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University of the Aegean

AGRIMA

Advancing Circular Economy with Innovative Packaging from Agricultural Waste

Summer Camp | July 7 – 11, 2025 | Marseille, France

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Agri-food Waste Management for Sustainable bio-economy through Higher Education curricula and upskilling

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Goals

AGRIMA aims to foster universities' **capacity building** for the **green transition** through **innovative practices and higher education curricula updating in agri-food waste management** for the **circular bioeconomy**.

AGRIMA addresses:

1. **Advancing pedagogical methods** for industrial agri-food waste valorisation **based on business-academia synergies**.
2. **Integrating citizen science** in bio-economy-enhanced waste valorisation as a means of **civic engagement and environmental advocacy**.

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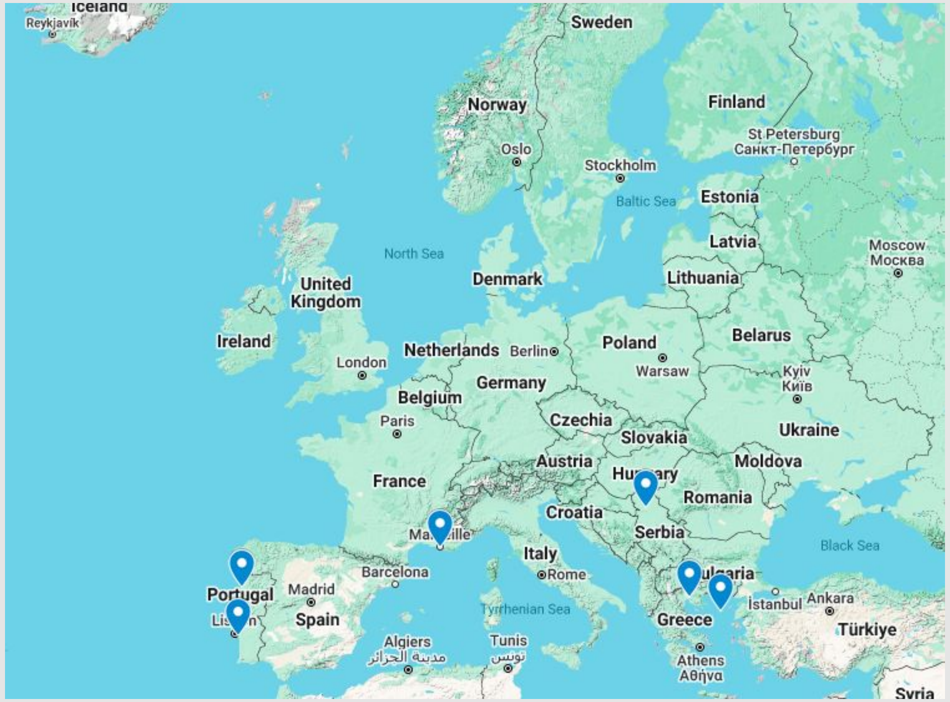
Partners



amU Aix Marseille Université

AVIPE Associação de Viticultores do Concelho de Palmela

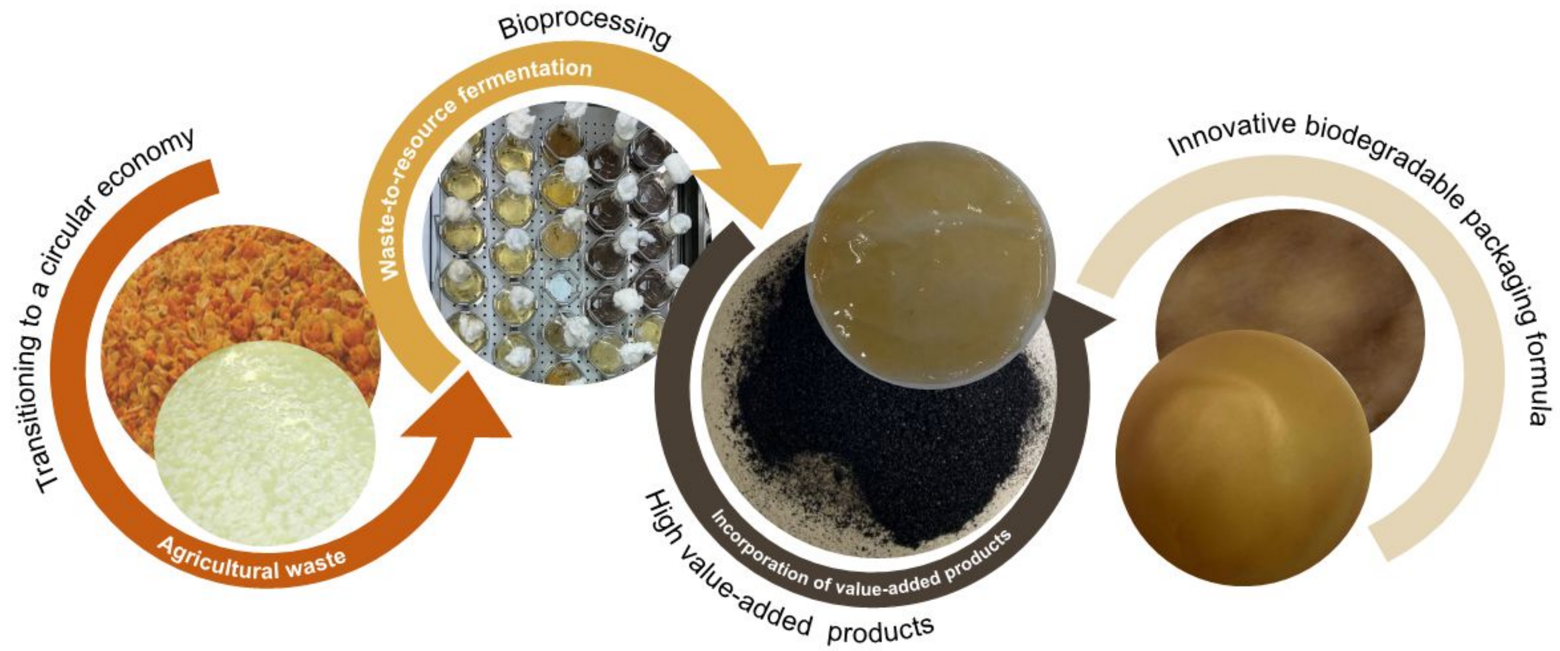
P.PORTO

UNIVERSITY OF NOVI SAD

UNIVERSITY OF THE AEGEAN

Web2Learn Open, social learning

Valorization of agri-food waste for circular economy applications

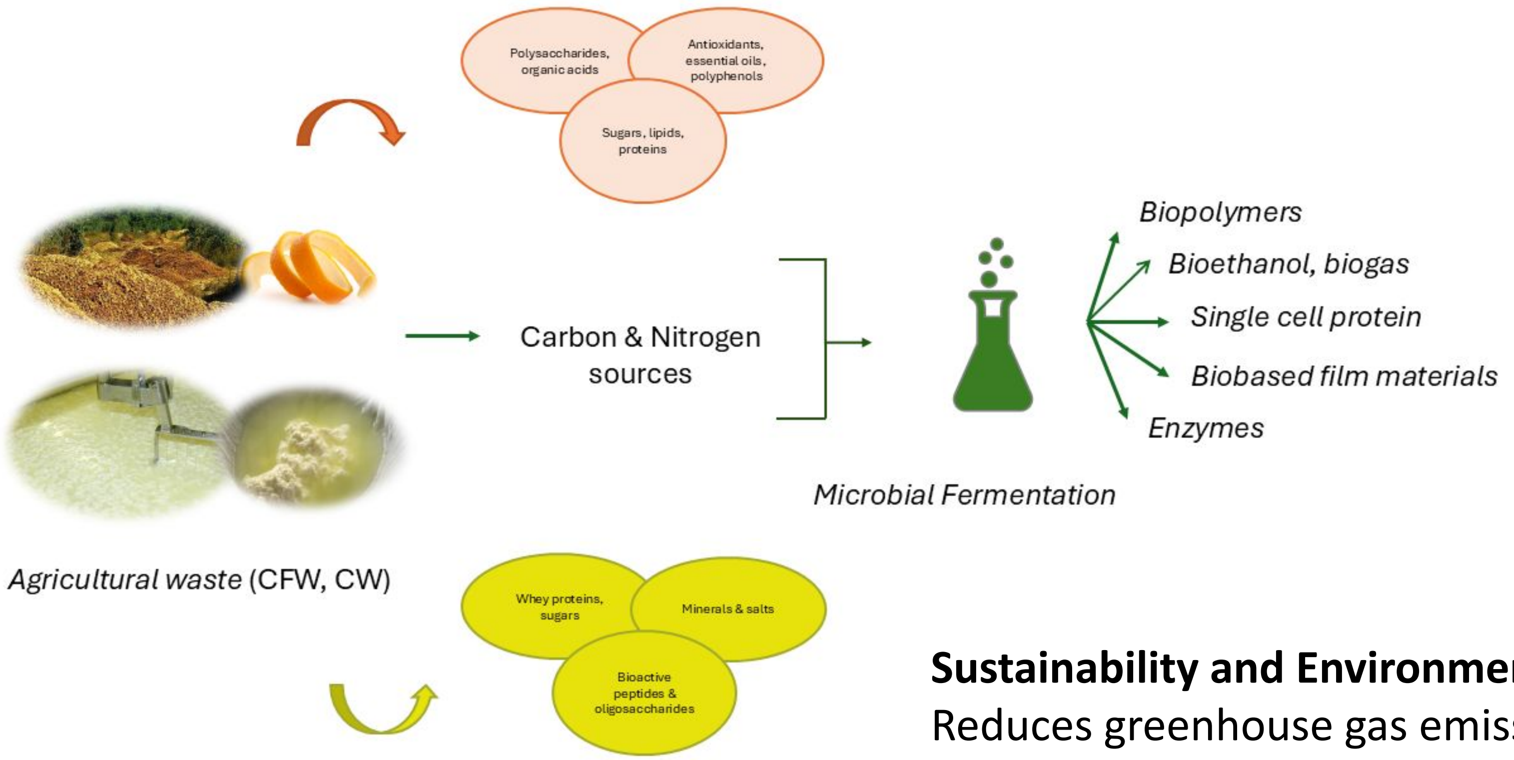


Global challenge: The agri-food sector generates vast amounts of organic waste, contributing to pollution and climate change.

Innovation opportunity: Transforming this waste into sustainable materials supports circular economy goals and reduces reliance on petroleum-based plastics.

Project aim: To develop biodegradable films from microbial biomass and melanin using food waste streams as carbon sources.

Valorization of agricultural waste



Sustainability and Environmental Impact

Reduces greenhouse gas emissions by diverting waste from landfills

Extracts valuable nutrients and energy, reducing reliance on fossil fuels

Challenges and Limitations of Waste Valorization

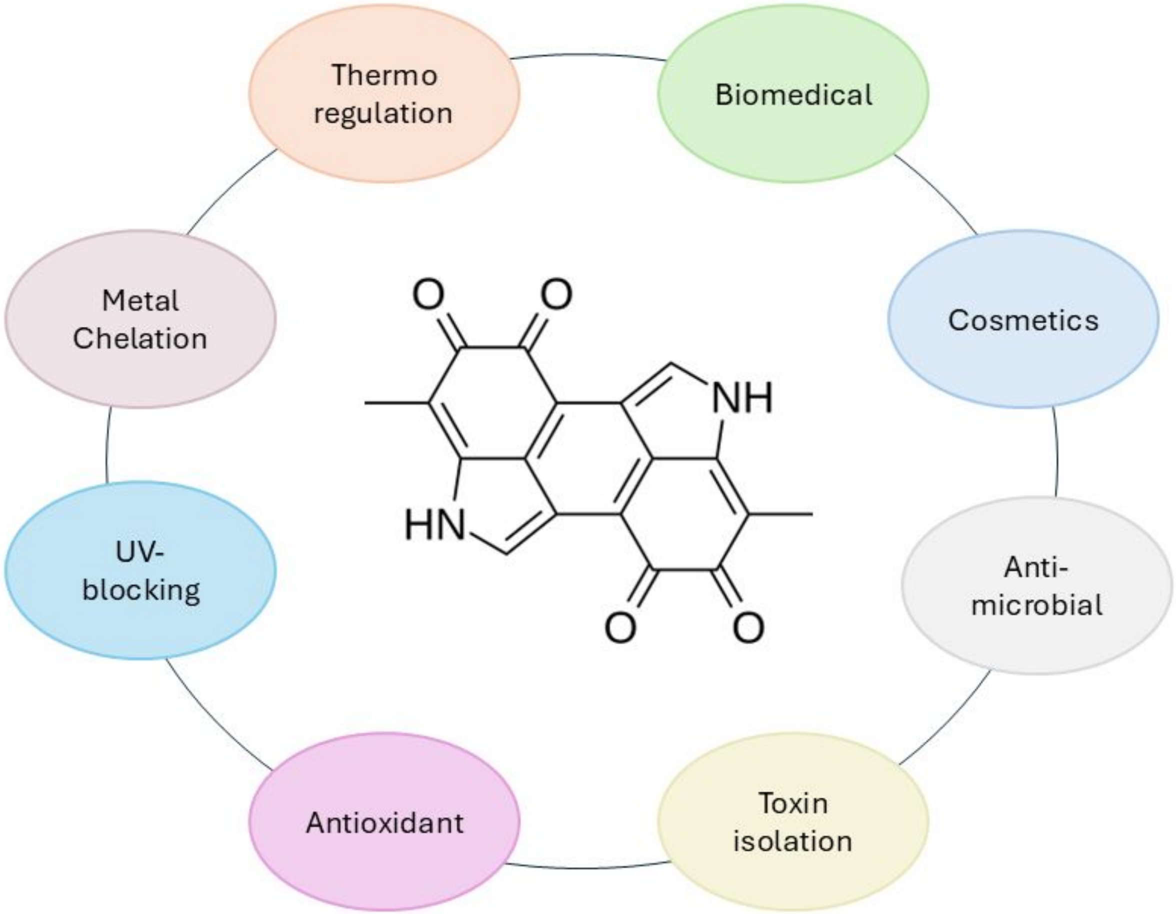
High moisture content and varied composition hinder processing

Significant initial investment required for infrastructure

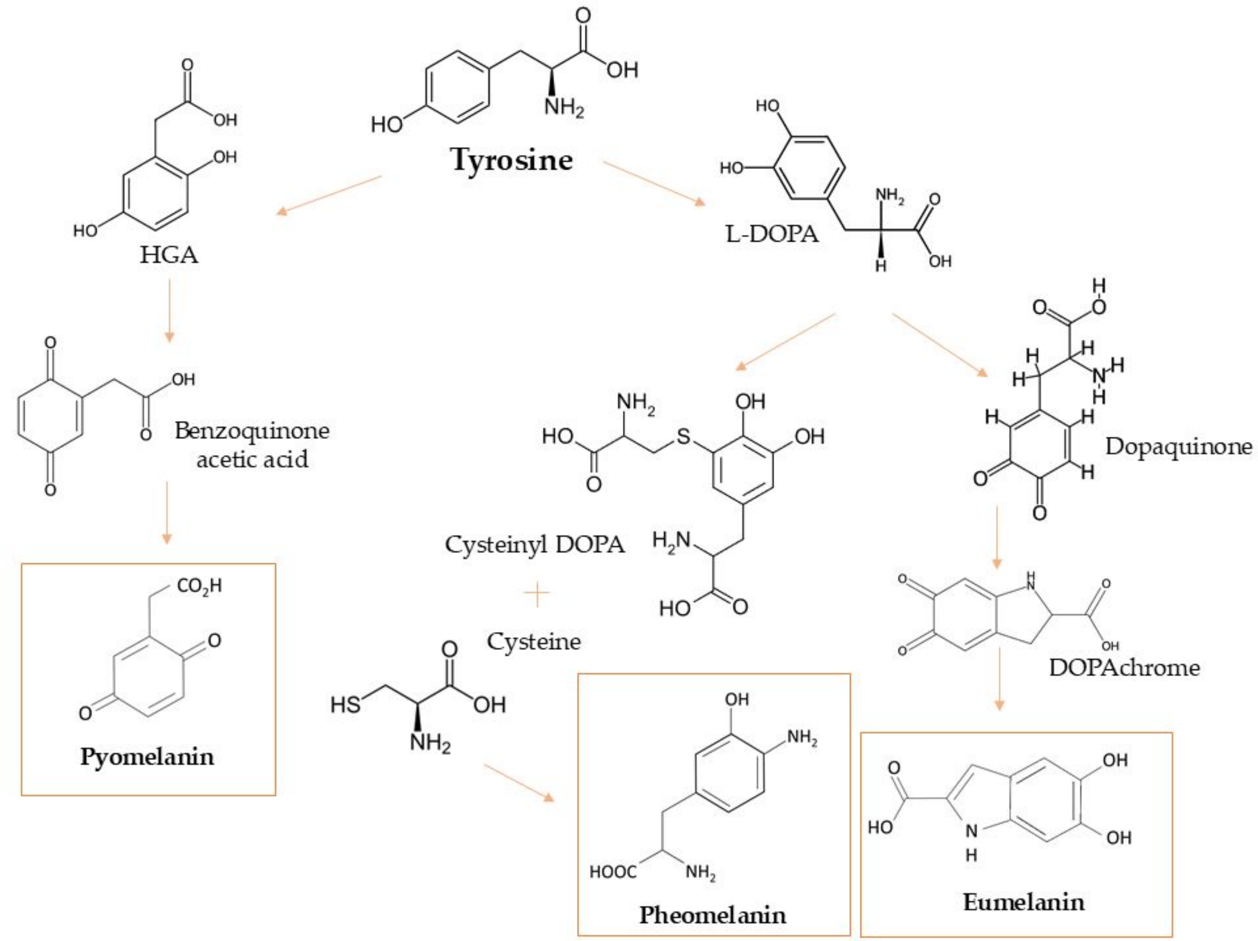
Consumer acceptance and regulatory constraints pose challenges

Citrus waste

- Processing waste: About 60% of fruit weight ends up as waste (peels, seeds, pulp), resulting ~16 million tons of waste per year.
- Management challenge: High water content (80-90%) accelerates breakdown, increasing environmental impact.
- Climate change: Improper disposal causes groundwater contamination and methane emissions from landfills.


Thermo regulation
Biomedical
Cosmetics
Anti-microbial
Toxin isolation
Antioxidant
UV-blocking
Metal Chelation
O
O
NH
HN
O
O

Microbial melanin biosynthesis



- DOPA metabolic pathway (tyrosine as precursor) or DHN pathway (malonyl-coenzyme A as precursor) pathway
  Main enzymes involved in melanogenesis: polyphenol oxidases or tyrosinases, lacuses and catechols oxidases with copper ions
- Hydroxylated aromatic compounds that accumulate due to enzymatic imbalances during the catabolic process, lead to the production of alternative types of melanin

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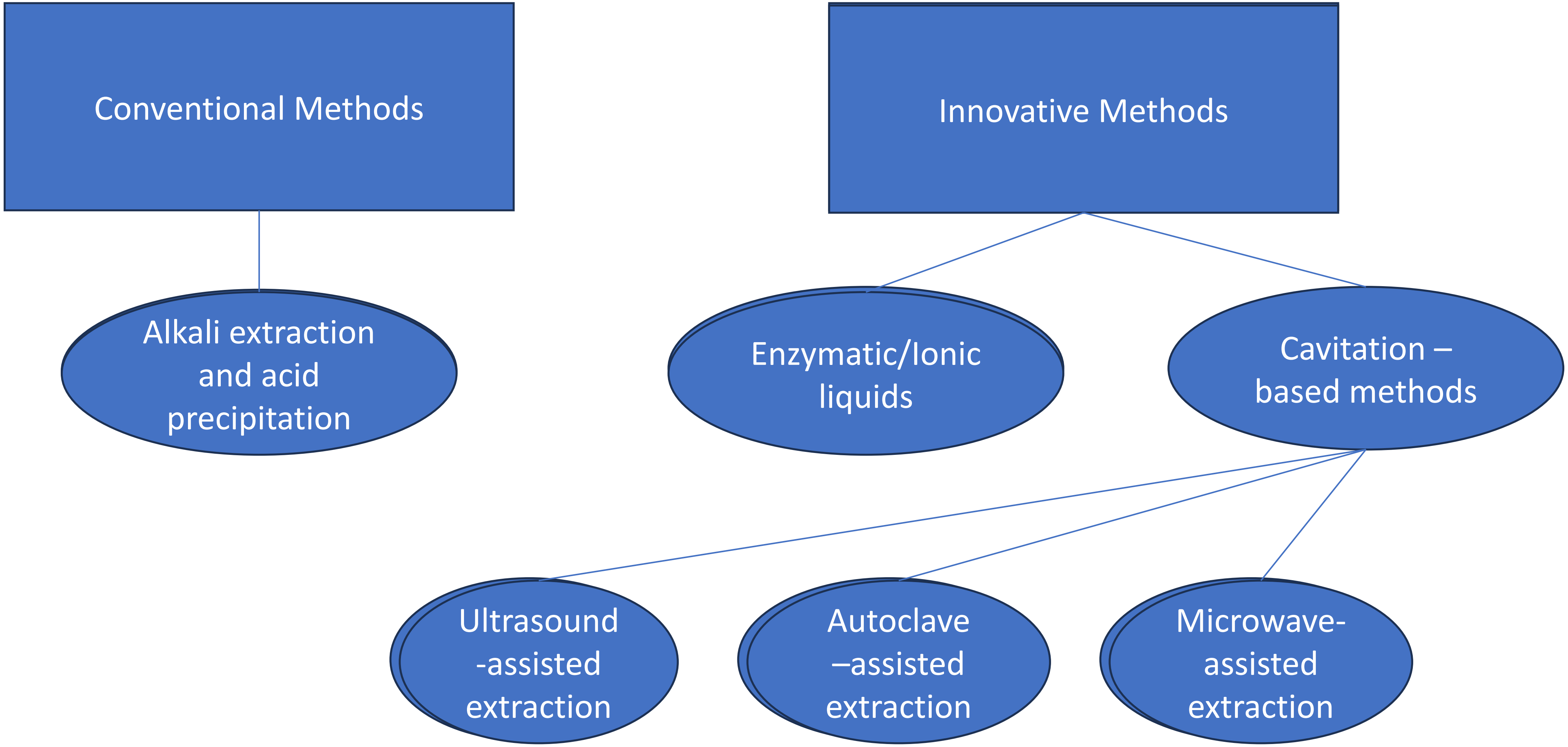
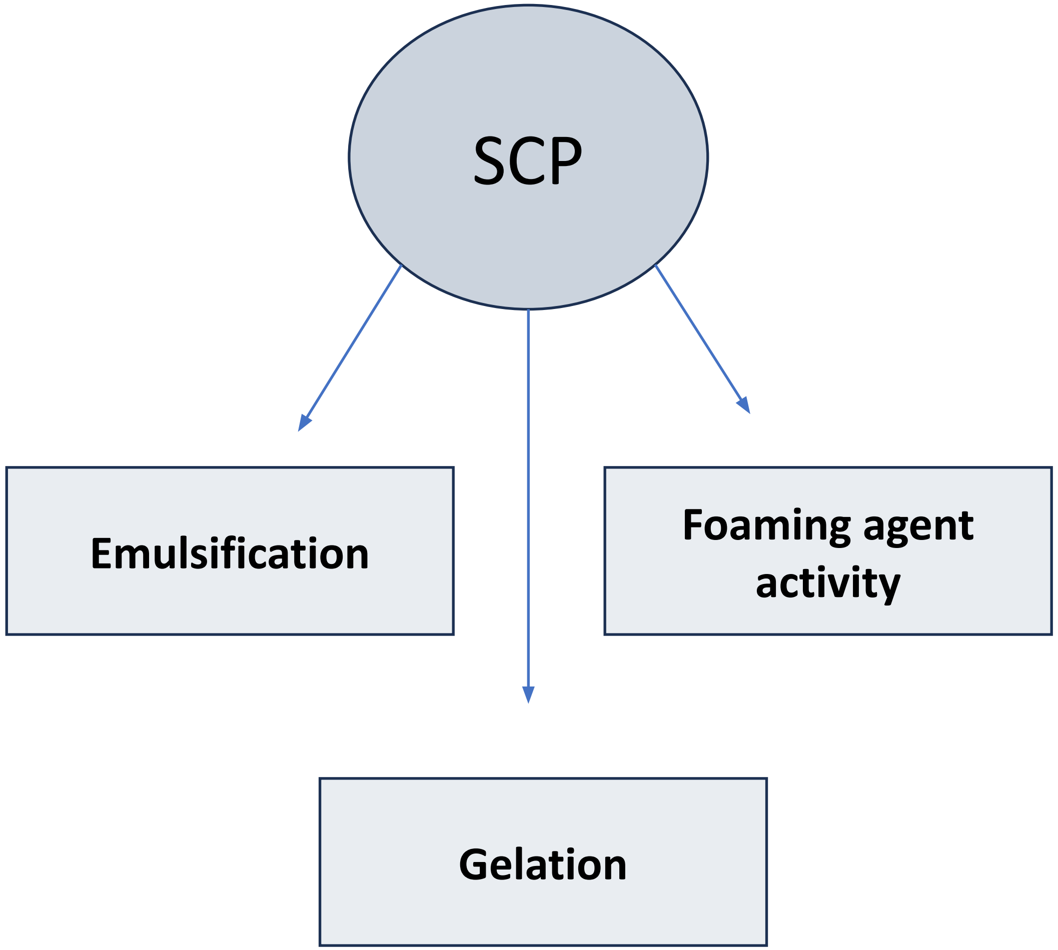
Microbial melanin extraction methods





Functional properties of SCP

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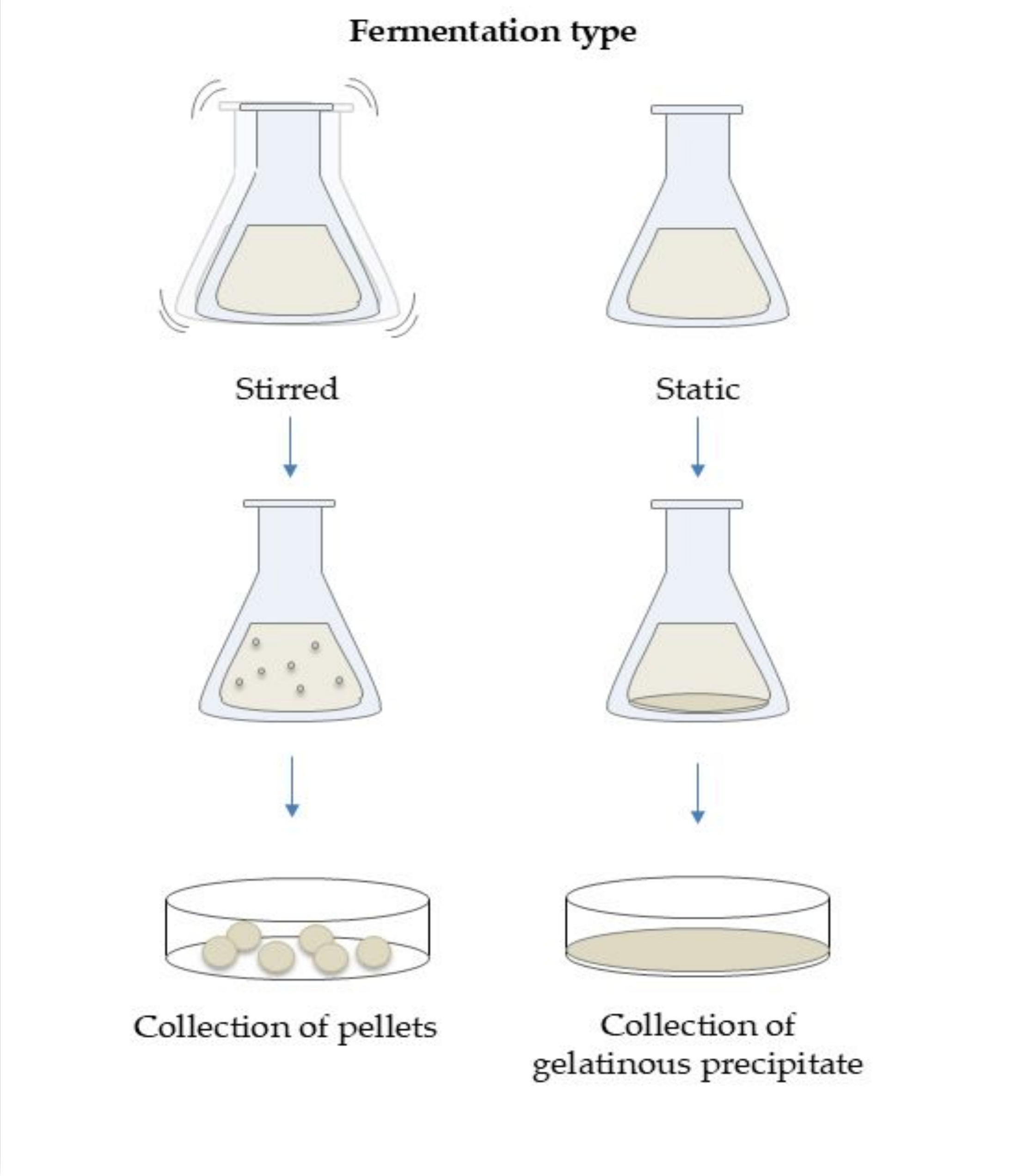

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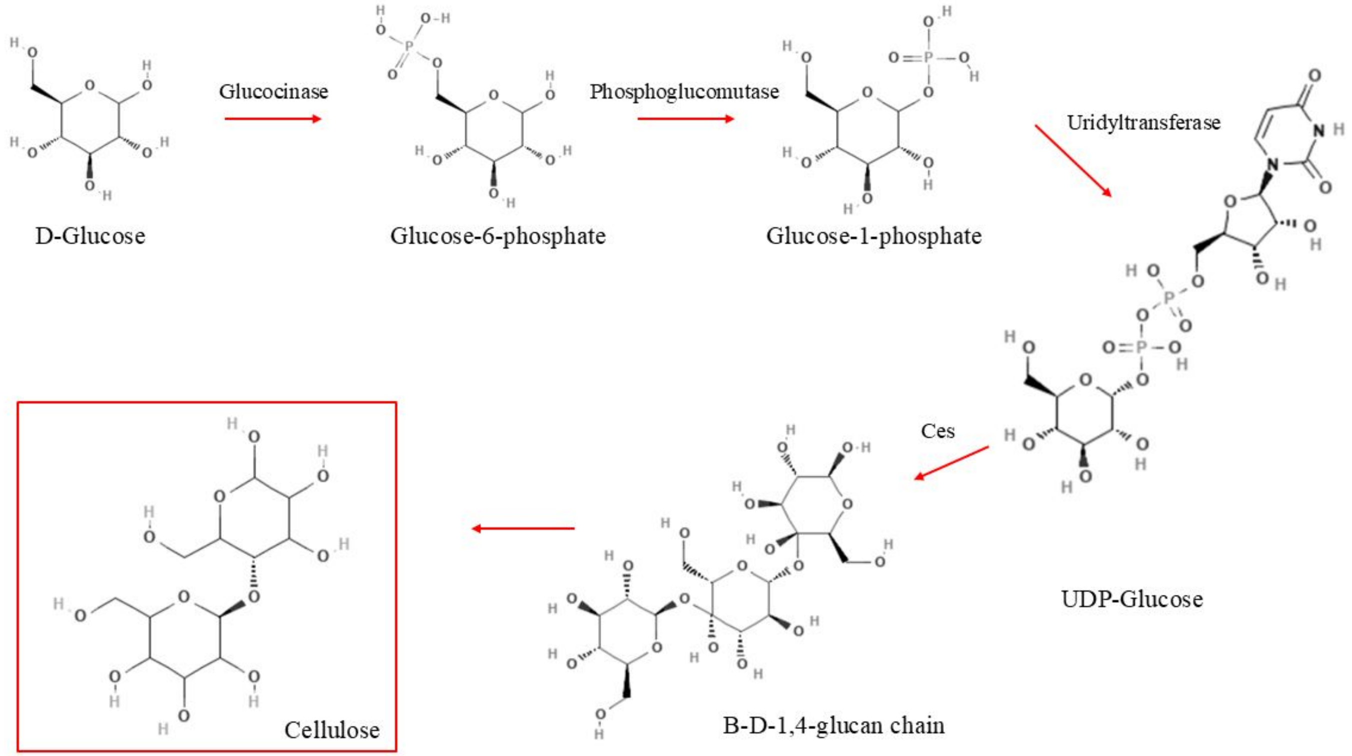

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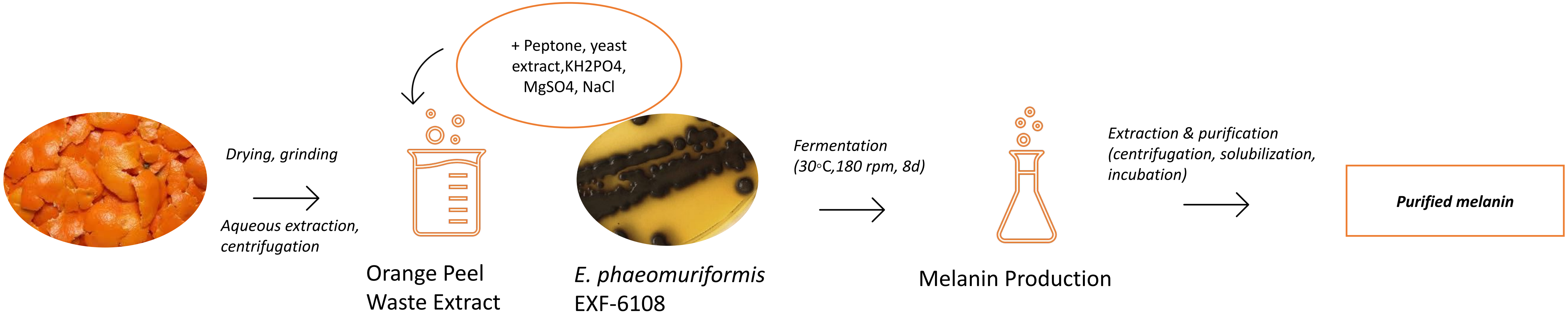

Batch fermentations in flasks: 8d, 180rpm, pH=6, T=30◦C, $V_{working}$=50mL, $V_{inoculum}$= 2% v/v.

- Nitrogen source: yeast extract (1.5 g/L), peptone (1.5g/L)
- Carbon source : aqueous extract of orange peel waste rich in free sugars
- Minerals: 1 g/L KH_2PO_4, 2 g/L MgSO4, 1 g/L NaCl

Extraction & recovery of melanin

- pH adjustment to 12 (solubilization, 1M NaOH), incubation overnight (70°C, 230rpm).
- pH raised to 2 (precipitation, 5M H_2SO_4), washing, purification (Medicell Membranes Ltd, 12-14 kDa molecular weight cut-off), drying (85°C, 24h).

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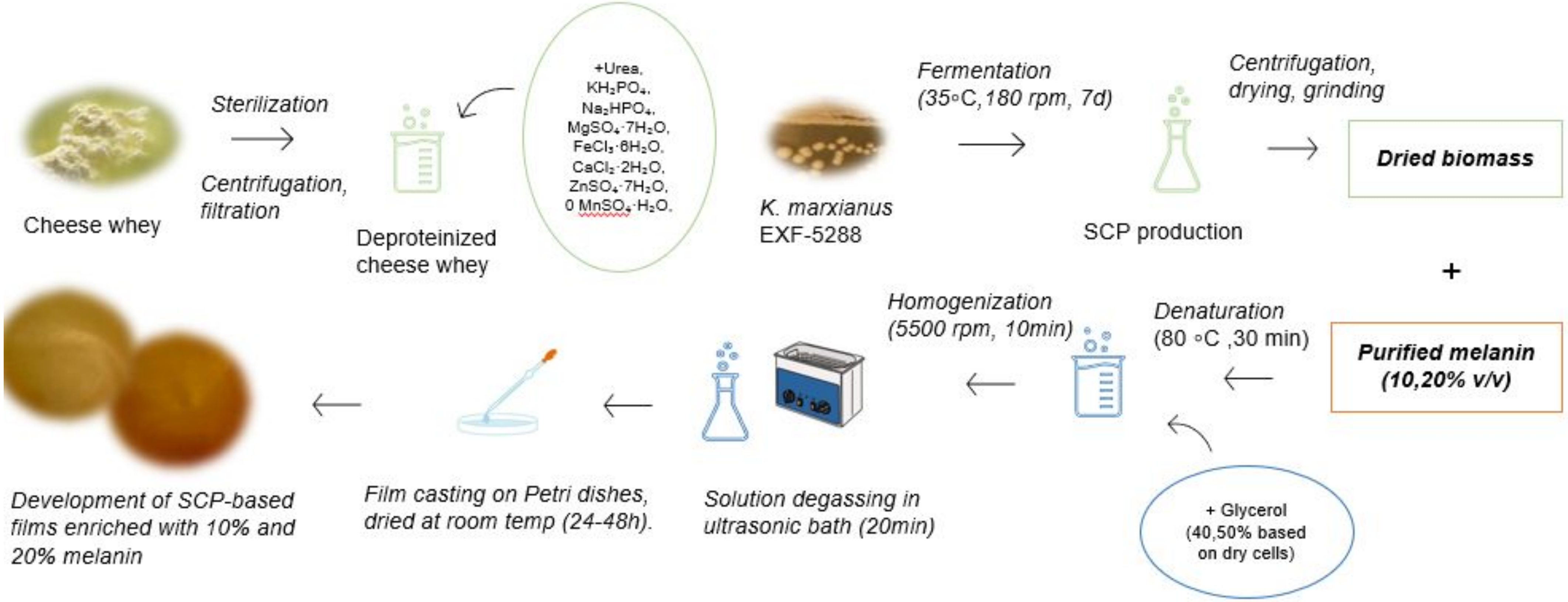
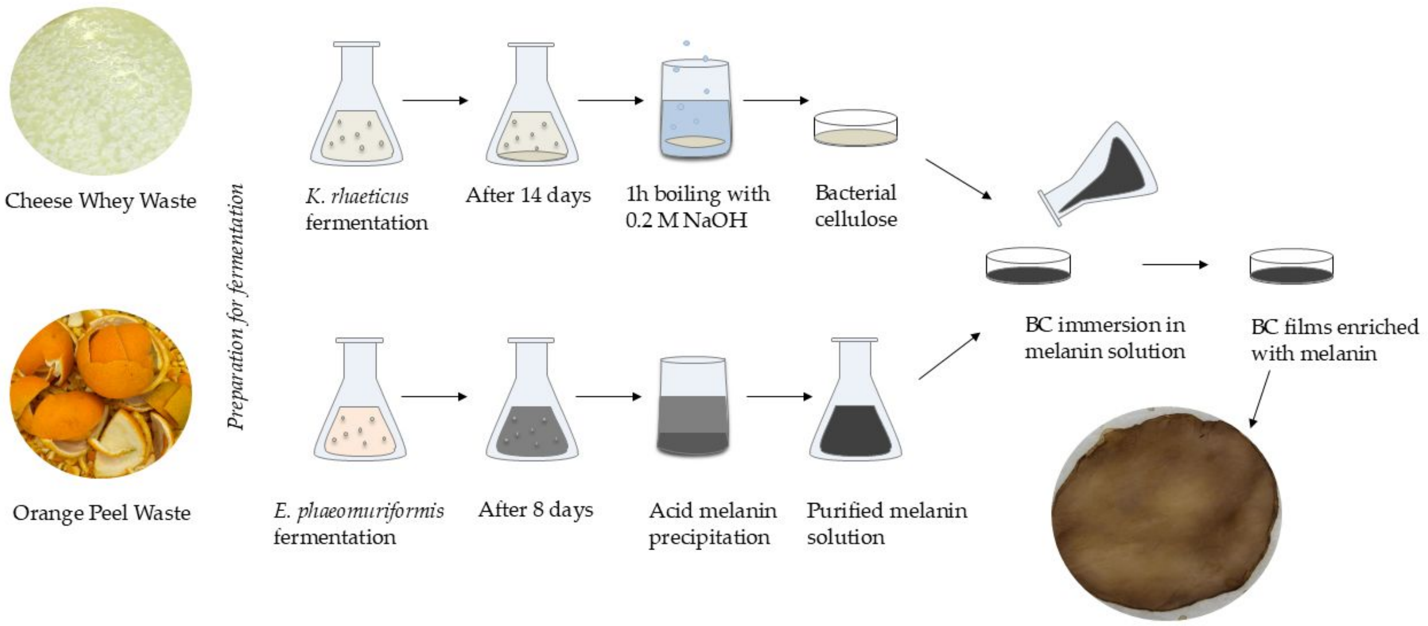


Cheese whey
Sterilization
Centrifugation, filtration
Deproteinized cheese whey
+Urea, KH2PO4, Na2HPO4, MgSO4·7H2O, FeCl3·6H2O, CaCl2·2H2O, ZnSO4·7H2O, 0 MnSO4·H2O.
K. marxianus EXF-5288
Fermentation (35◦C,180 rpm, 7d)
SCP production
Centrifugation, drying, grinding
Dried biomass
+
Purified melanin (10,20% v/v)
Denaturation (80 ◦C ,30 min)
+ Glycerol (40,50% based on dry cells)
Homogenization (5500 rpm, 10min)
Solution degassing in ultrasonic bath (20min)
Film casting on Petri dishes, dried at room temp (24-48h).
Development of SCP-based films enriched with 10% and 20% melanin



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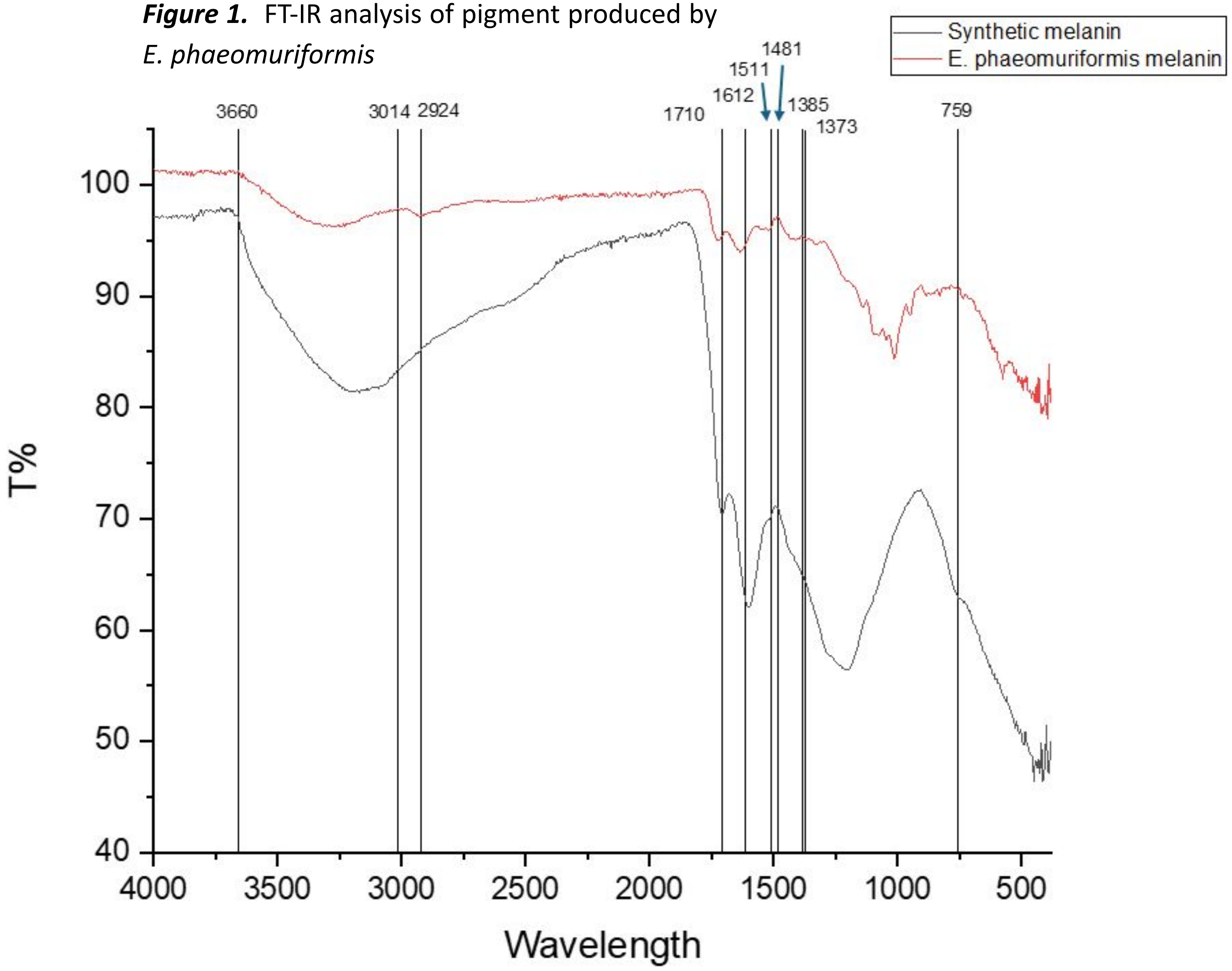

Figure 1. FT-IR analysis of pigment produced by *E. phaeomuriformis*

Film development trials

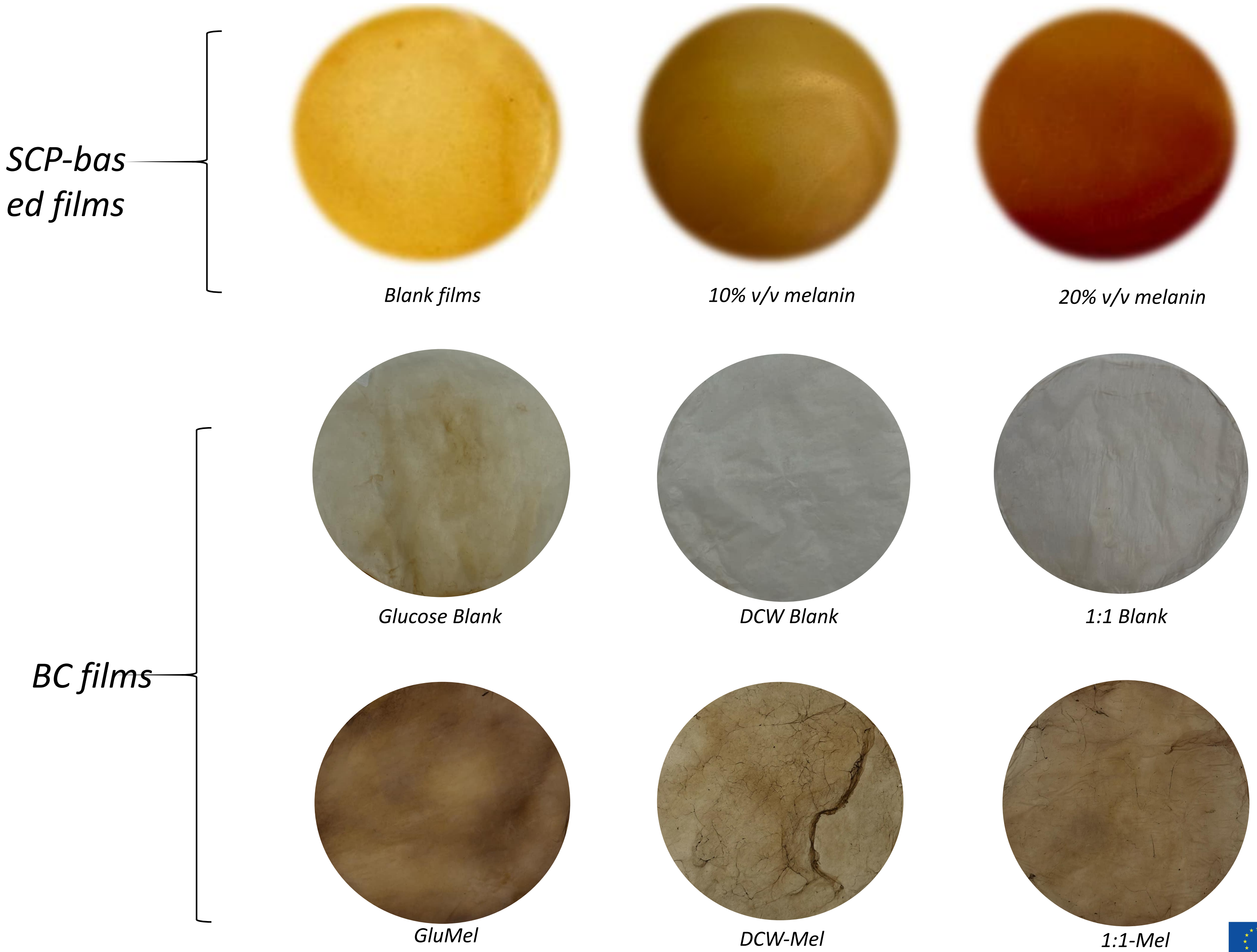

Figure 2. *Effect of melanin and substrate on BC and SCP-based films appearance*

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Table 1. Effect of different carbon sources on BC production using K. rhaeticus UNIWA AAK2

Carbon Source	Time (h)	TDW (g/L)	Consumed FAN (%)	Consumed sugars (%)
Glucose	336	1.20 ± 0.00[a]	43.50	77.06
1:1	336	0.40 ± 0.00 [b]	47.63	50.98
DCW	336	0.20± 0.00 [c]	48.85	37.39

Different letters within the same columns indicate statistically significant differences ($p<0.05$).

- DCW did not enhance BC production
  Satisfactory consumption of total sugars > 75% in the case of glucose
  Low consumption of total sugars with the addition of DCW

- Max TWD: 1.2 g/L **Glucose**
- Min TWD 0.2 g/L **DCW**

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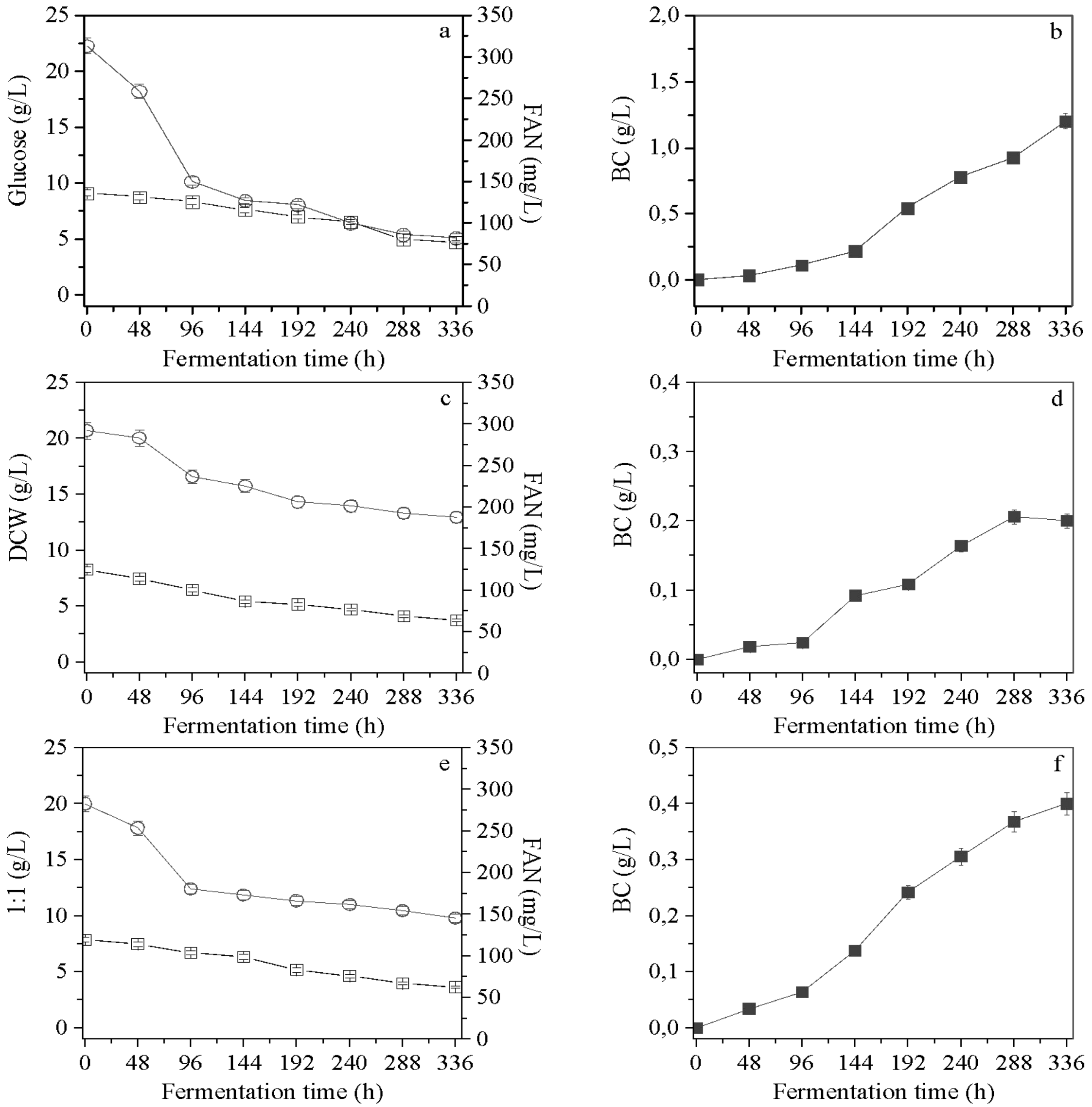

- Sugar consumption is most intense in the first 96 h, especially in **glucose**.
  The greatest BC production occurs between 144-240 h, and almost stabilizes after 288 h.
- FAN consumption does not vary greatly in any of the fermentations.

Figure 3. *Evaluation of BC production using glucose (a, ○), DCW (c, ○) and 1:1 mixture (e, ○) from Komagataeibacter rhaeticus UNIWA AAK2. FAN (□),* dry BC weight from glucose (b,■), DCW (d, ■) and 1:1 ratio (f,■)

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Table 2. Effect of melanin in SCP-films produced by K. marxianus EXF-5288

SCP-based films	L*	a*	b*	C*	h*	Film Opacity
Blank	61.83 ±2.01[b]	11.30 ±1.35[b]	42.70±2.02[a]	44.17±2.32[a]	75.23±0.94[a]	0.07±0.00[a]
10MEL	43±1.92[a]	21.40±1.50[a]	41.53±1.45[a]	46.77±0.65[a]	62.73 ±2.41[b]	0.18±0.00[b]
20MEL	44.5±2.41[a]	21.07±1.16[a]	41±3.32[a]	46.20±2.41[a]	62.63±2.41[c]	1.06±0.00[c]

Different letters within the same columns indicate statistically significant differences ($p<0.05$).

- Statistically significant differences were observed between the samples in the L*, a*, h* parameters and film opacity of the films; however, no significant changes were found in the b* and C* parameters.
- Different letters within the same columns indicate statistically significant differences between the samples ($p > 0.05$).

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Table 3. *Effect of melanin and substrate on BC-films produced by Komagataeibacter rhaeticus UNIWA AAK2*

Film	L*	a*	b*	C*	h*	Film opacity
Glucose	77.20 ± 0.50[a]	4.40 ± 0.37 [a]	16.43 ± 2.10 [a]	17.03 ± 2.10 [a]	74.90 ± 0.73 [a]	0.76 ± 0.03 [a]
1:1	83.87 ± 0.66 [b]	3.67 ± 0.09	0.50 ± 0.16 [b]	3.70 ± 0.08 [b]	10.80 ± 1.44 [a,b]	0.56 ± 0.03 [b]
DCW	84.07 ± 0.73 [b]	3.43 ± 0.12 [a]	1.23 ± 0.68 [b]	3.63 ± 0.33 [b]	341.57 ± 7.85 [b]	0.32 ± 0.01 [c]
GluMel	37.37 ± 1.77[c]	10.43 ± 0.41 [b]	15.00 ± 1.82 [a,c]	18.27 ± 1.72[a]	55.03 ± 2.26 [a]	1.16 ± 0.01 [d]
1:1-Mel	50.50 ± 2.12 [d]	10.70 ± 0.94 [b]	18.13 ± 0.54 [a]	21.07 ± 0.66 [a]	59.37 ± 2.37 [a]	0.78 ± 0.01 [e]
DCW-Mel	51.57 ± 2.95 [d]	10.33 ± 0.84 [b]	16.47 ± 1.67 [c,a]	19.43 ± 1.86 [a]	57.80 ± 0.91 [b,a]	0.42 ± 0.02 [f]

Different letters within the same columns indicate statistically significant differences ($p<0.05$).

- Statistically significant differences between different substrates and melanin addition for opacity.
- Melanin reduces brightness and enhances red/yellow shades.

  Melanin creates more intense colors and increases opacity.

- Melanin reduces films' brightness.

  1:1-Mel and GluMel films have more distinct a* and b*.

  Films with melanin have more intense C*

  DCW films have greener tones, while the rest have warmer tones.

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Table 4. Effect of melanin enrichment on moisture content, S, S.I. and WVP of BC from *K. marxianus EXF-5288*

SCP-based films	M. C. (%)	S (%)	S.I. (%)	WVP (g*mm/m^2*d*kPa)
Blank	20.71±0.27^b	48.31±0.30^a	5.23±0.29^a	8.15±3.21^a
10MEL	18.68±0.32^a	38.86±0.91^b	7.08±0.35^b	9.19±3.51^a
20MEL	18.39±0.39^a	36.84±0.61^c	9.03±0.18^c	9.49±4.76^a

Different letters within the same columns indicate statistically significant differences (p<0.05).

- Statistically significant differences were observed between the samples in moisture content, solubility, and swelling index; however, no significant changes were found in water vapor permeability.

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BC films charachterization (4): Moisture content, solubility, swelling index, water vapor permeability

***Table 5.** Effect of different carbon sources and melanin enrichment on moisture content, S, S.I. and WVP of BC from K. rhaeticus UNIWA AAK2*

Film	S.I. %	Solubility %	Moisture content %	WVP (gmm/(m²dkPa))
Glucose	607.71 ± 6.74[a]	43.10 ± 4.15 [a]	20.00 ± 1.61 [a]	3.37 ± 1.38 [a]
1:1	633.33 ± 12.57 [b]	26.09 ± 3.04 [b]	13.79 ± 0.71[b]	1.27 ± 0.52 [b]
DCW	262.50 ± 8.02 [c]	12.50 ± 2.38 [c]	14.29 ± 1.88 [b]	0.95 ± 0.59 [c]
GluMel	680.72 ± 11.65 [d]	53.89 ± 2.93 [d]	9.71 ± 0.71 [b]	8.10 ± 3.19 [d]
1:1-Mel	540.00 ± 5.46 [e]	50.00 ± 4.84 [a]	28.26 ± 3.40[c]	7.94 ± 3.11 [d]
DCW-Mel	491.43 ± 4.71 [f]	56.10 ± 4.48 [d]	18.75 ± 1.18 [a]	1.86 ± 0.72 [c]

Different letters within the same columns indicate statistically significant differences ($p<0.05$).

- Statistically significant differences in all comparisons between melanin-free and melanin films
  Statistically significant differences in all comparisons between carbon sources, except moisture content

- **Moisture content:** No clear correlation to melanin, max: 1:1-Mel (28.26%).
  Solubility: Increased by addition of melanin, max: DCW-Mel (56.10%).
  Swelling: Increased with the addition of melanin, max: GluMel (680.72%).
  WVP: Strongly influenced by carbon source and melanin, max: 8.10 GluMel.

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Table 6. *Effect of different carbon sources and melanin enrichment on mechanical properties of BC from K. marxianus EXF-5288.*

SCP-based films	TS (MPa)	E%
Blank	1.24±0.01[b]	3.41±0.10[a]
10MEL	0.57±0.11[a]	3.86±3.07[a]
20MEL	0.41±0.17[a]	2.78±0.53[a]

Different letters within the same columns indicate statistically significant differences ($p<0.05$).

- Statistically significant differences were observed between the samples in tensile strength; however, no significant changes were found in elongation at break.

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Table 7. *Effect of different carbon sources and melanin enrichment on mechanical properties of BC from K. rhaeticus UNIWA AAK2.*

Film	TS	E%
Glucose	4.40 ± 1.13 [a]	0.98 ± 0.21 [a]
1:1	12.75 ± 6.58 [a]	3.29 ± 1.32 [a]
DCW	3.70 ± 1.21 [a]	1.71 ± 0.40 [a]
GluMel	6.04 ± 4.21 [a]	1.33 ± 1.00 [a]
1:1-Mel	1.78 ± 1.51 [a]	1.02 ± 0.43 [a]
DCW-Mel	8.54 ± 5.40 [a]	2.39 ± 1.15 [a]

Different letters within the same columns indicate statistically significant differences ($p<0.05$).

- Max TS: 12.75 g/L **1:1**
- Max E%: 3.29 g/L **1:1**
- Min TS: 1.78 g/L **1:1-Mel**
- Min E%: 0.98 g/L **Glucose**

- Not statistically significant differences in film comparison with melanin addition

  Not statistically significant differences in film comparison between different carbon sources

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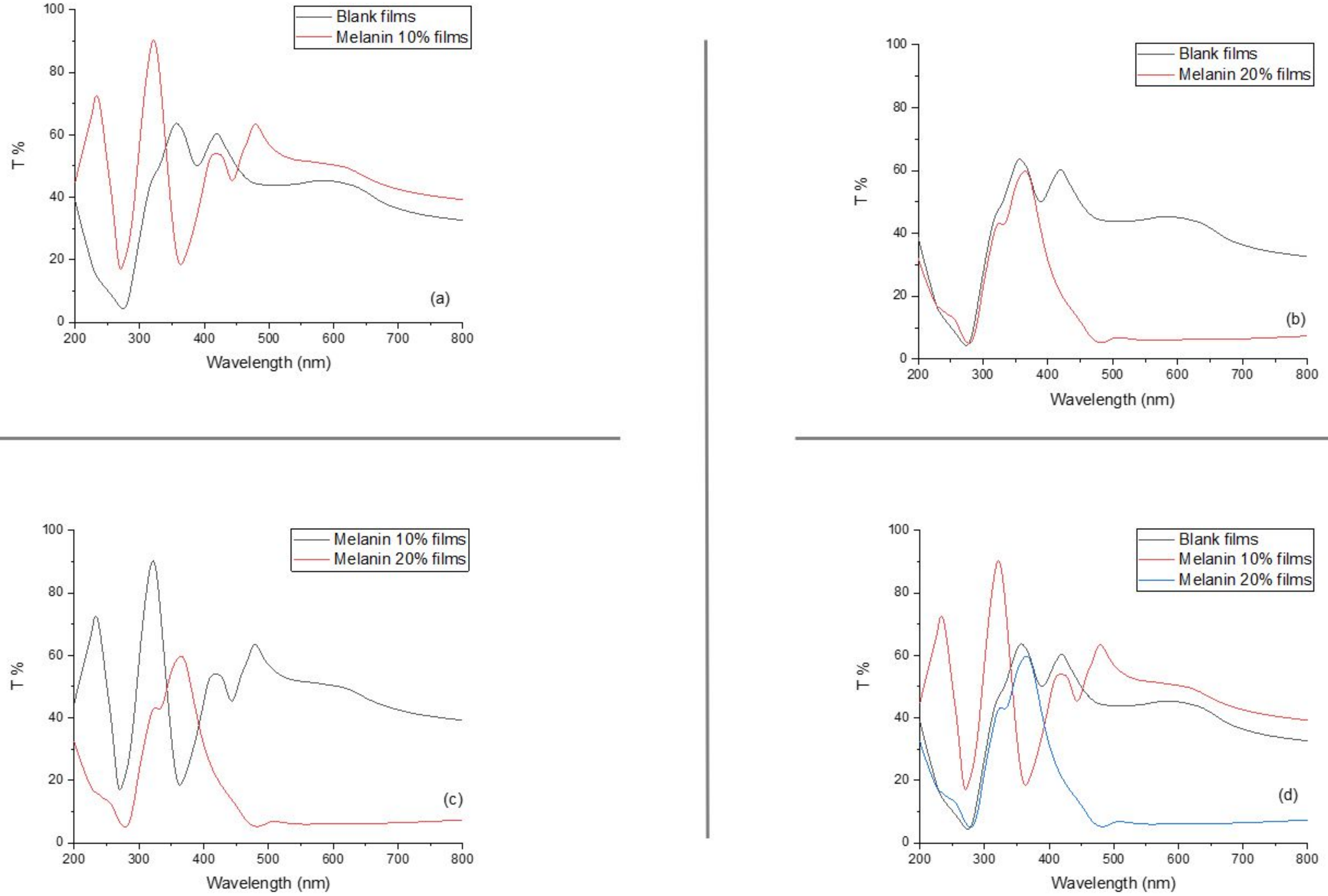

- Blank films: High UV transmittance across all regions
- 10MEL films: Partial UV-blocking, less effective in UVA.
- 20MEL films: Maximum UV-blocking, near-zero UVC/UVB transmittance.

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Figure 4. *Effect of melanin enrichment on UV-Vis transmission of SCP-based films from K. marxianus EXF-5288*

BC films charachterization (6): UV Transmission

- Melanin-enhanced films exhibit significantly reduced transmittance in UVA range
- The inclusion of melanin decreases transmittance in the UVB range
- The inclusion of melanin decreases transmittance in the UVC range in the cases of 1:1 and DCW.
- Glucose films exhibits the lowest UV transmission of all carbon sources

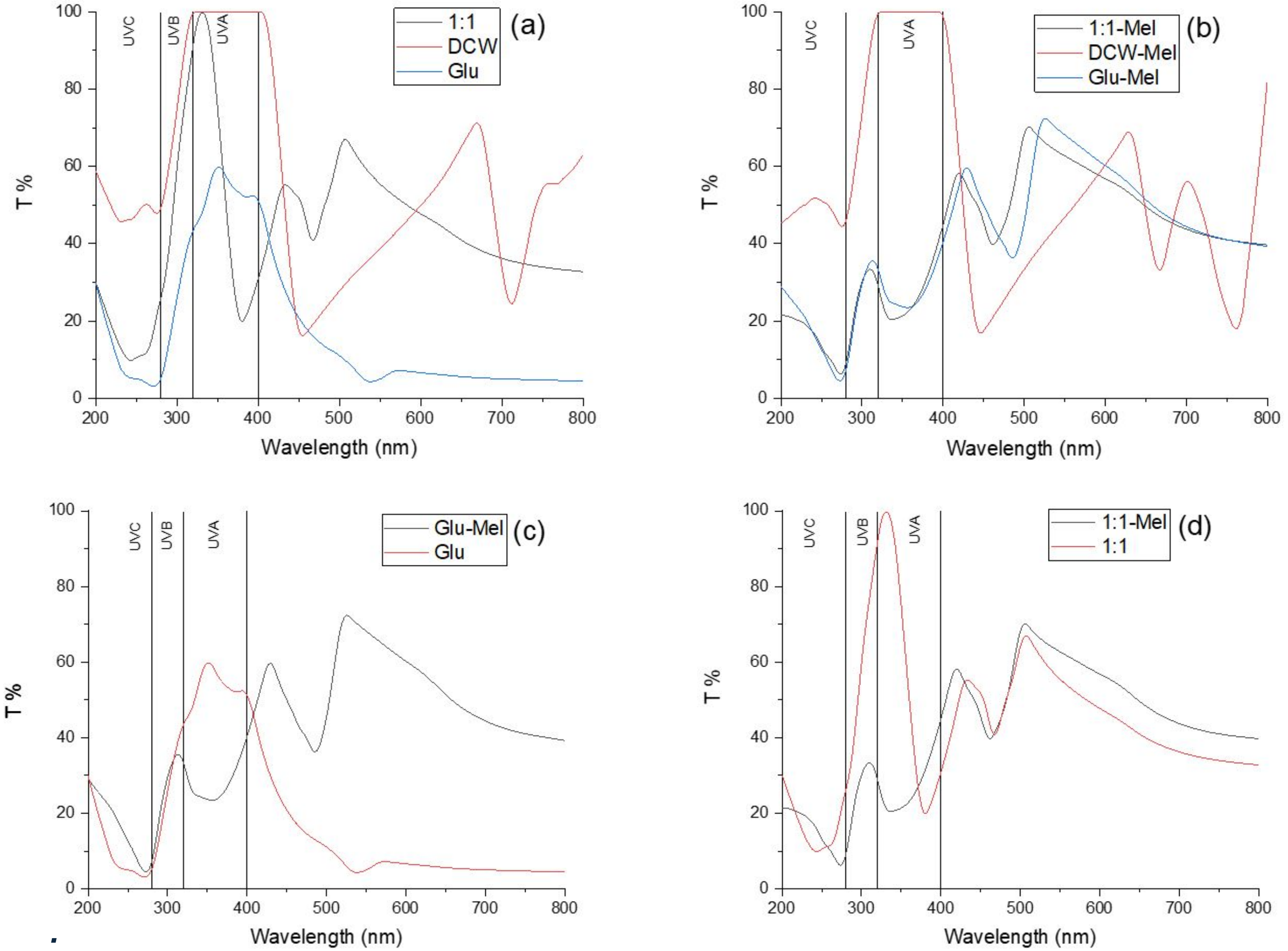
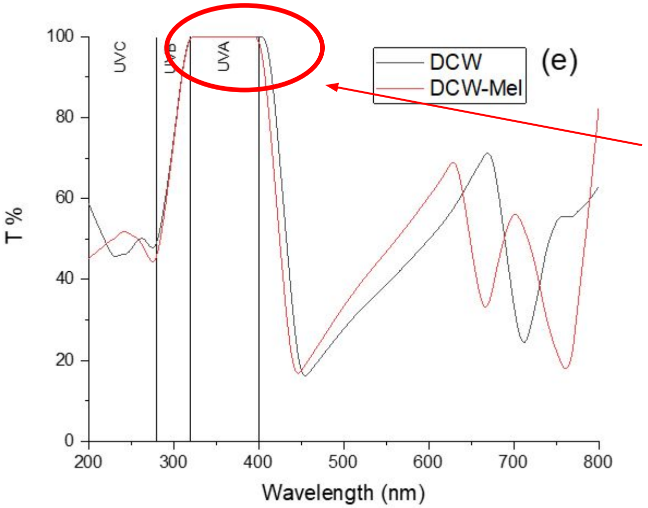


Figure 5. *Effect of different carbon sources and melanin enrichment on UV-Vis transmission of BC from K. rhaeticus UNIWA AAK2*



- DCW exhibits great transmission in the UVB range

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- Glucose is the ideal carbon source for BC production (1.2 g/L), compared to DCW (0.2 g/L).
- Melanin enhances the color and UV-blocking properties of BC films, making them darker and more vibrant.
- Melanin significantly affects moisture content, solubility, swelling index, and water vapor permeability but does not alter tensile strength or elongation.
- Melanin-enriched BC films could be used for UV-protection applications, such as cosmetics, protective clothing, and packaging materials.
- In the future: optimization of fermentation conditions and exploration of sustainable carbon sources like agri-food waste are needed to improve BC production.

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- **Optimize** fermentation and film-casting conditions
- **Explore** alternative food waste streams as carbon sources
- **Enhance** film properties with natural additives or blends
- **Evaluate** environmental & economic impact
- **Ensure** safety & regulatory compliance for real-world use
- **Develop** pilot-scale prototypes for packaging & biomedical use
- **Engage** consumers and stakeholders for feedback & acceptance
- **Establish** industry partnerships and IP protection

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