

COST Action CA24111



1st ENFiber Training School “Fibre 101”, taking place
on 10–11 September 2026 in Ljubljana



ENFiber

European Network for Valorising Food
Processing Waste into Sustainable Fibers

FIBRE UPGRADING FOR COLORANTS AND ENZYMES

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Bioproducts Production and Purification Lab

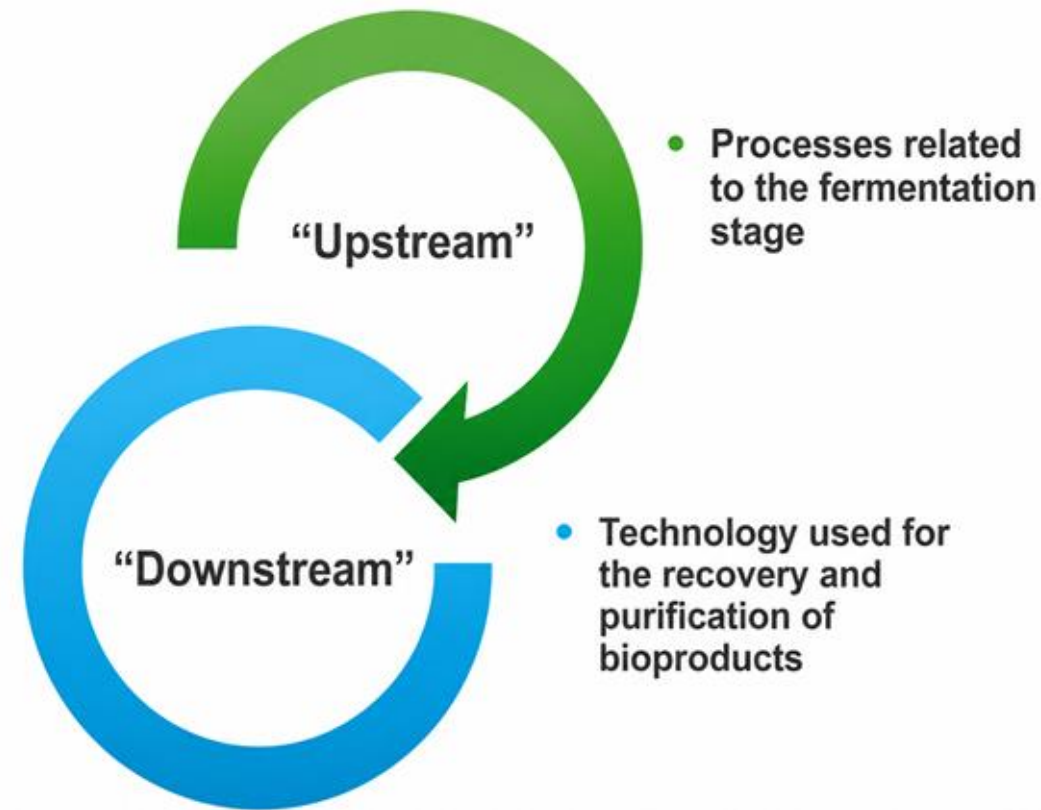


Research interest

- ❖ Upstream process: production of biomolecules, mainly, colorants and enzymes by microorganism.
- ❖ Downstream process: cell disruption, extraction using process.
- ❖ Stability of biomolecules.

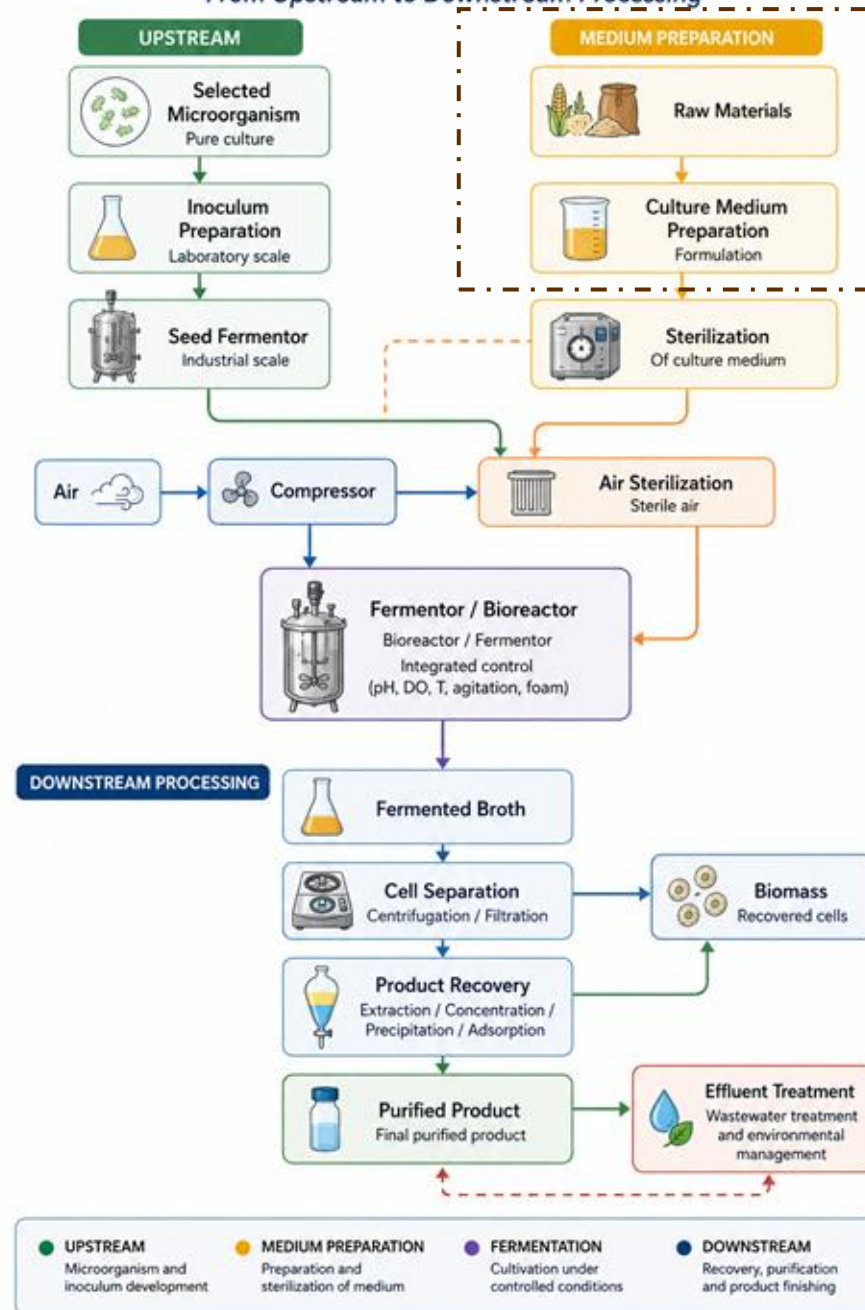
Microbial Bioprocess

Substrate + Microorganism → Product



BIOPROCESS FLOW DIAGRAM

From Upstream to Downstream Processing

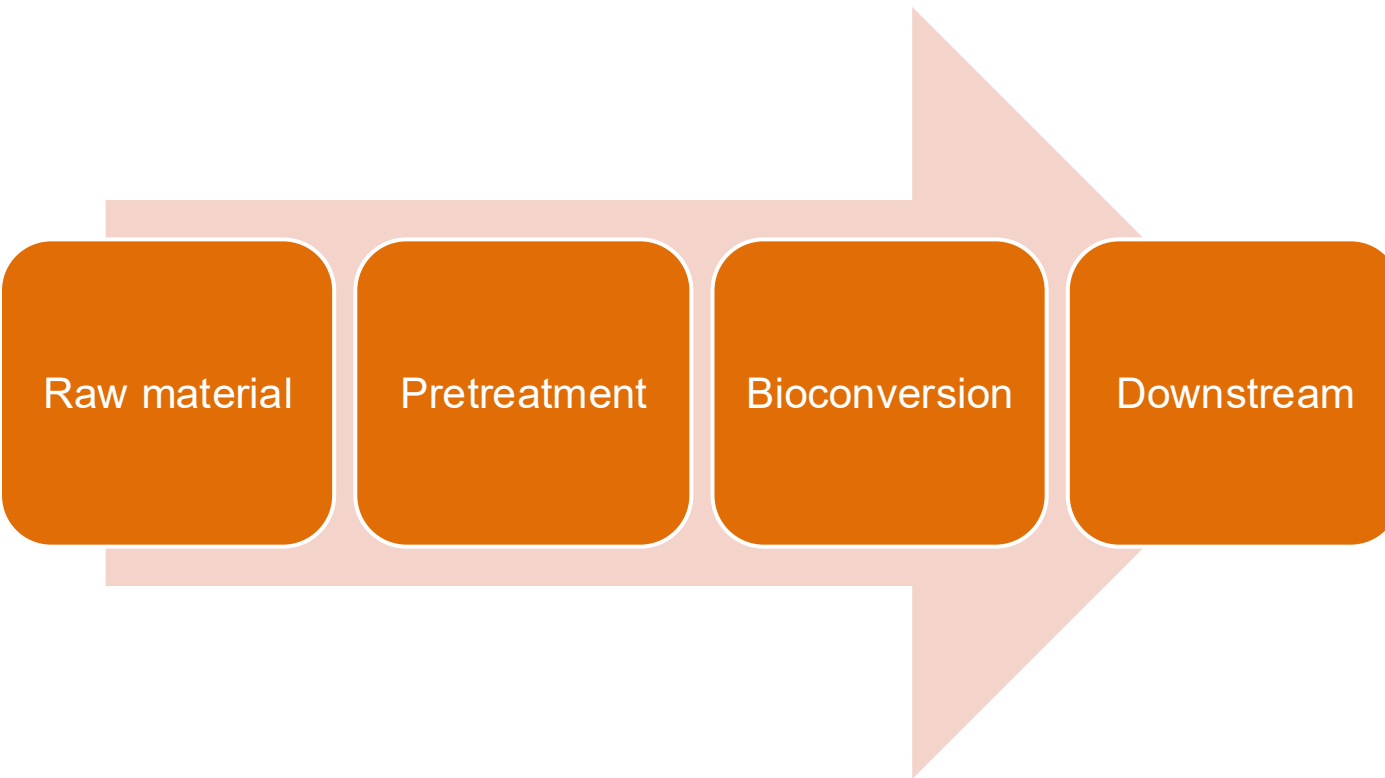


Fibrous biomass



Contextualization

Key challenges when using fibre raw materials in bioprocesses



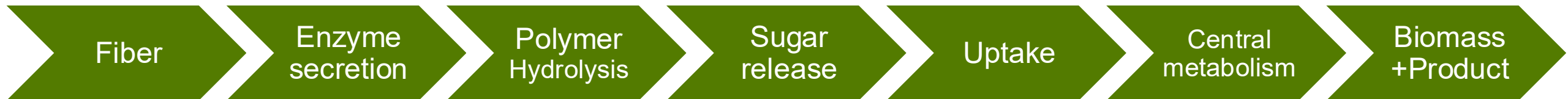
- **Raw material:** composition, particle size, moisture, variability;
- **Pretreatment:** nutrient accessibility vs. inhibitor formation;
- **Bioconversion:** mixing, rheology, mass transfer and oxygen transfer;
- Downstream:** solid–liquid separation and product recovery.

Main engineering challenge: achieving efficient nutrient utilization while maintaining adequate mixing and mass transfer without compromising downstream processing.

Soluble glucose



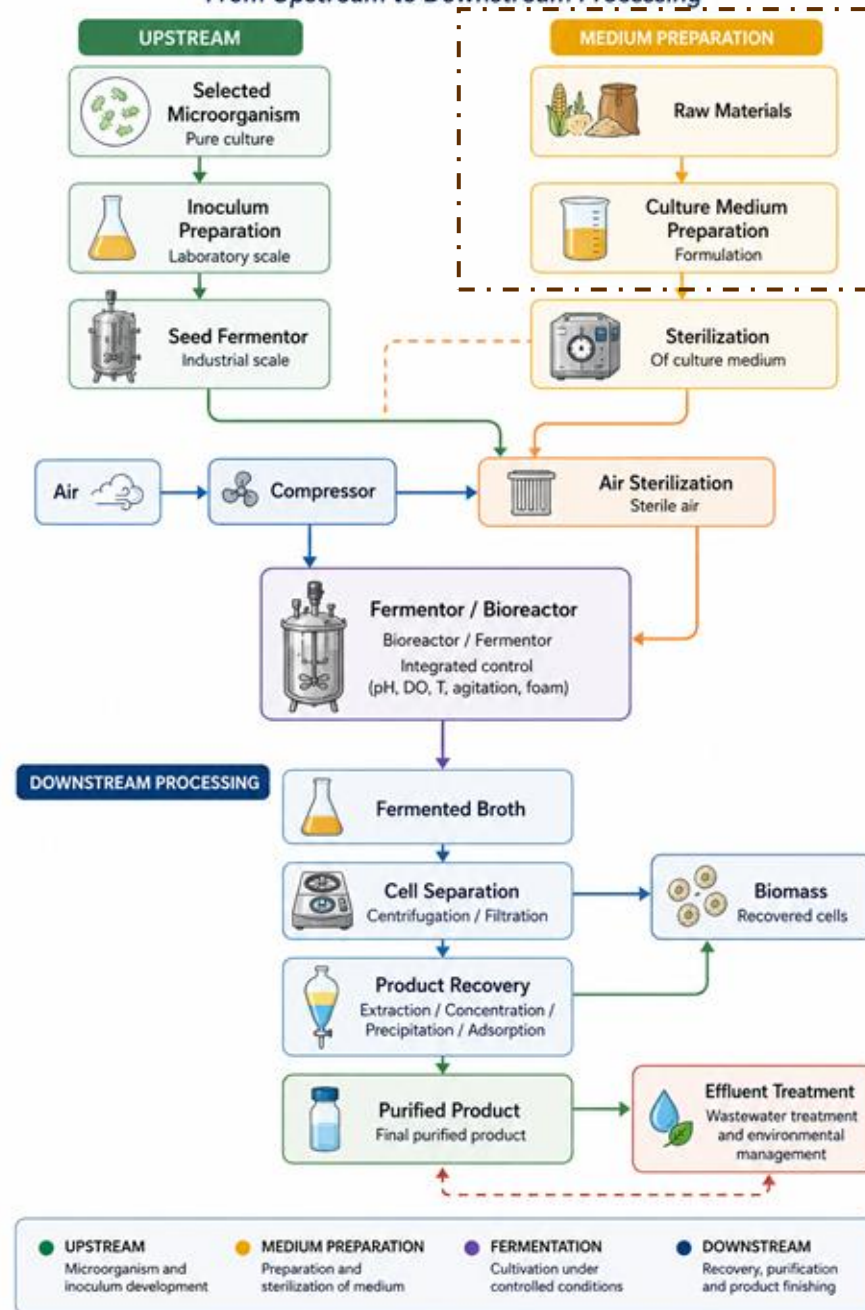
Fibre substrate



Growth on fibre substrates may induce fungal cell-wall remodeling and morphological adaptations, which can ultimately affect broth rheology, mass transfer, and the recovery of intracellular metabolites.

BIOPROCESS FLOW DIAGRAM

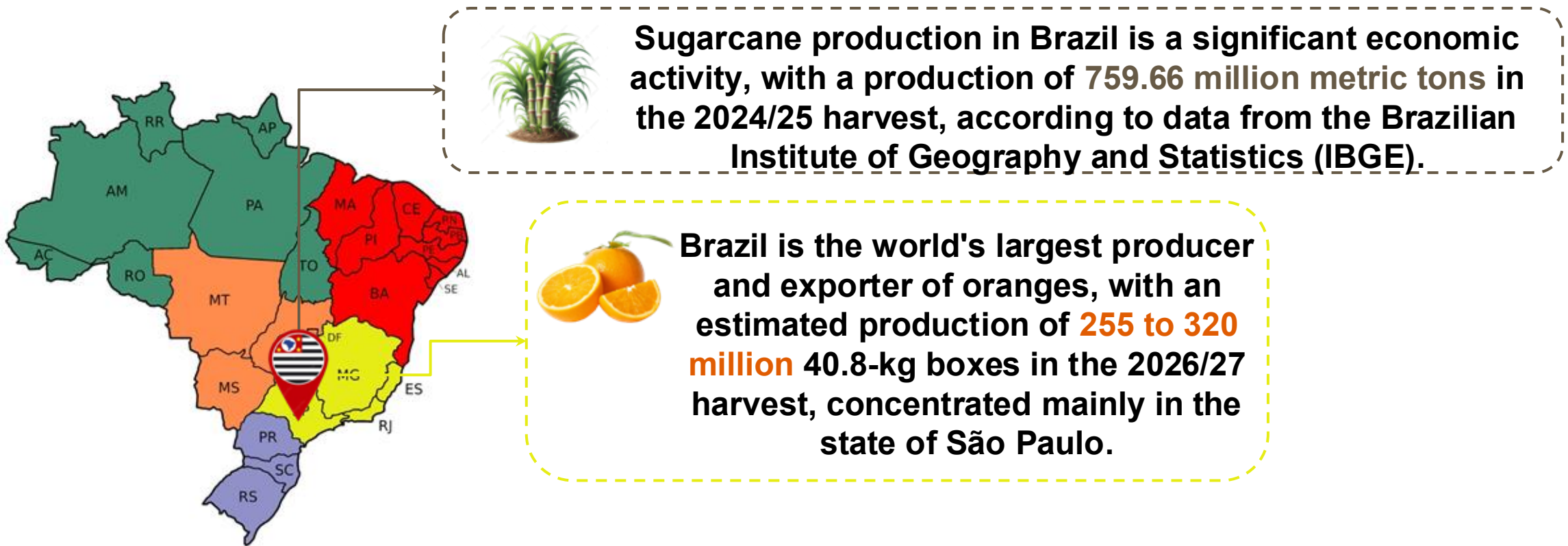
From Upstream to Downstream Processing

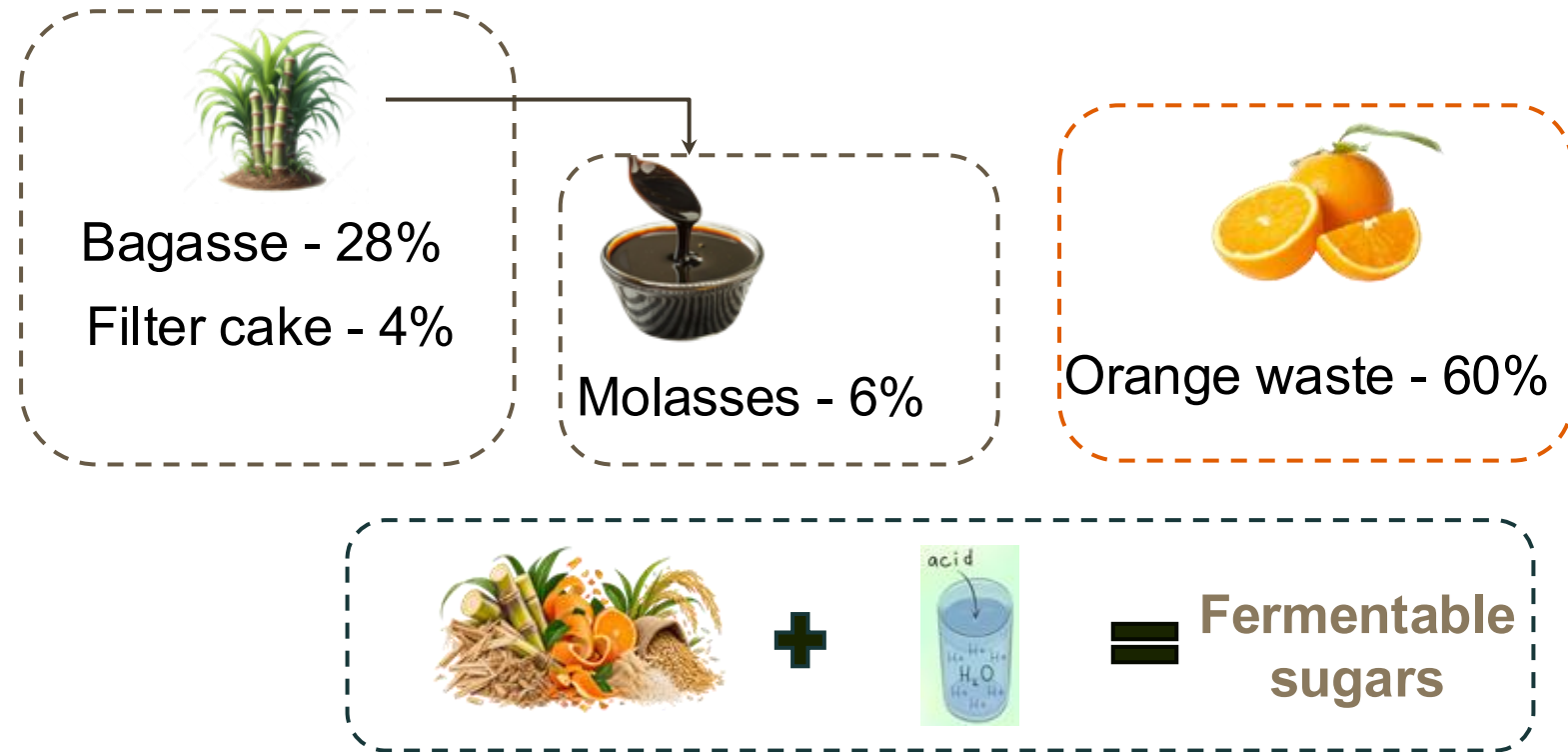


Fibre biomass



Contextualization





Studies of Bioppul research group



Caio de Azevedo
Lima

Sustainable Chemistry and Pharmacy 35 (2023) 101204



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Process development and techno-economic analysis of co-production of colorants and enzymes valuing agro-industrial citrus waste

<https://doi.org/10.1016/j.scp.2023.101204>

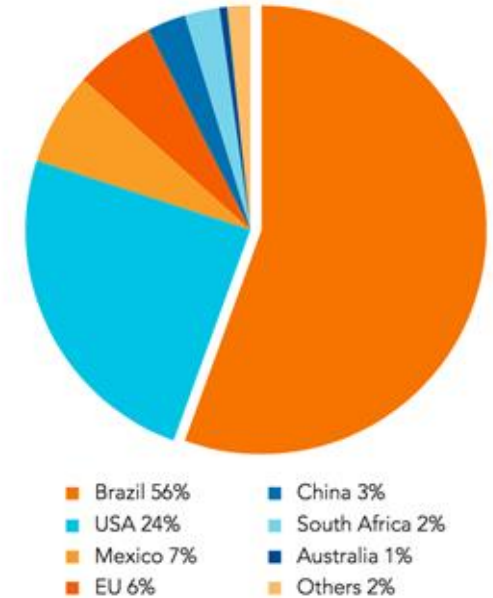


Figure. The major orange-growing regions. Source: Orange book Tetrapark

Figure. World orange juice production, 2014/15. Source: FAS-USDA

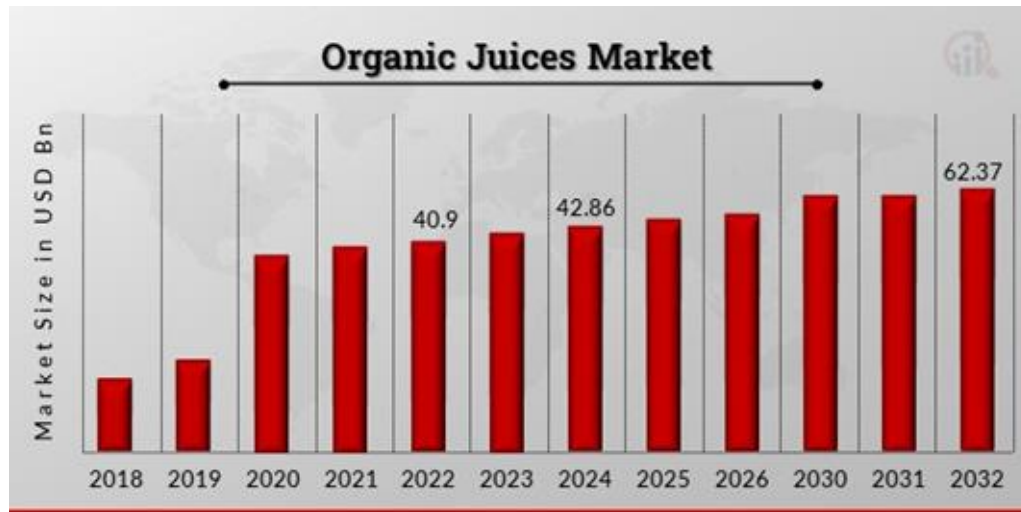


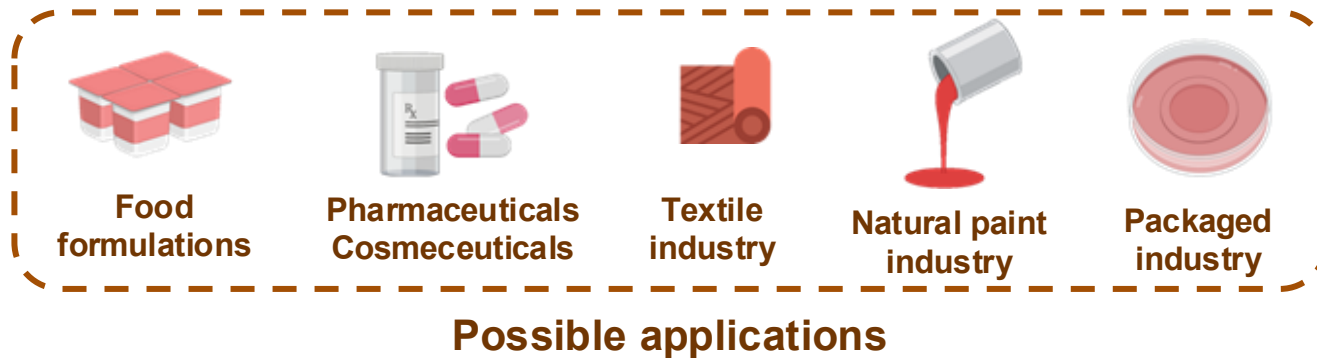
Figure. Organic Juices Market. Source: Market Research Future



42% of waste rich in Sugars



Figure. General products derived from whole oranges. Source: SunBase, Florida



HIGH-ADDED VALUE MOLECULES
NATURAL COLORANTS

NATURAL COLORANTS

Insect



Microorganisms

Bacteria



Algae



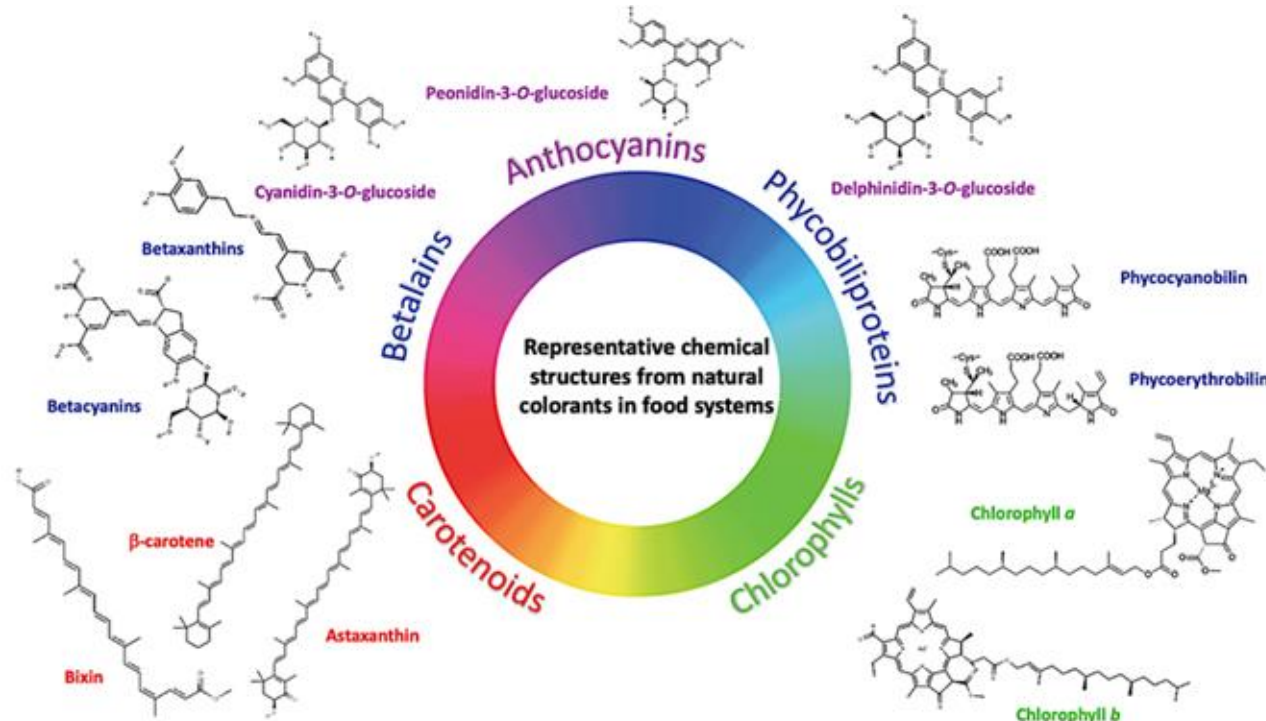
Yeas



Filmentous
fungi

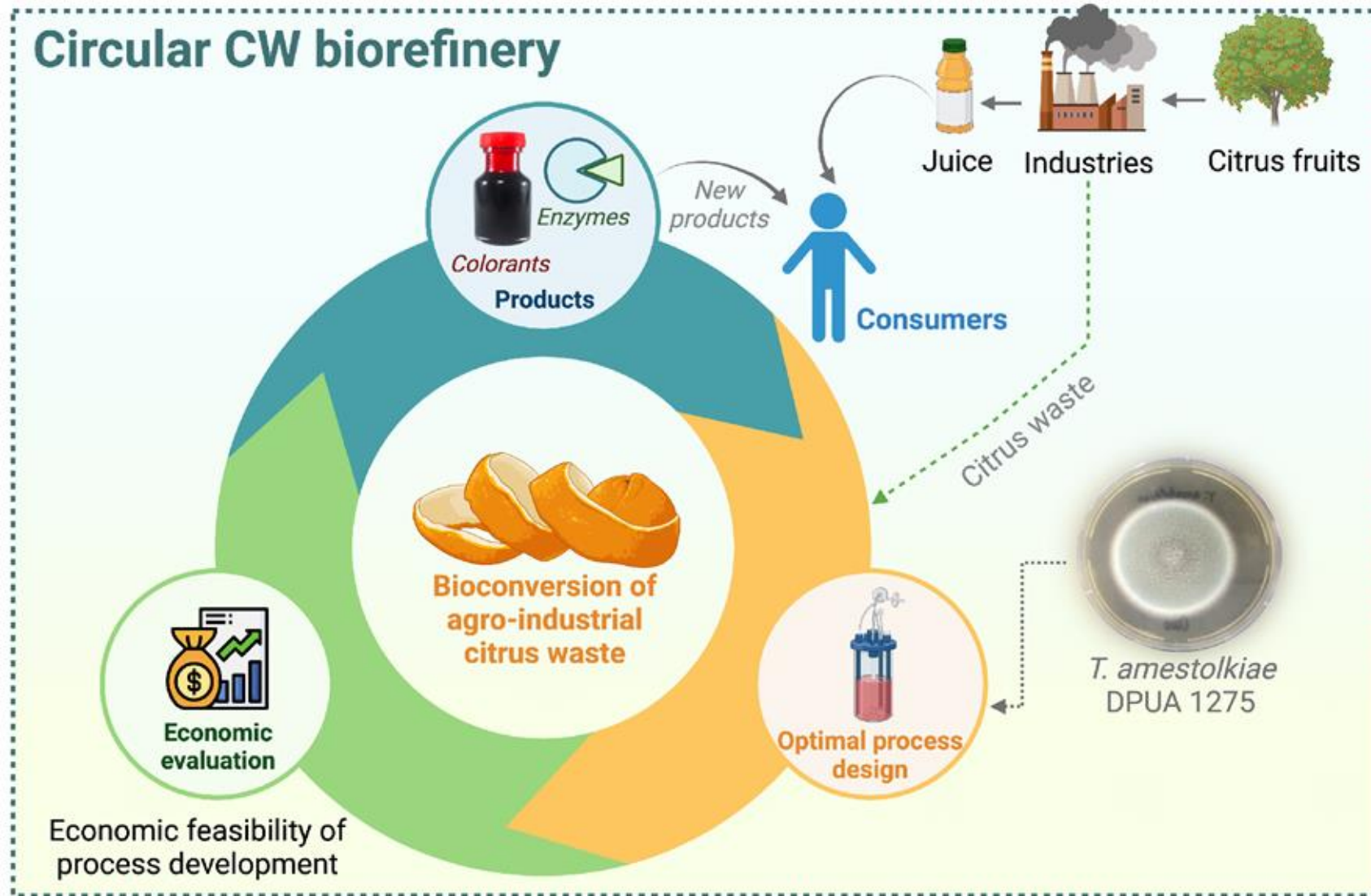


Plants

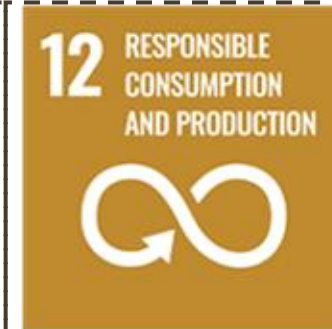
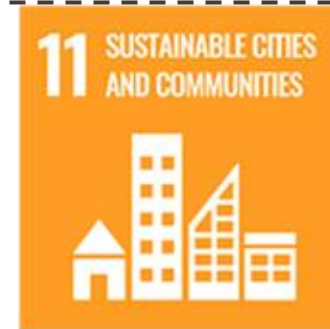
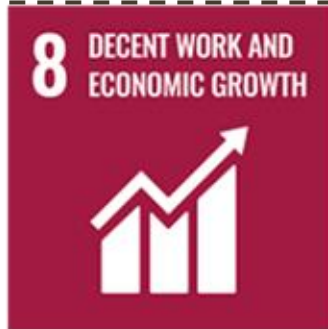


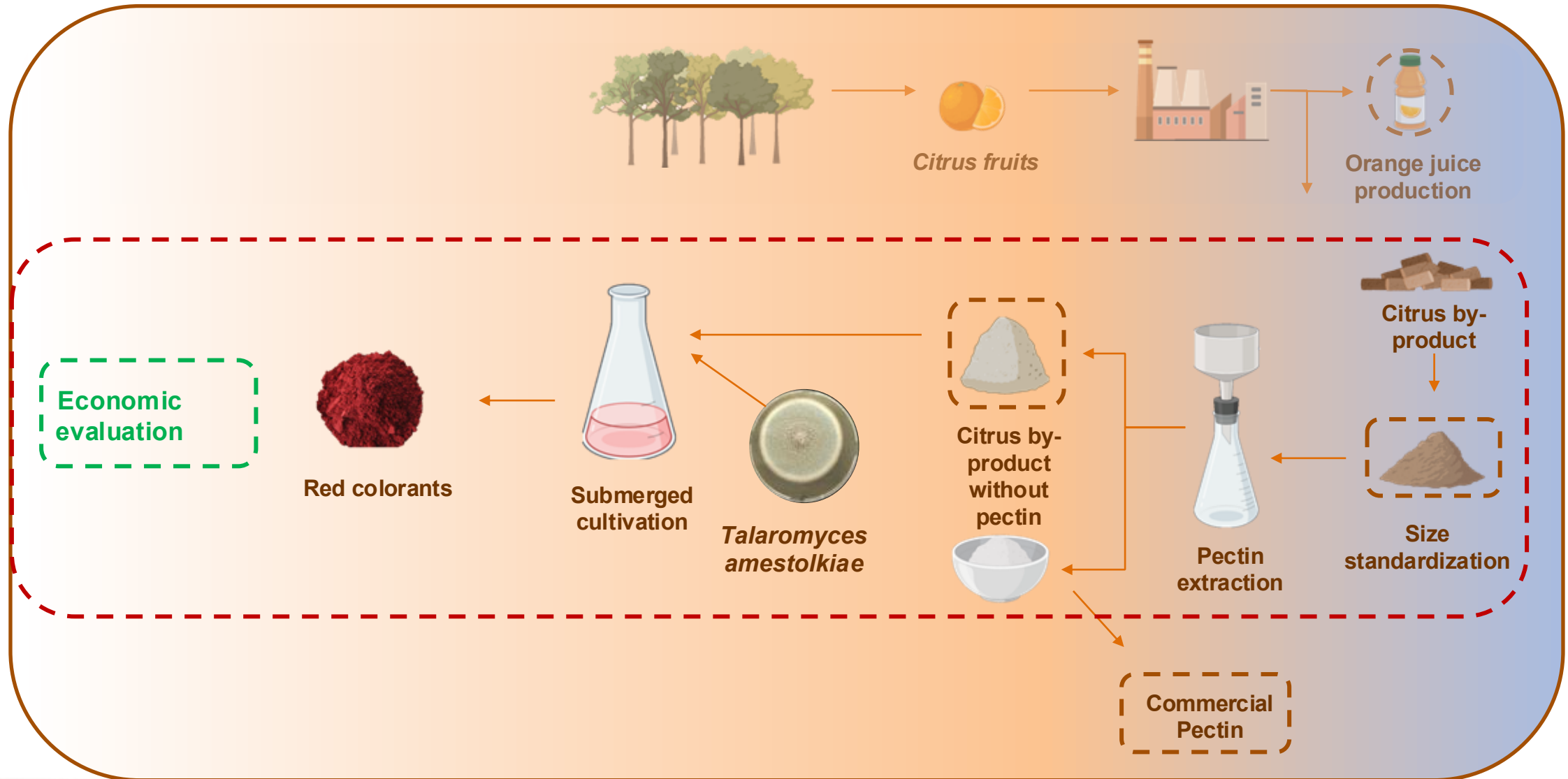
Advantages of microbial colorants:

- can secure the production of the metabolite concerned, followed by controlled conditions, without the influence of free external factors and seasonal raw material supply;
- often are more stable and soluble than similar products extracted from plants or animals;
- capable of minimizing batch-to-batch variations.



SUSTAINABLE DEVELOPMENT GOALS





Materials and methods - Economic evaluation

The calculations to obtain production costs were made considering small scale (laboratory scale) and fixed the percentage:

- 15% - Labors;
- 4% - utilities and waste management
- 81% - materials/consumables and the capital input

$$\frac{\text{Material - Based Production Cost}}{\text{Gram}} = \frac{\sum_{i=1}^n \frac{\text{Use of material}_i}{\text{Batch}} \times \frac{\text{Price of material}_i}{\text{Unit of material}_i}}{\frac{\text{Product Generated}}{\text{Batch}}}$$

$$\text{Total Return (in USD per gram)} = [C_{\text{colorant}} \times \$_{\text{colorant}} + C_{\text{pectin}} \times \$_{\text{pectin}}] - [\alpha * \text{cost per gram}]$$

Results - Chemical composition of citrus by-product

Table 1. Chemical composition of citrus by-product (CB) and citrus by-product without pectin (CB/PEC). Data expressed in percentage (%w.w⁻¹, weight dry).*

Components	CB (%)	CB/PEC (basic pulp) (%)
Lignin	21.58 ± 1.45 ^a	22.35 ± 0.56
Cellulose	11.94 ± 0.16 ^a	23.59 ± 0.56
Xylan	6.50 ± 0.09 ^a	11.29 ± 0.39
Arabinosyl group	4.94 ± 0.13 ^a	8.43 ± 0.11
Acetyl group	2.10 ± 0.52 ^a	2.25 ± 0.14
Proteins	6.56 ± 0.31	ND*
Pectin	4.99 ± 0.29	ND*
Ash	6.58 ± 0.11 ^a	8.87 ± 0.16
Lipids	2.08 ± 0.19 ^a	12.23 ± 0.83
Extractives	25.04 ± 2.14 ^a	1.68 ± 1.03
Total	92.29 ± 5.39 ^a	90.69 ± 3.78

ND=not detected; Data are showed as mean ± standard deviation of three replicates; Means identified with different letters (a, b) on the same line differ significantly ($p < 0.05$). Pulp yield = 54% (w.w⁻¹). #Percentage of untreated loss in relation to treated shown in parentheses.

Results - Red colorant production using citrus by-product

The culture media composition varied according to the experiment, as described below (g.L⁻¹): MSG (Monosodium Glutamate): 25.0; G (Glucose): 10, S (Salts): CaCl (0.015), MgSO₄ (0.012) and FeSO₄ (0.010).

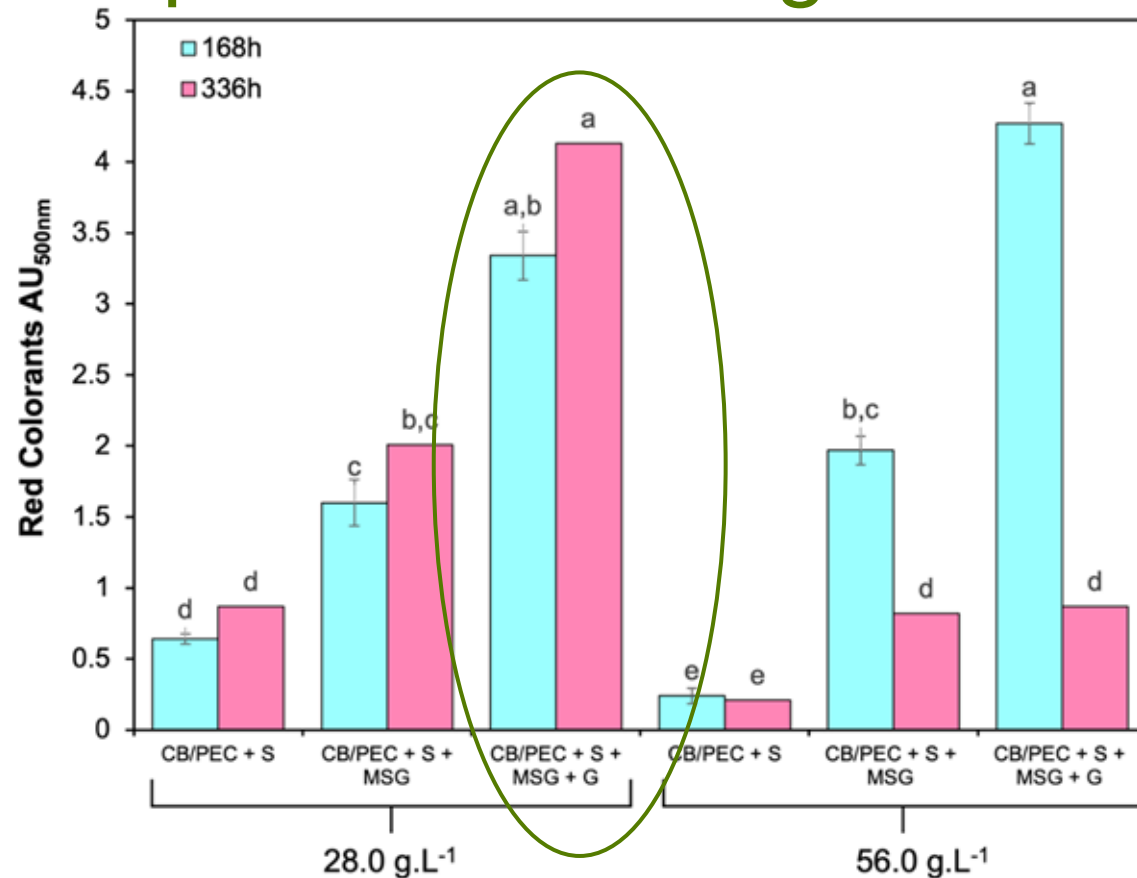


Figure. Production of red colorant by *T. amestolkiae* using citrus by-product without pectin (CB/PEC) in the culture medium carried out at 30°C; 150 rpm in orbital shaker for 168h/336h. Means identified with different letters between all the bars are differ significantly (p<0.05).

Results - Enzymes production using citrus by-product

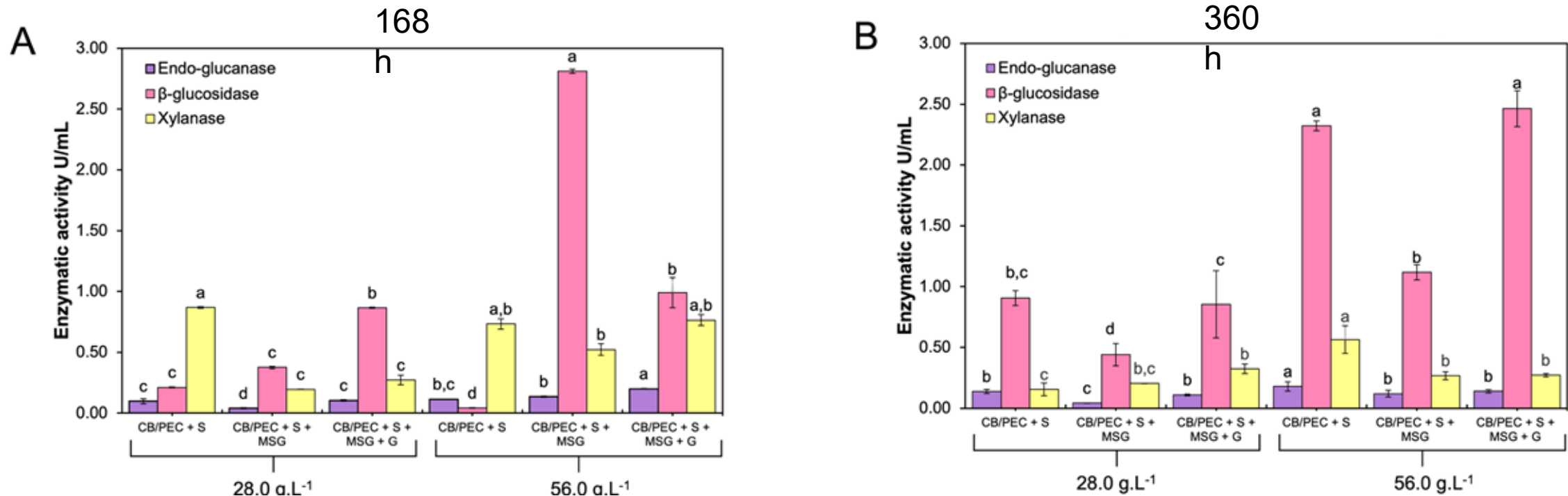
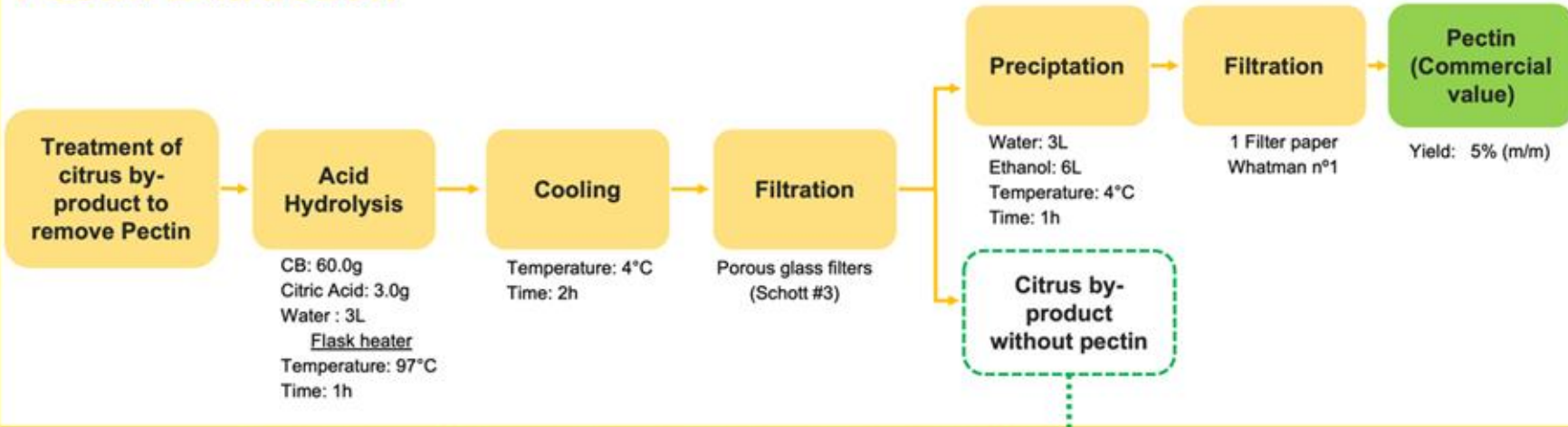


Figure. Enzymatic activity of different enzymes presented in the fermented media of *T. amestolkiae* using citrus by-product without pectin (CB/PEC) in the culture medium carried out at 30°C; 150 rpm in orbital shaker for 168h (A) and 336h (B). *MSG: Monosodium glutamate; G: glucose; S: Salts. Means identified with different letters between all the bars are differ significantly ($p < 0.05$).

Pectin Extraction



CB/PEC-Medium – Colorant Production

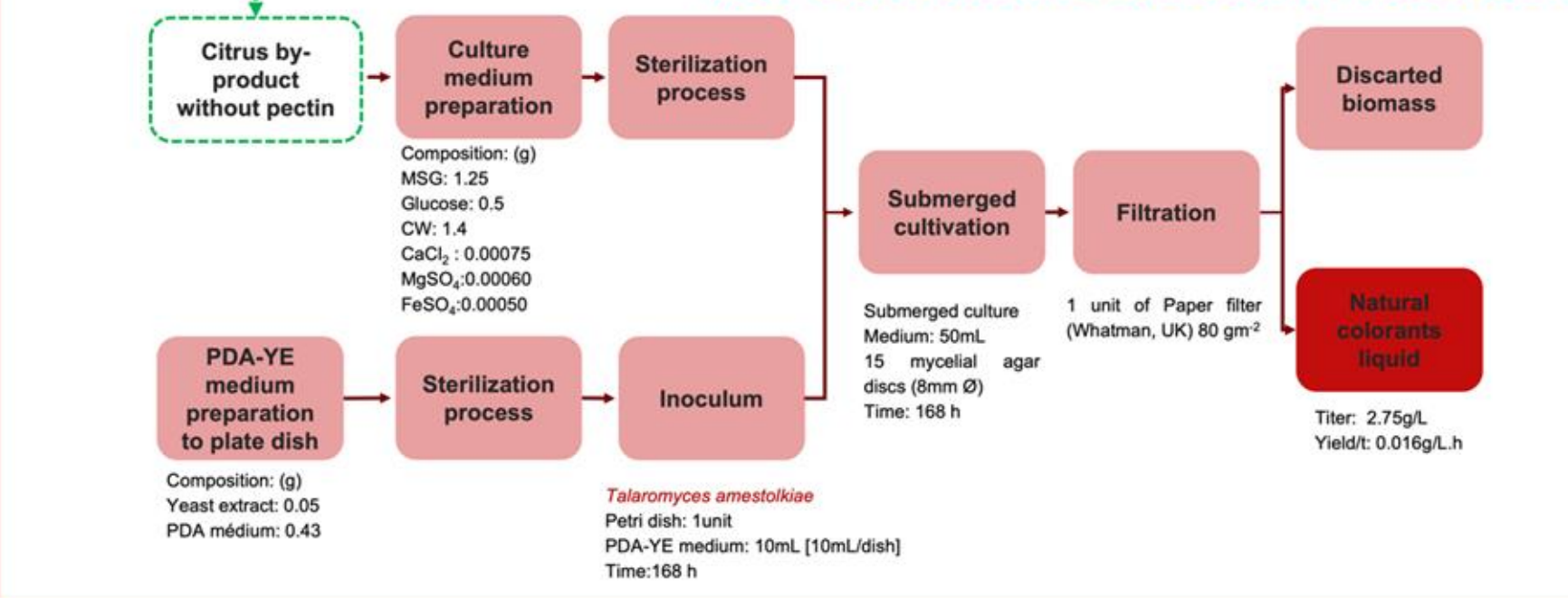


Figure. Flowchart of pectin extraction and red colorant production by *T. amestolkiae* in submerged culture.

Results - Economic evaluation

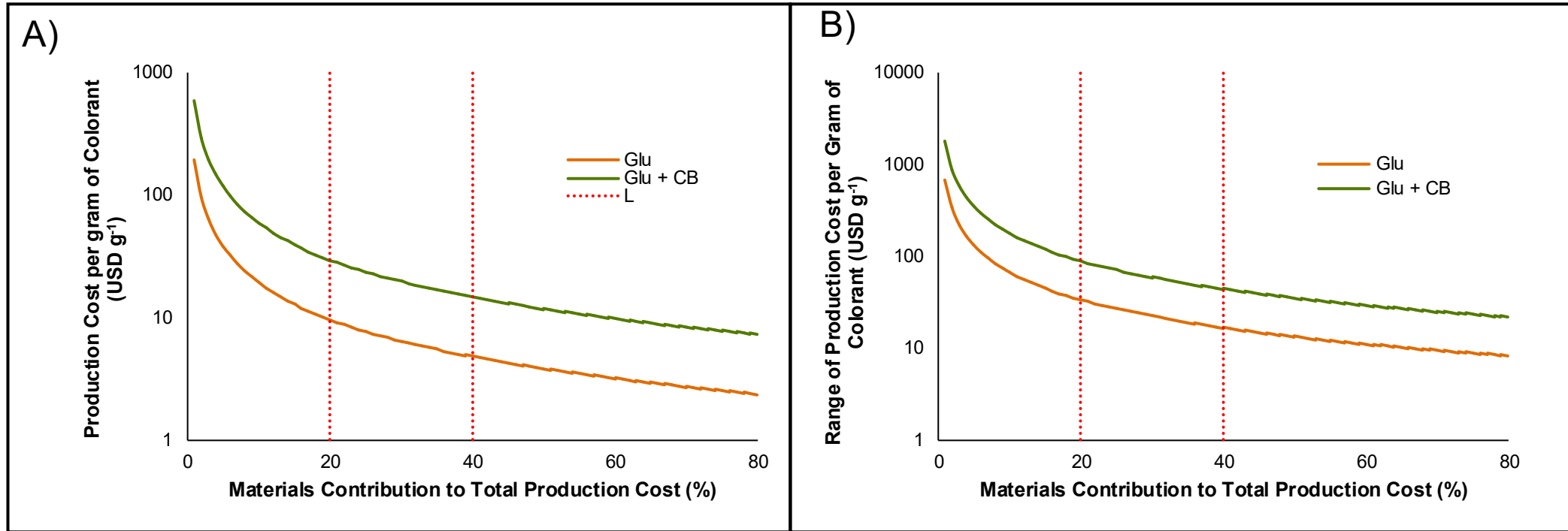


Figure. Results for the calculation of production cost per gram of colorant (a) and the variation on that cost (maximum minus minimum cost) when considering the ranges for the variables studied (b) for all the combinations of materials and capital contribution. Vertical red dotted lines indicate the typical contribution for materials.

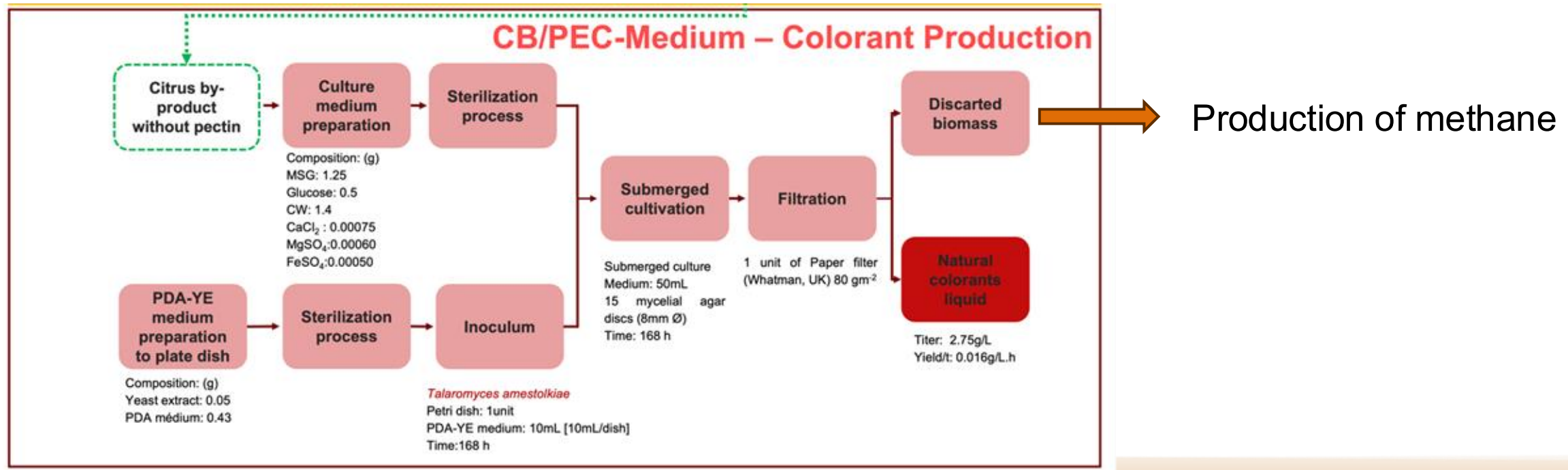
Conclusions

- Citrus by-product contains polysaccharides that can be used as carbon source to produce enzymes and natural colorants by bioconversion using the microorganism *T. amestolkiae*.
- The economic analysis has shown that current production of this colorant needs to be improved, especially if citrus waste will be used for its production.

One of the key parameters for the analysis was the multiplier applied to the production costs. It is imperative to improve the production titer during the fermentation to significantly decrease production costs to obtain a positive return.

Current studies

- ❖ Other studies with the citrus by-product in new cultivation conditions improved around 20% the actual production.
- ❖ Studies of economic analysis and life cycle assessment are being conducted to show the impact of the new process developed which included the production of methane with the whole biomass generated after the bioprocess.



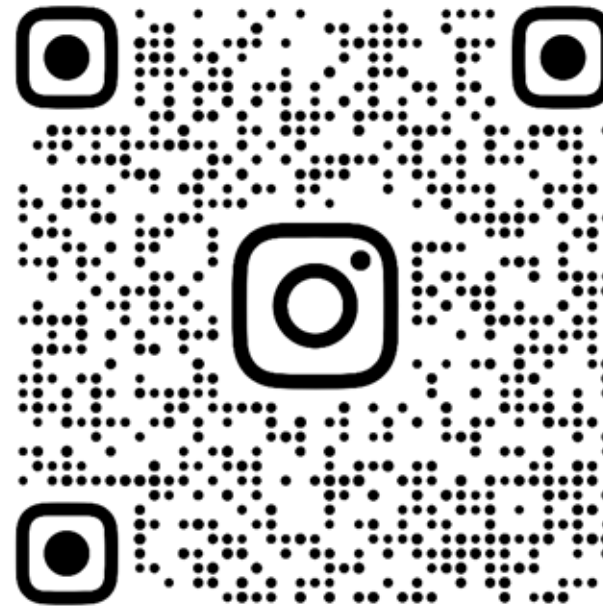
Final remarks

- Fibers and other renewable raw materials can serve as nutrient sources for microbial bioprocesses, contributing to more sustainable production.
- Cultivation conditions may need to be adapted, since these alternative substrates can affect microbial growth and product formation.
- Bioprocess optimization is often necessary to improve substrate utilization, productivity, and process performance.
- With appropriate process optimization, fibers (waste) can become viable feedstocks for sustainable and economically feasible bioprocesses.

ACKNOWLEDGEMENTS



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