 2017; Suarez Ruiz et al., 2020

Starch extraction

Cultivation of starch-enriched *Chlorella vulgaris* CCALA924.

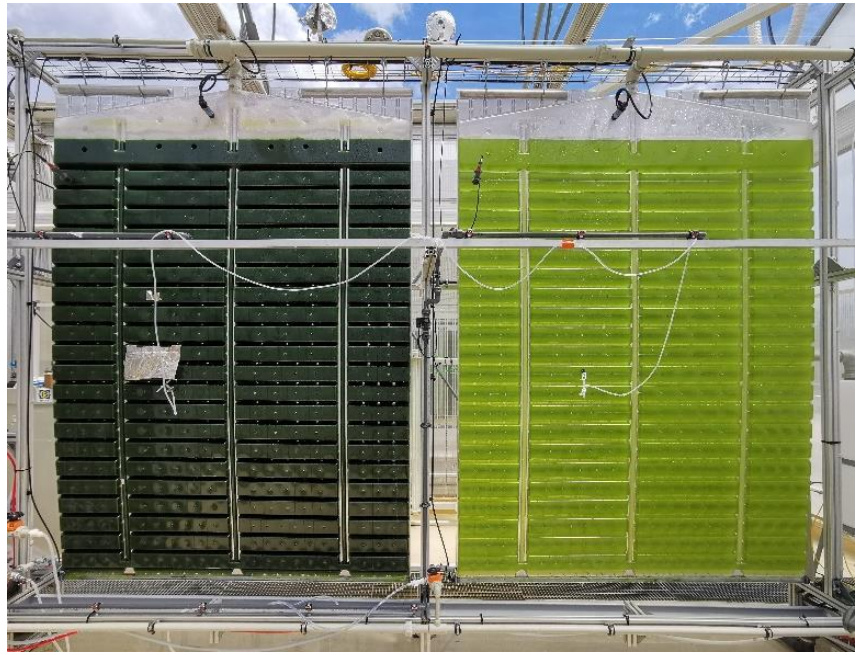
Culture stage 1
Replete growth in PBR

→

Culture stage 2
Medium deprivation & starch accumulation

→

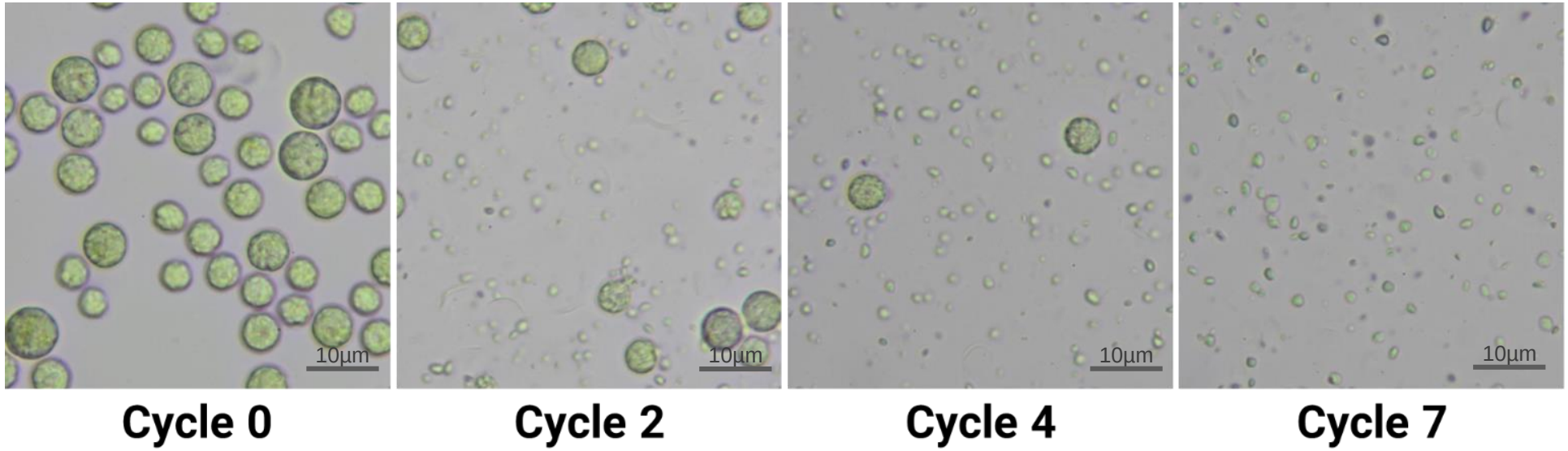
Cell harvest
Membrane filtration & centrifugation



Chlorella vulgaris CCALA924
180L flat panel airlift photobioreactor

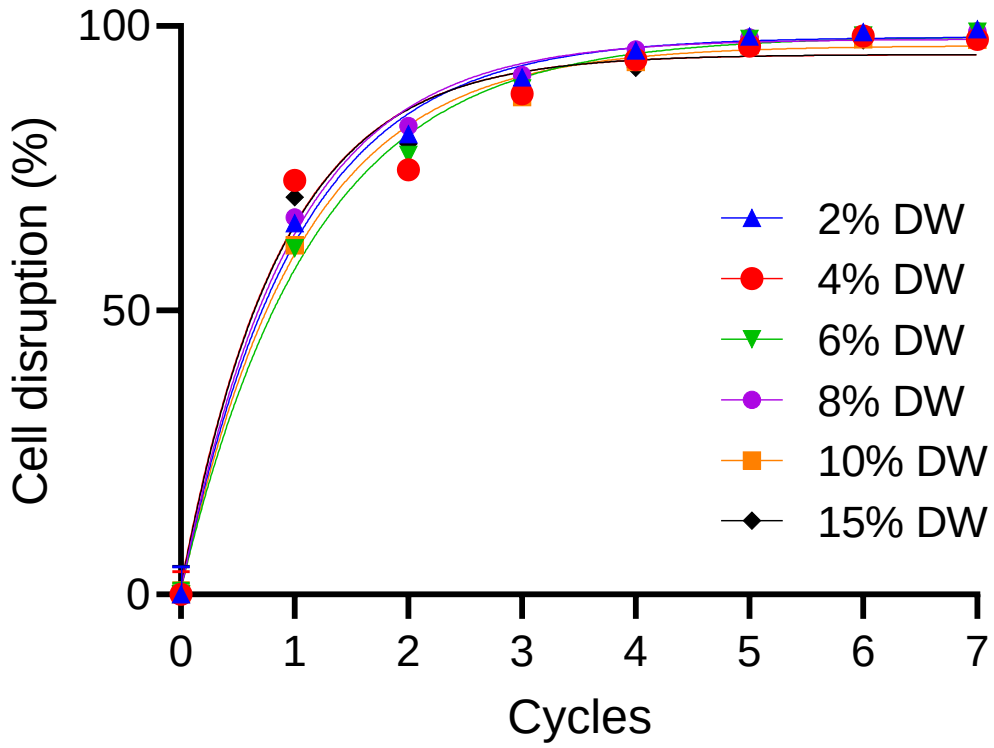
Downstream process of microalgae for starch extraction.

High pressure homogenization (HPH) released the starch granules from the cells.



Disruption of biomass along HPH cycles. *Chlorella vulgaris* cells & cell lysate, optical microscope, x1000.

Five cycles of HPH led to total cell disruption for concentration up to 15%DW.



Starch extraction was conducted on 430 g_{DW} of starch-enriched biomass.

Unfrozen biomass

Cell disruption

High pressure homogenization

250 MPa, 5 cycles

CF2 cell disruptor

Cell lysate

Starch separation

Centrifugation

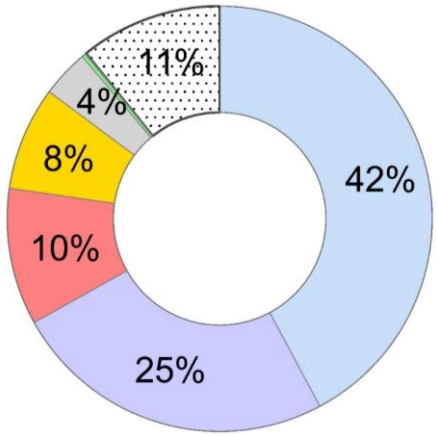
15 900g

Avanti J-265 XP

Fractionated biomass

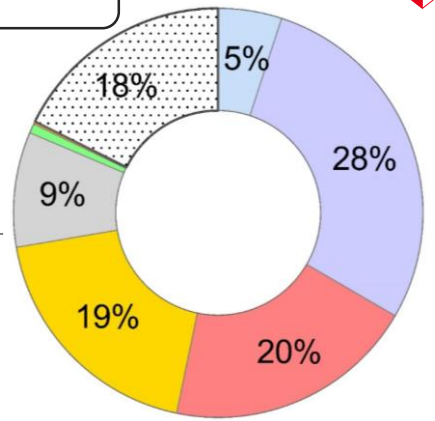
Repartition of biomass compounds after starch separation:

Biomass broth

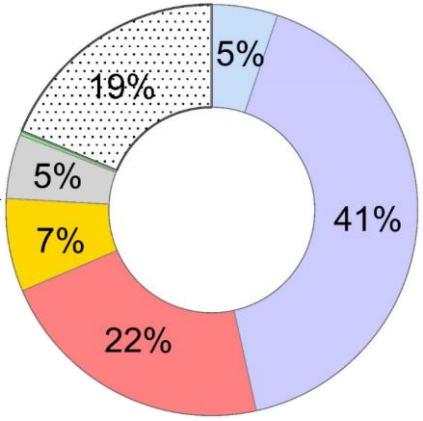


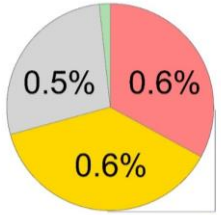
Centrifugation

Supernatant

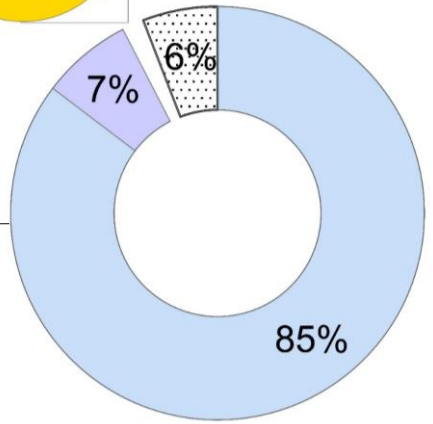


Green pellet



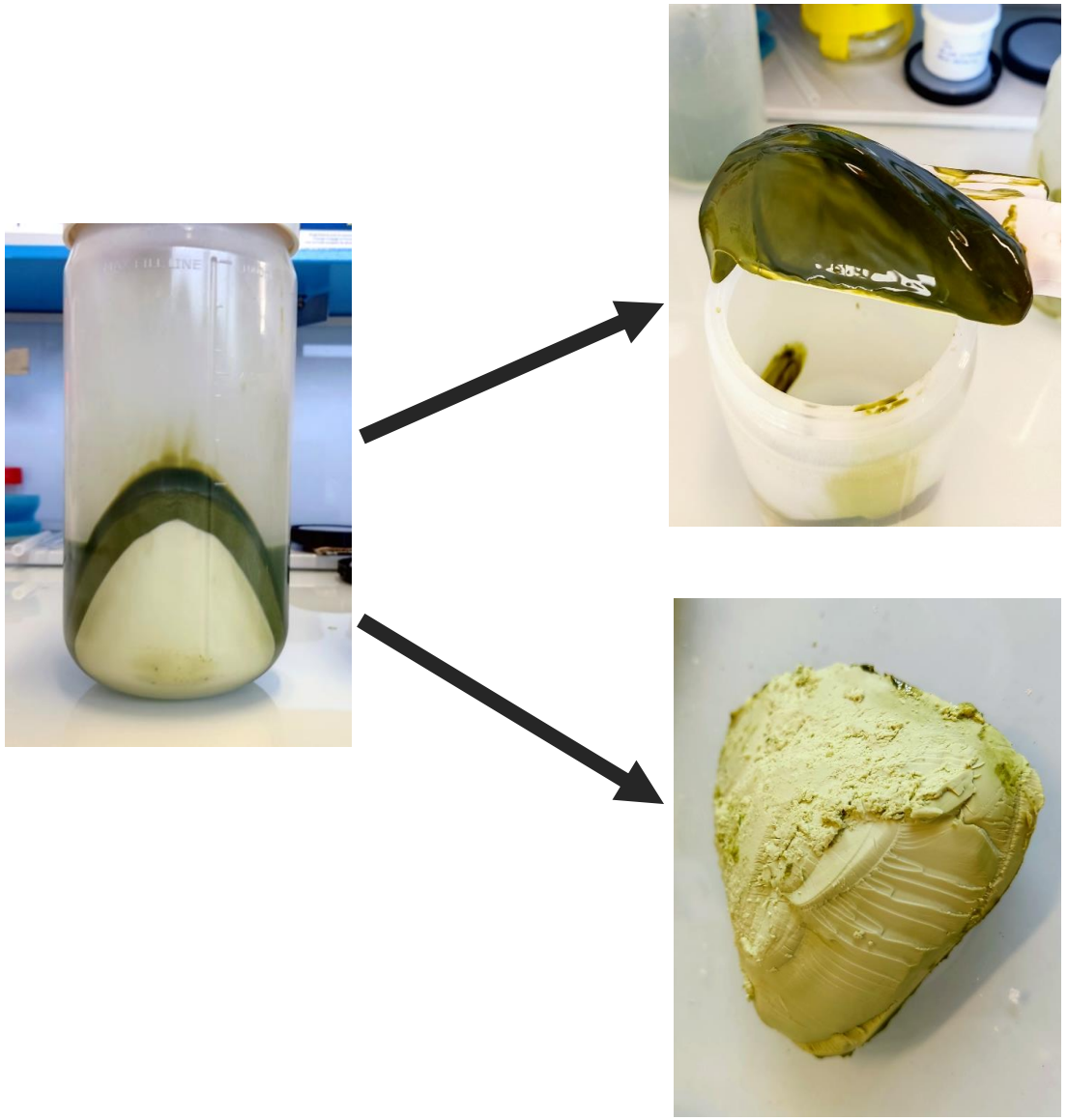


Starch pellet



- Starch
- Non-glucose carbohydrates
- Protein
- Lipids
- Ashes
- Other

Starch pellet recovery.

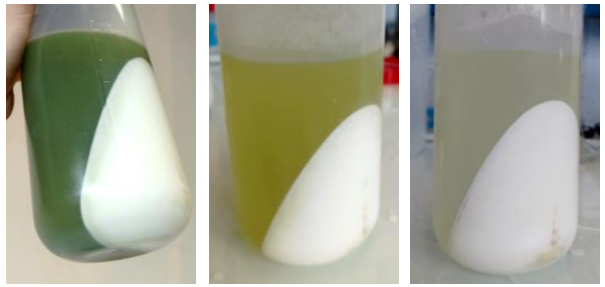


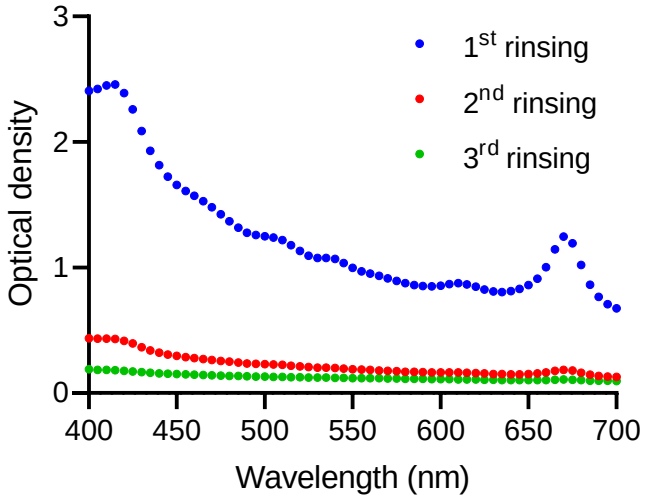
Step 3: Purification with simple water rinsing (no organic solvent).

Raw starch

Starch suspension

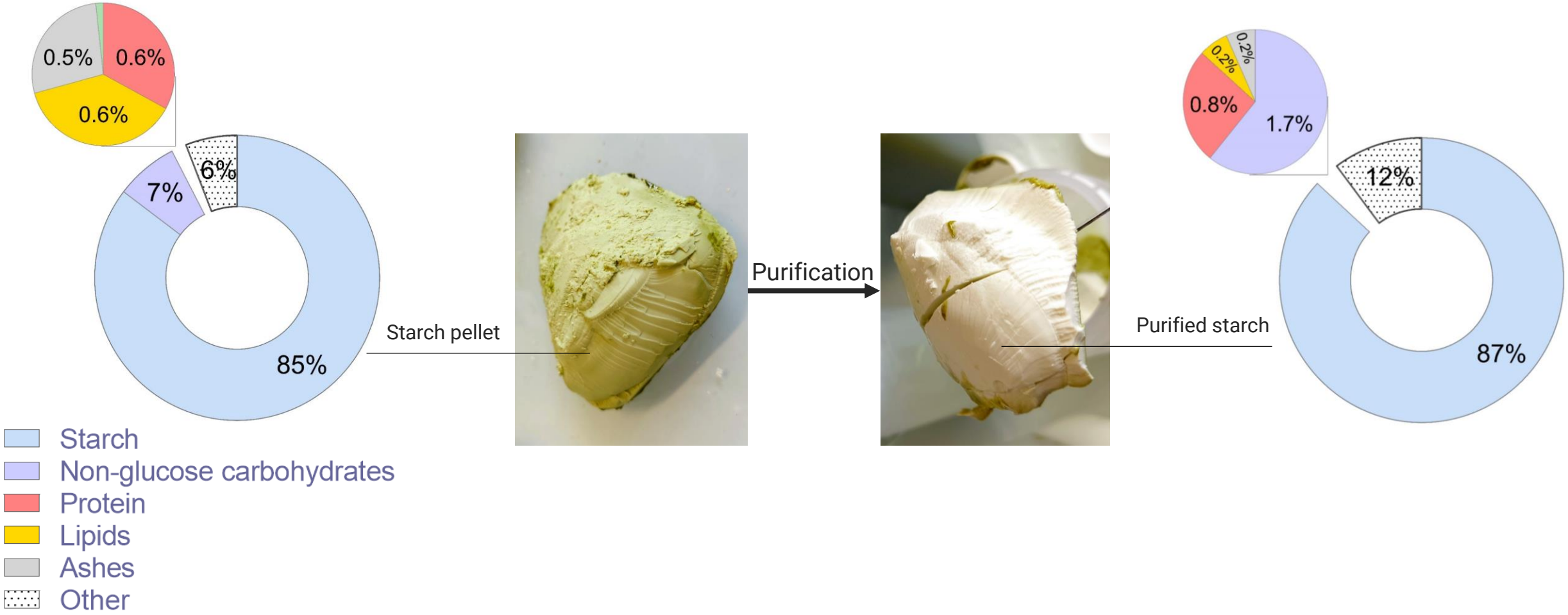
Water rinsing + centrifugation
3 times



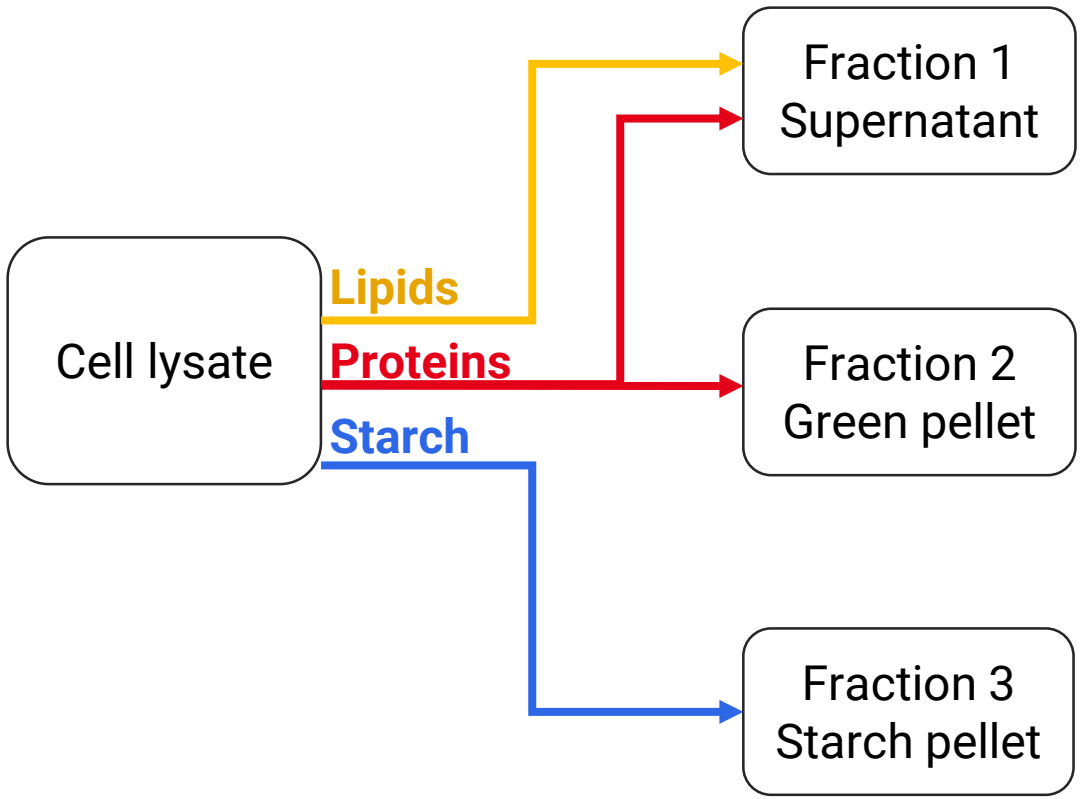


Purified starch

Purification removes green colour and non-glucose carbohydrates.



The starch extraction process opens biorefinery perspectives.



	lipids	proteins	starch
Fraction 1 Supernatant	80±1 %	61±5 %	3±1 %
Fraction 2 Green pellet	16±0 %	36±8 %	2±1 %
Fraction 3 Starch pellet	4±0 %	3±1 %	95±10 %
Total:	100%	100%	100%

Final yield of starch extraction & purification is 98.5%.

Unfrozen biomass

430.3 gDW
of biomass

42±3 % starch

181±14 g pure starch

Raw starch

216.3 gDW
of unpurified starch

85±5 % starch

184±11 g pure starch

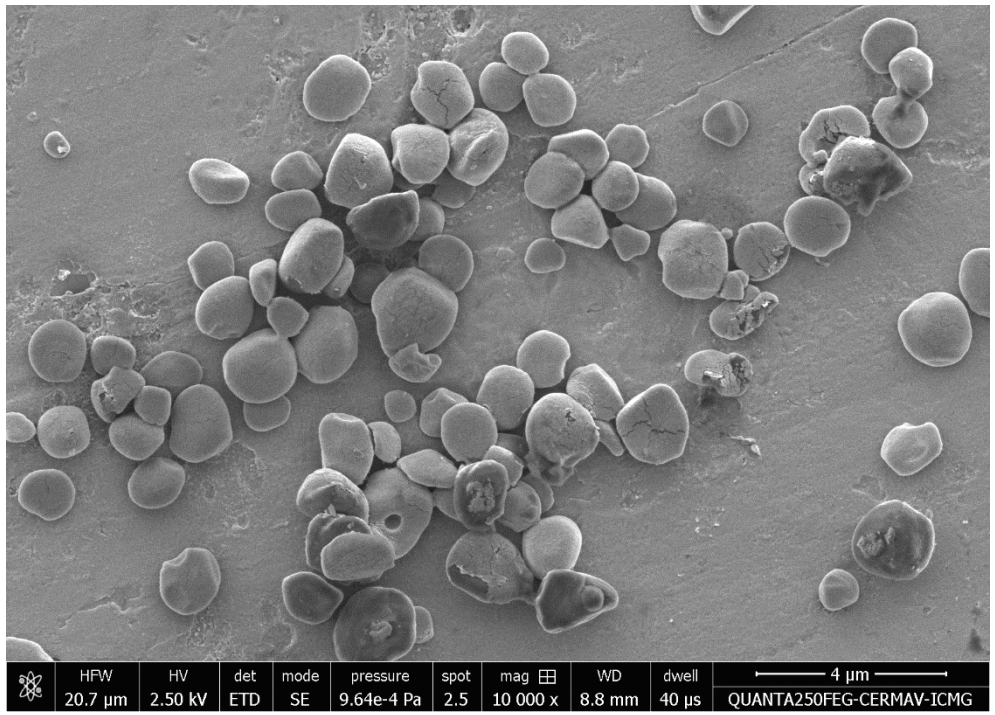
Purified starch

205.8 gDW
of purified starch

87±3 % starch

179±6 g pure starch

The extracted starch retained its integrity and displayed the usual properties of native starches.

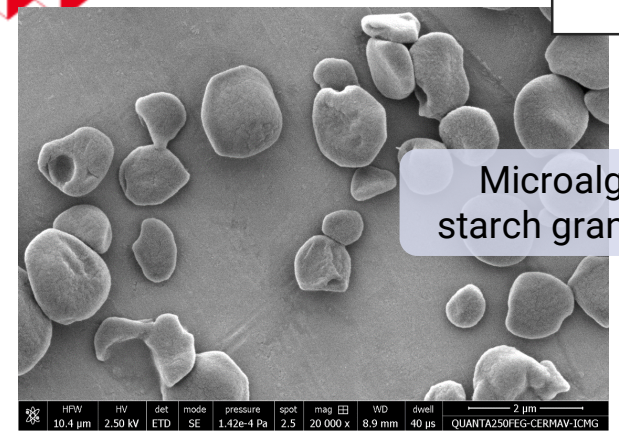


Chlorella vulgaris starch granules.

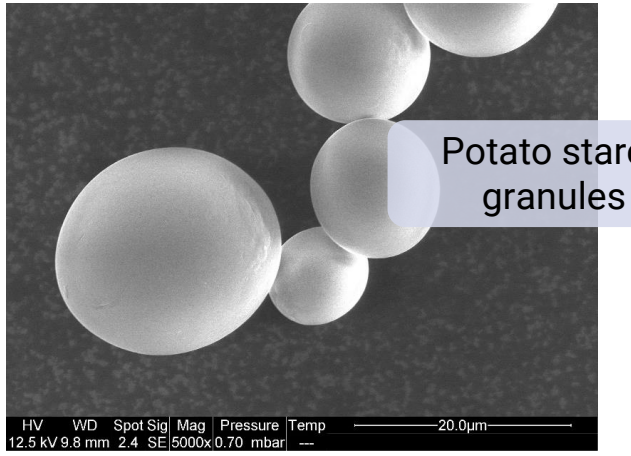
Granules mean diameter	1.5 µm
Crystallinity type	Type A
Distance between crystalline lamella	9.5 nm
Amylose / amylopectin content	13% / 87%
Gelatinization temperature in water	65 °C

Starch plasticization

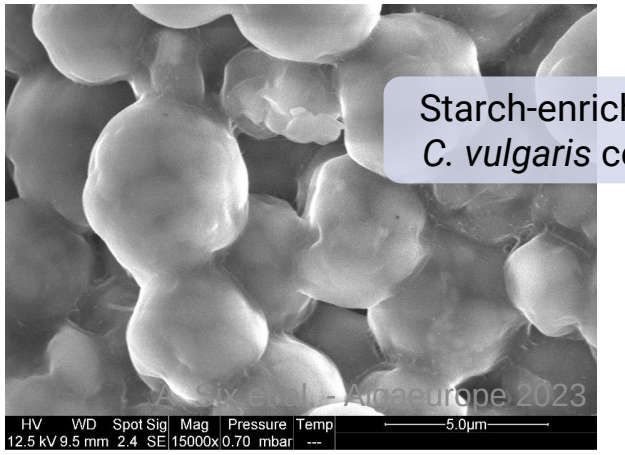
Process of starch plasticization into thermoplastic starch (TPS)



Microalgal starch granules



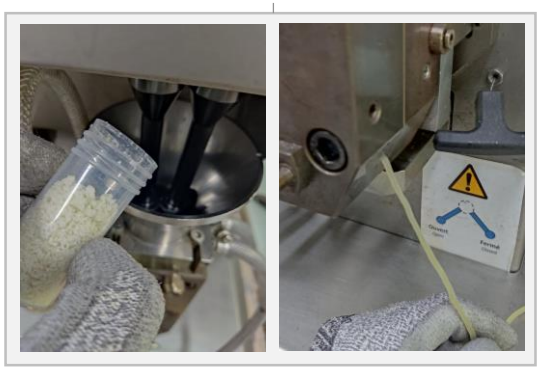
Potato starch granules



Starch-enriched *C. vulgaris* cells

Plasticizer addition
Glycerol 30% final wt

Extrusion
Microcompounder
120 or 140 °C



TPS* extrudates

Injection
Sheath 140 °C, Mold 40 °C

TPS dogbones

Dogbones were obtained after extrusion and injection at 140°C, with important shrinkage.

Potato TPS

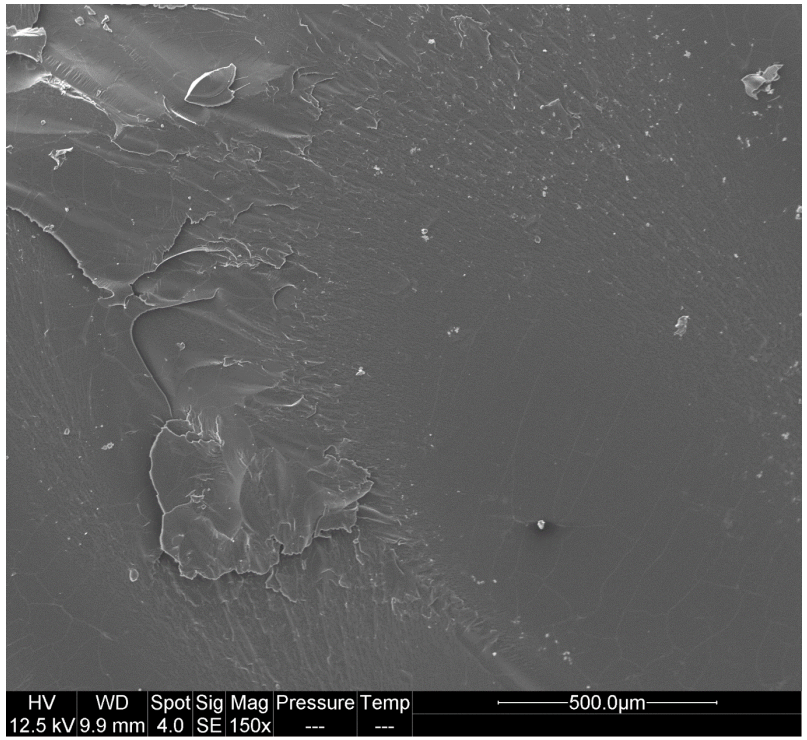
Microalgal TPS

Plasticized biomass

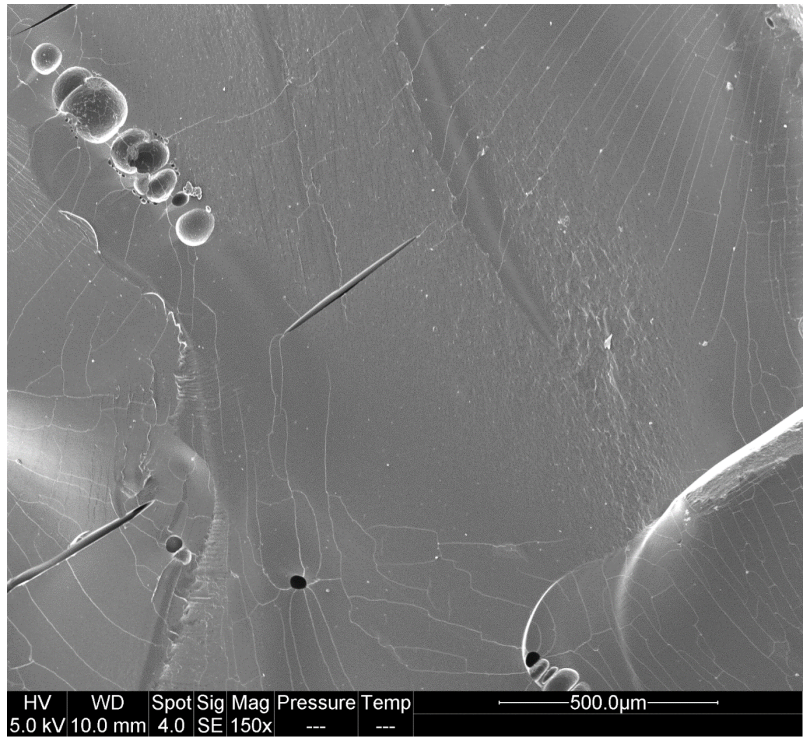
Commercial TPS

SEM revealed homogenously plasticized matrix for potato and microalgal TPS.

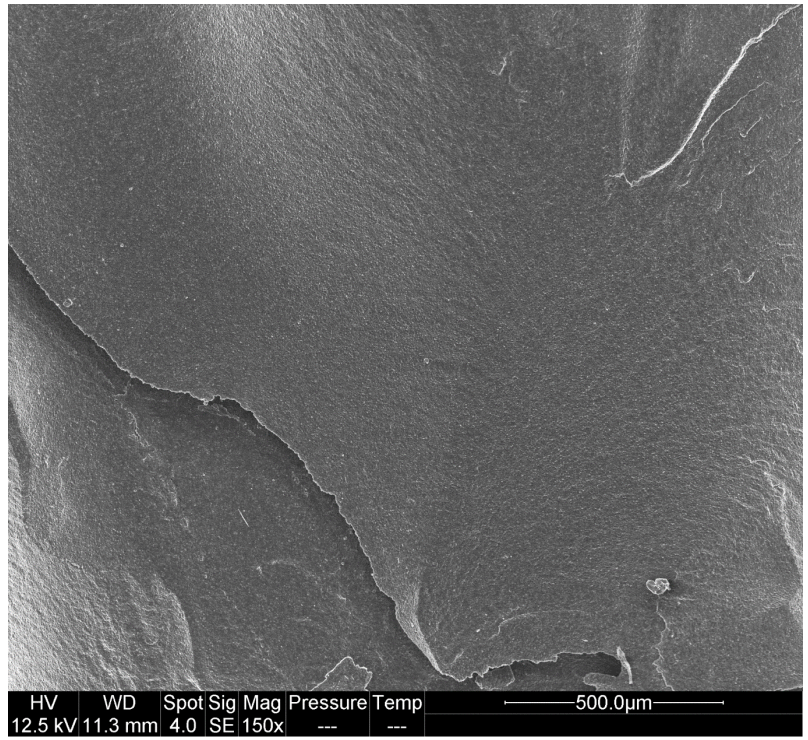
Potato TPS



Microalgal TPS

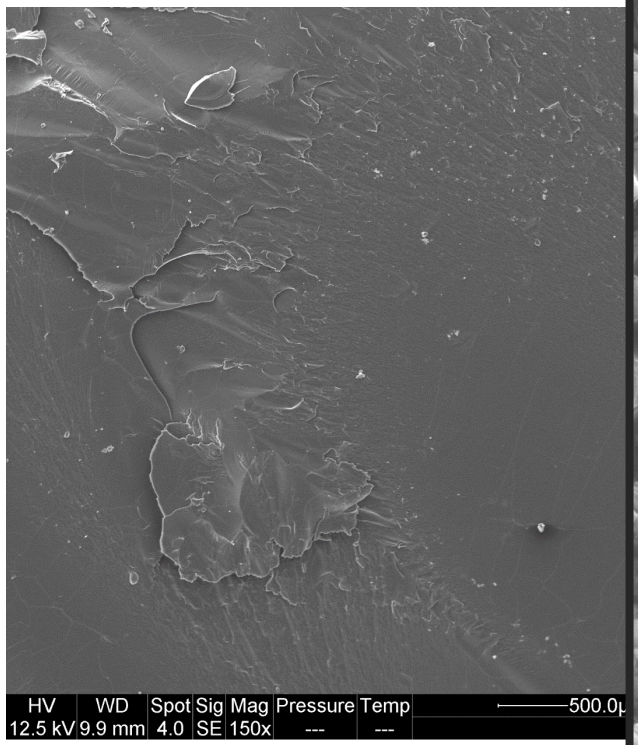


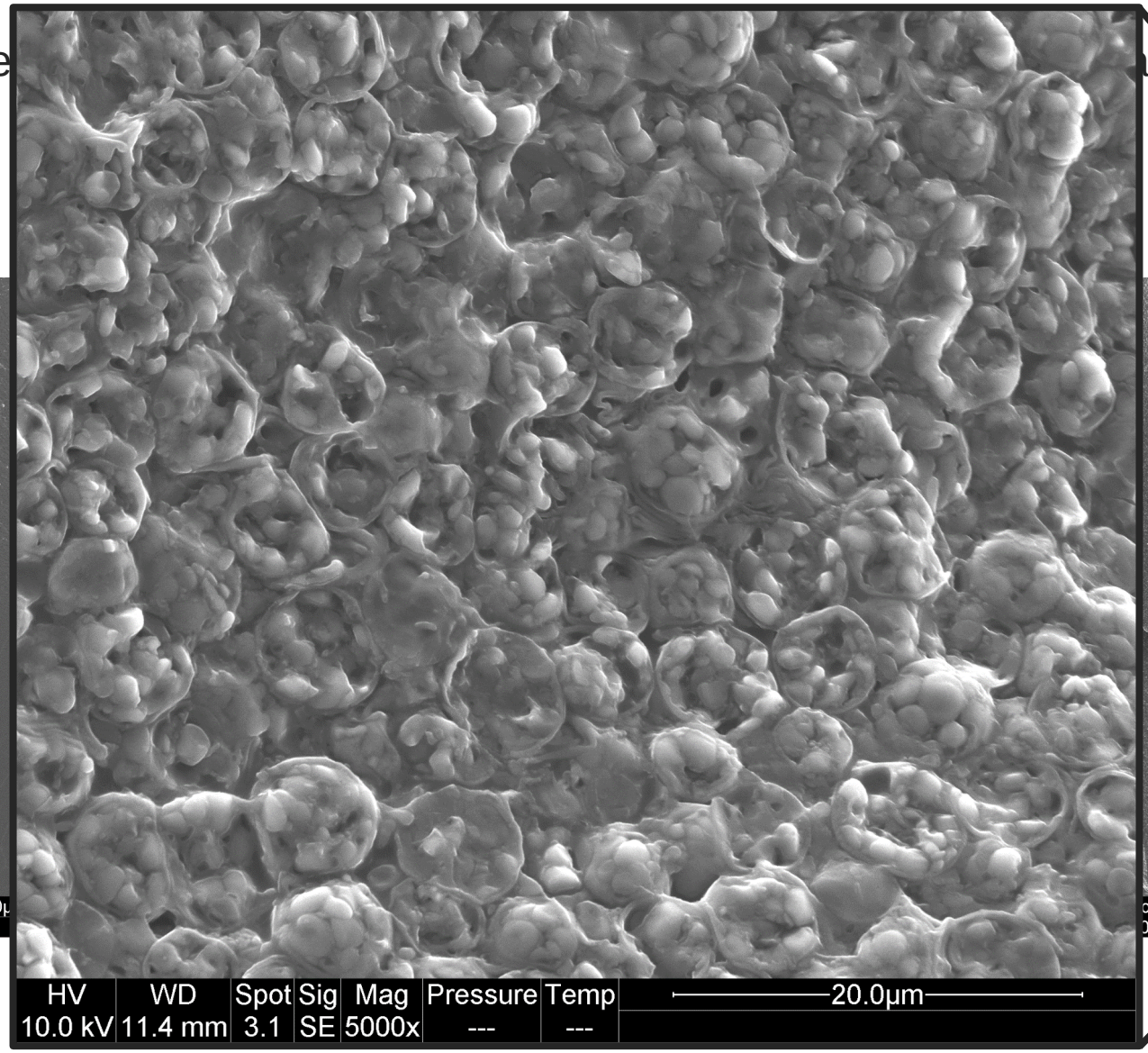
Plasticized biomass



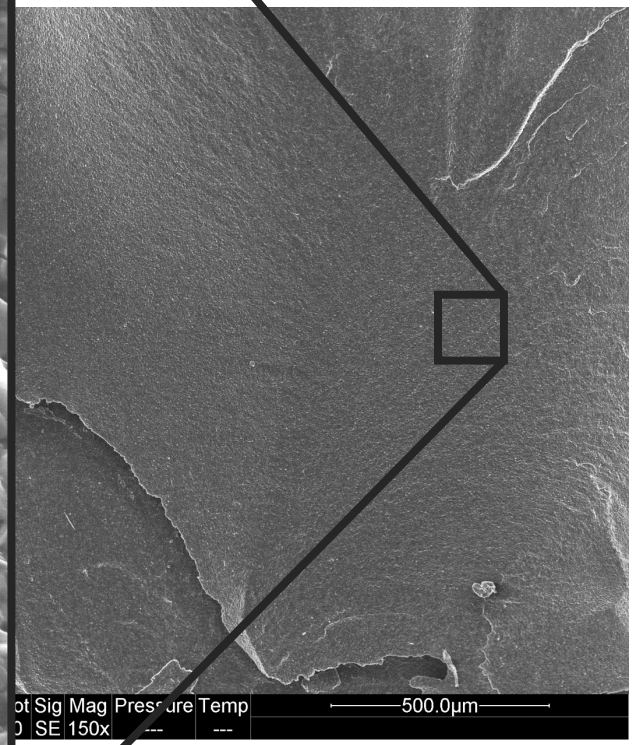
SEM reve … lgal TPS.

Potato TPS

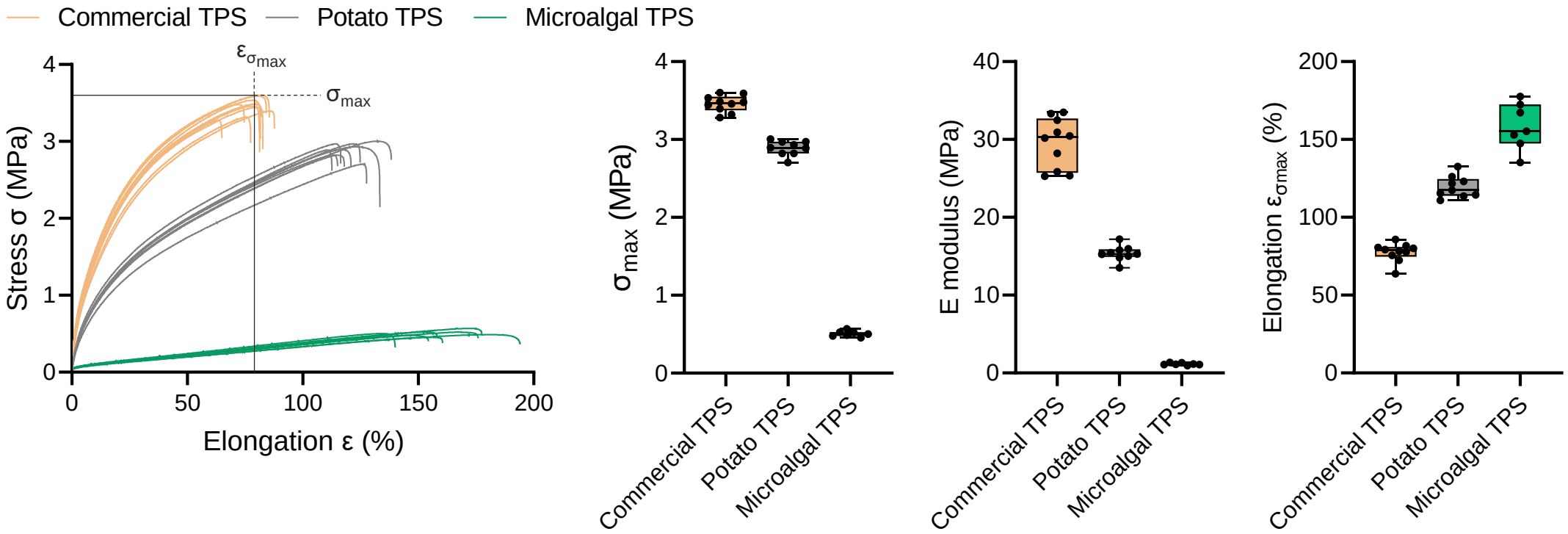




Plasticized biomass



Microalgal TPS was softer and elongated more than control TPS.



*TPS: thermoplastic starch

Conclusion & perspectives

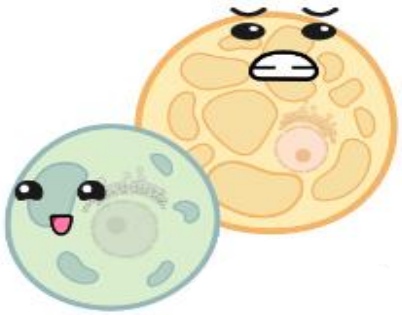
Achievements:

- ✓ Extraction of 206 g_{starch} with 98% recovery and 87% purity (no use of Percoll or any organic solvent).
- ✓ Plasticization of microalgal starch using industrially-scalable processes.

Perspectives:

- Reduce the energy consumption of the extraction process.
- Explore the biorefinery potential of the separated fractions & remove green color from the purified starch.
- Understand why microalgal TPS is softer than other TPS.

Thank you



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SUPPLEMENTARY SLIDES

Methodology used in this study:

Cell disruption

Mechanical disruption: High Pressure Homogenizer, 250 MPa (CF2 Cell Disruptor, Constant Systems Ltd.)

Quantification of cell disruption: Cell counting (Multisizer 4, Beckman Coulter™)

Biomass composition analysis

Starch: Enzymatical D-glucose quantification (Enzytec starch kit)

Non-glucose carbohydrates: [D-glucose]-[Total carbohydrates] (Dubois protocol)

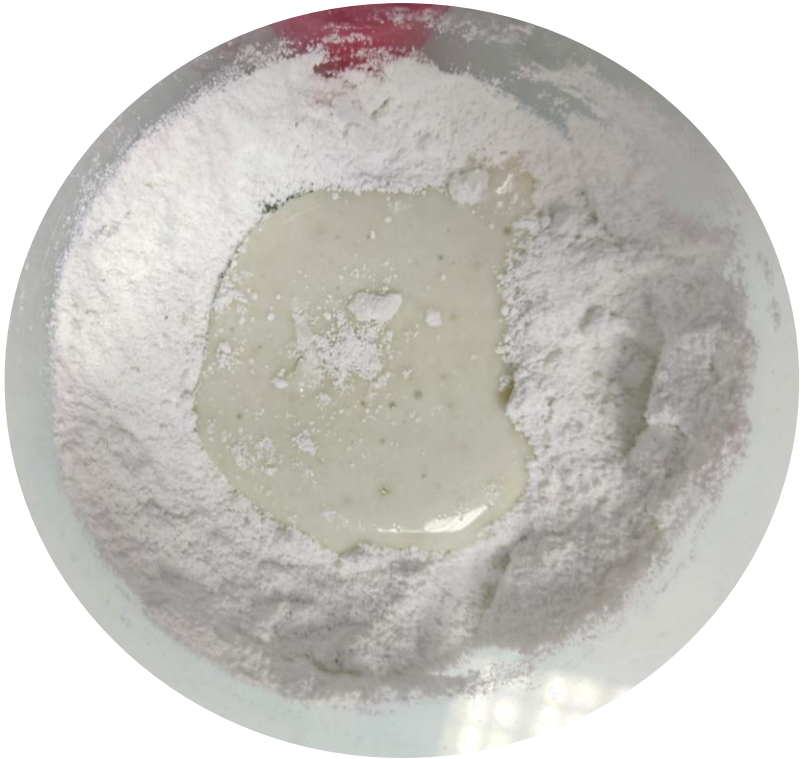
Protein: Total nitrogen quantification and N-to-protein ratio of 5.04[1]
(total organic carbon analyzer TOC-L, Shimadzu™)

Lipids: Total lipids by GC-FID (GC-2010 Pro AOC-20i/AOC-20s, Shimadzu™)

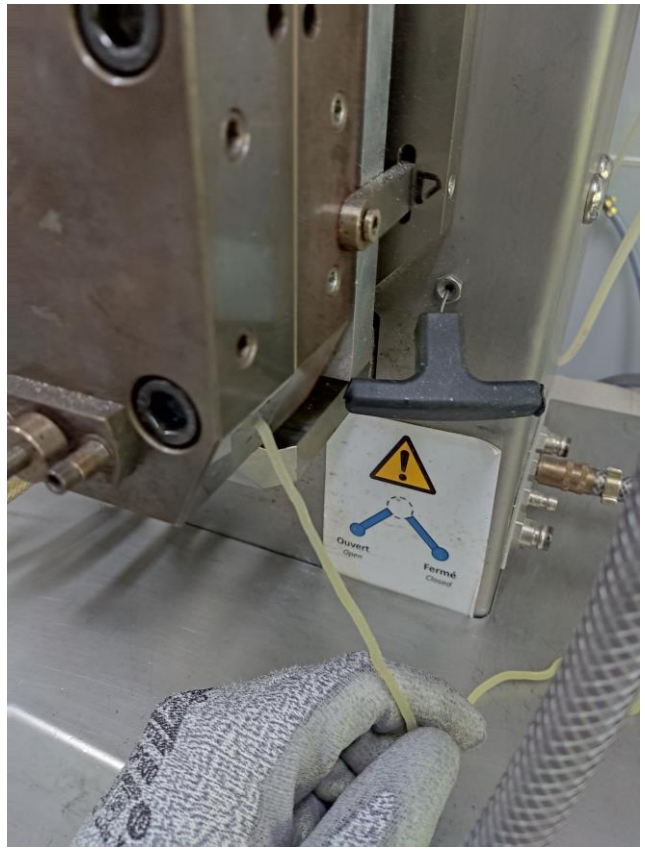
Ashes: Residues after ignition 2h at 550°C (oven Nabertherm)

[1] Templeton, 2015

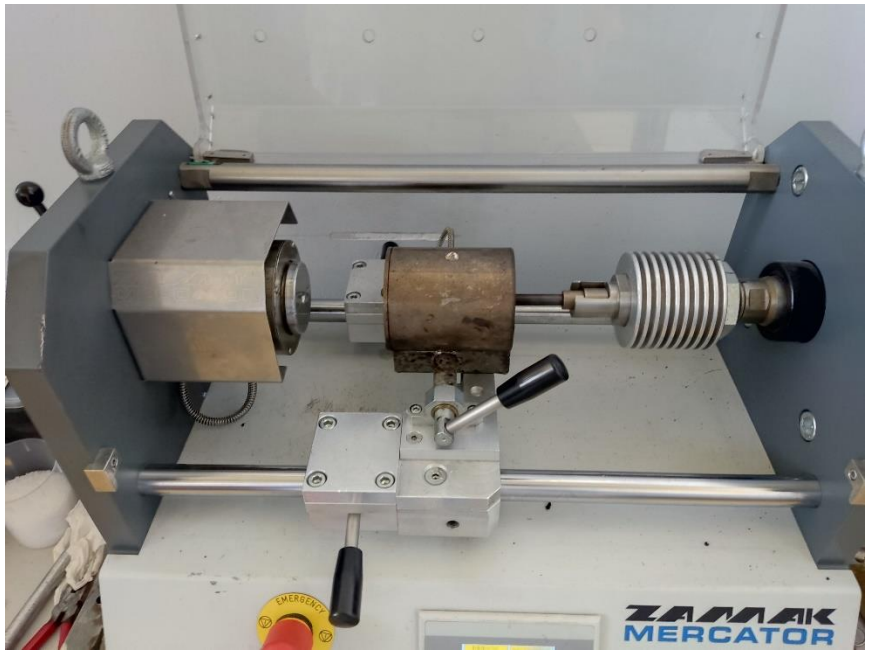
The white microalgal starch turns back to green when adding glycerol.



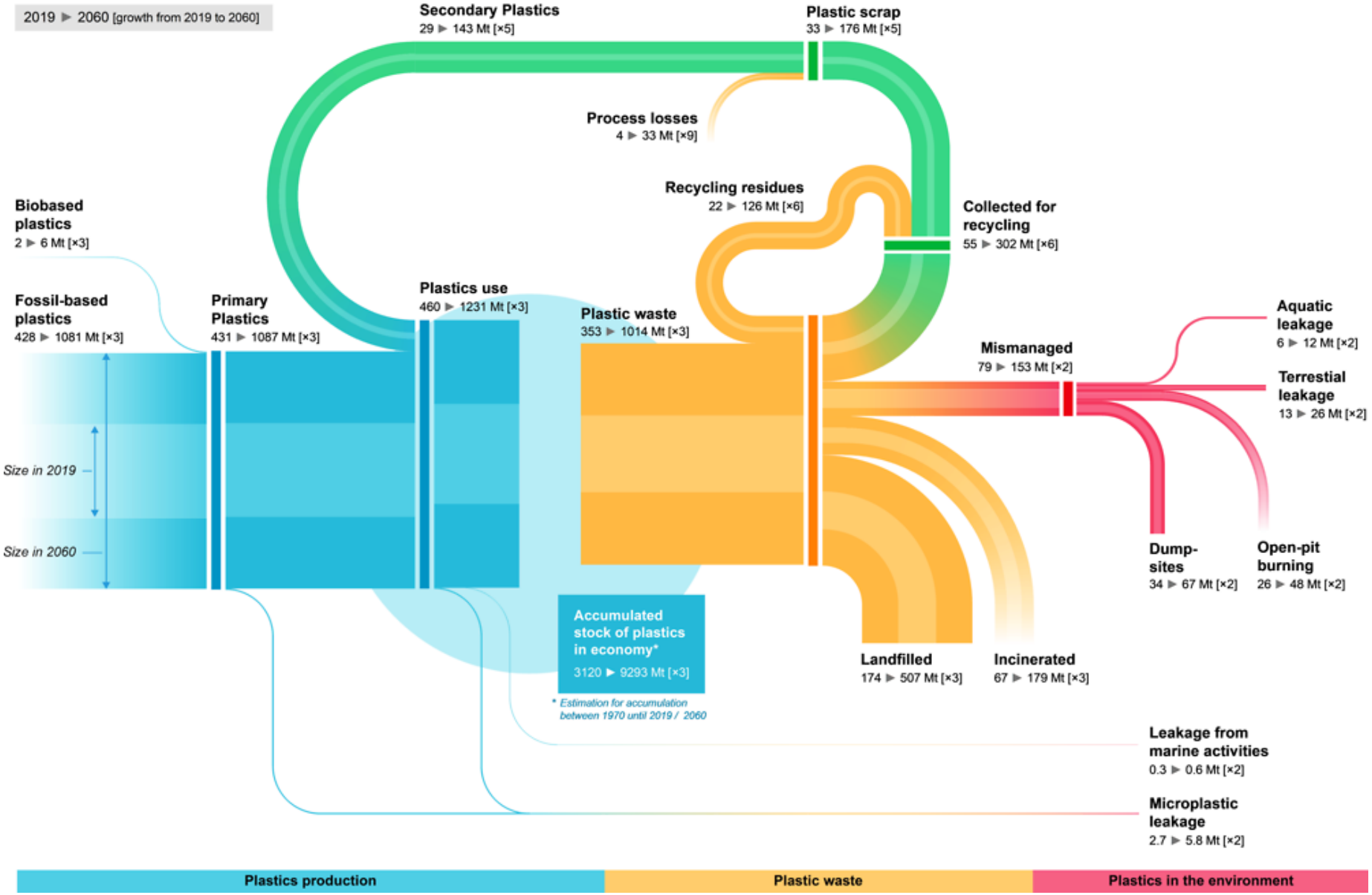
Extrusion process of the starch+glycerol mix.



Injection process of the TPS extrudates.



Supplementary slide



OCDE predictions of plastic fate for 2060.