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Novel biostimulant bacterial EPS production via Solid-state fermentation as a valorisation strategy for agri-food waste

Enric Garcia Muchart
Laura Mejias Torrent
Oscar Martínez-Avila

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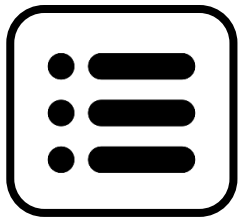
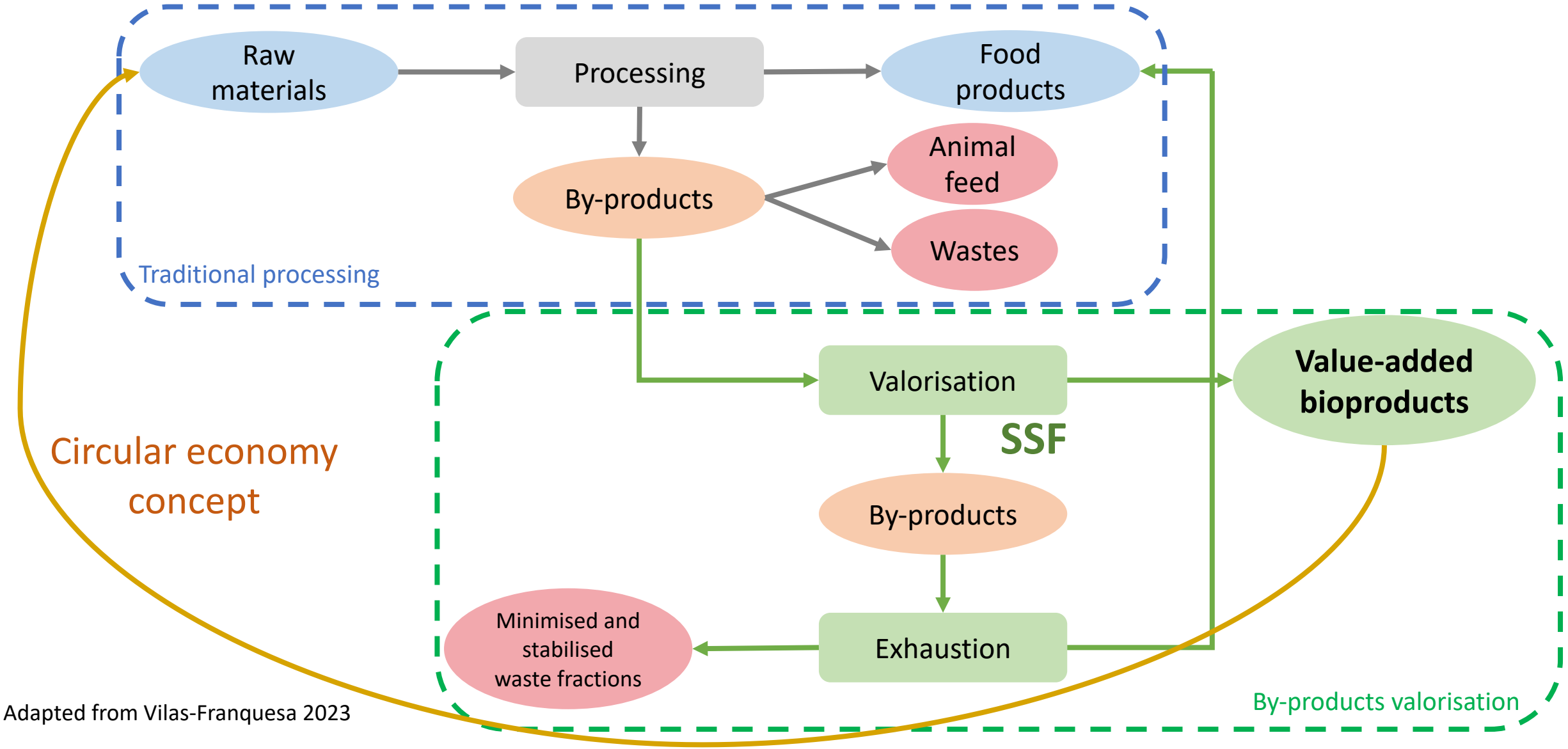
ACCIÓ Generalitat de Catalunya



GENERAL CONTEXT

Circular economy and valorisation concept

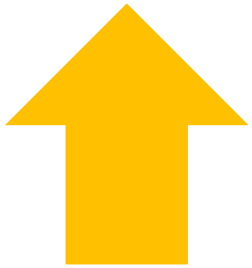


Adapted from Vilas-Franquesa 2023

GENERAL CONTEXT

Biostimulants

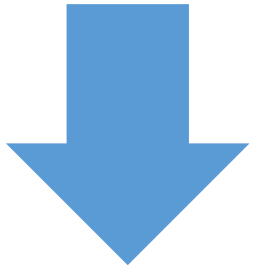
To find a balance



Efficient use of land

Improvements of yields

High-quality products



Minimize the impacts of agricultural practices on human health and environment

Chemical fertilizers: massive use which produces negative effects on the environment derived from their energy-intensive **production**, **processing** and **application**.

Organic fertilizers: alternative source of nutrients. They provide significant advantages as substitute of chemical counterparts, but **some nutrients can not be easily used by plants** due to the chemical bounding they have.

BIOSTIMULANTS

"a substance and/or microorganism whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality" Regulation (EU) 2019/1009

GENERAL CONTEXT

Biostimulants

A substance is defined as **biostimulant** based on its **function**, instead of what it contains.

Regulation (EU) 2019/1009

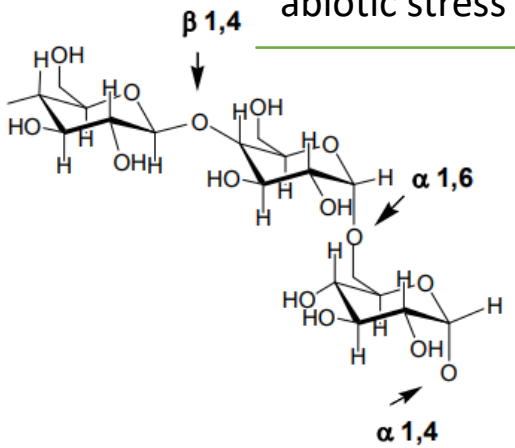
6 main groups

- Humic and fulvic substances
- Protein hydrolysates and other N-containing compounds
- Seaweed extracts and botanicals
- Chitosan and other polymers
- Inorganic compounds
- Beneficial fungi and bacteria

Exopolysaccharides (EPS)

Effects of EPS

- Improvement of the soil particle aggregation and quality
- Increasement of the nutrient uptake and water retention capacity of plants
- Efficiency conferring resistance to abiotic stress



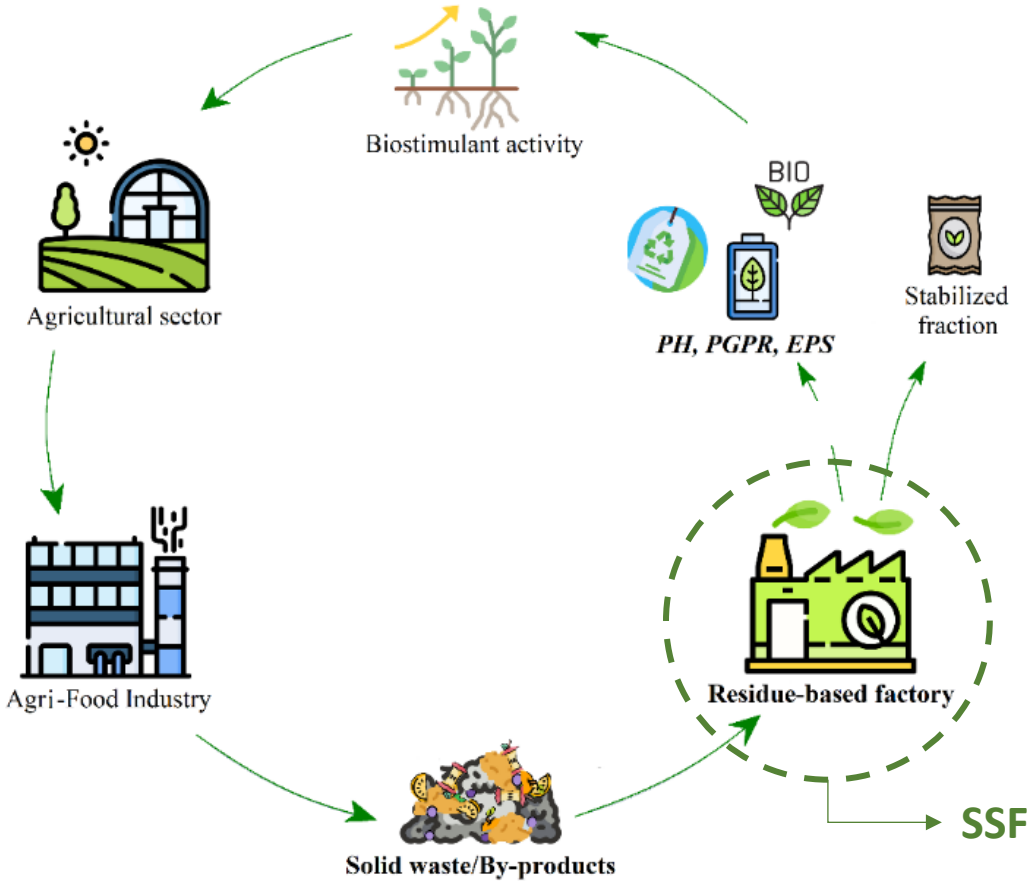
Produced from **bacteria**

In **sugar-rich** substrates

Sucrose, glucose...

Agri-food by-products

OBJECTIVES



Two-fold general objective:

- **Produce EPS** (value-added bioproduct) from specific bacteria through **SSF** using **agri-food waste** as substrate (valorisation).

Specific objectives:

- Determine the **best conditions** to maximize EPS production at **lab-scale**.
- Suggest a **value-chain new perspective** for the agri-food industry.

AGRI-PROSUME project

Initial date: 01/09/2021
Final date: 31/08/2025

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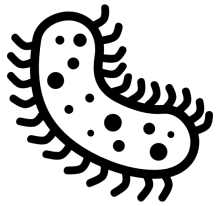
MATERIALS AND METHODS

Microorganisms

Bibliographical research + Representative variety

Bacterial strains



	Bioproducts	Optimal growth conditions
Azotobacter beijerinckii (CECT 9204)	N-fixing bacteria + **EPS** production	30ºC, 48 h, aerobic
Leuconostoc mesenteroides (DSM 20484)	**EPS** production	30ºC
Geobacillus thermodenitrificans (DSM 465)	**EPS** production	60ºC, sugars, aerobic facultative
Alicyclobacillus acidocaldarius (CECT 4328)	Amylases + **EPS** production	55ºC, 72 h, aerobic, low pH
Burkholderia cepacia (CCM 2656)	PHA + **EPS** production	30ºC

MATERIALS AND METHODS

By-products / Substrates

Beet and Ginger Juice Waste (BJW & GJW)

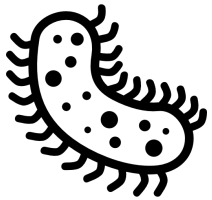
Vegetable Milk Waste (VMW)

Apple Pomace (AP)

Pomegranate Peels and Seeds (PP & PS)

EXPERIMENTAL DESIGN

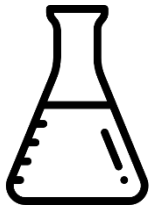
Screening experiments → **Time-course** dynamics → **Optimisation** of operational parameters



Bacteria

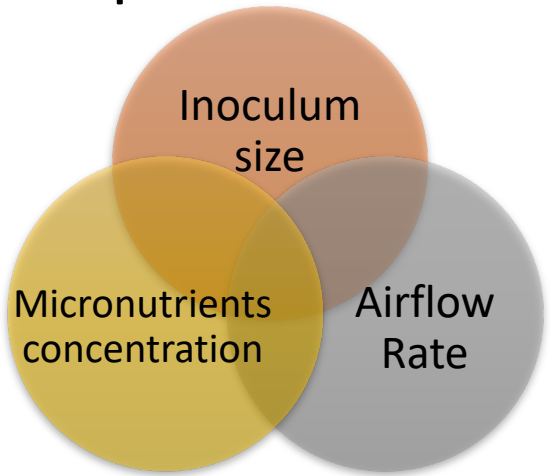
X

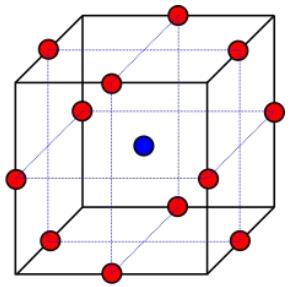
By-products



8 days

0, 24, 48, 72, 96, 120, 144, 168, 192h





Box-Behnken design

MATERIALS AND METHODS

Lab-scale SSF system

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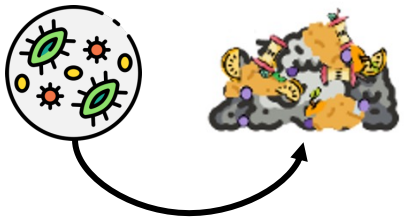
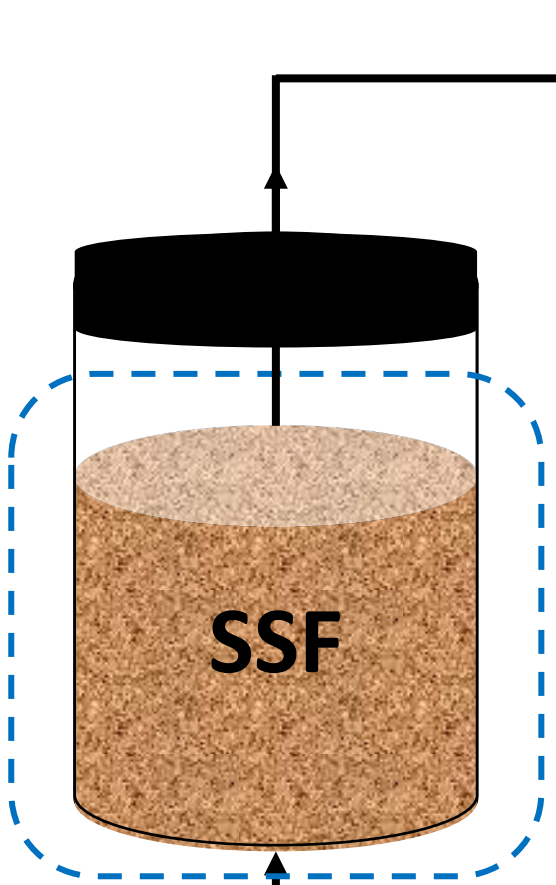
Sterile materials and conditions

100 – 150 g Inoculated substrate





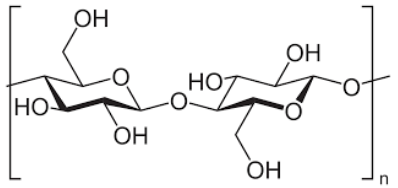
Oxygen online monitoring

Air flow

Water bath to control **temperature**

4-8 days of fermentation

EPS bioproduct



Offline analysis of response parameters:

1. Crude **EPS** production
2. Bacterial **biomass** production
3. **Reducing sugars** consumption
4. **pH** variation
5. **TS** and **VS**

MAIN RESULTS

Characterisation

	pH	Red. Sugars (g g^{-1} DM)	C/N ratio	WHC (mL g^{-1})
Beet Juice	5.0	0.24	34.2	1.37
Ginger Juice	4.3	0.35	58.9	1.87
Apple Pomace	3.7	0.43	67.8	1.12
Pomegranate Peels	3.8	0.13	106.7	0.20
Pomegranate Seeds	4.3	0.09	19.2	0.93
Vegetable Milk Waste	4.5	0.06	9.3	0.37

Good conditions for EPS production in SSF system:

- Acid **pH** values
- High **C/N** ratio
- Elevated content of available **sugars**
- Enough **WHC** to retain the inoculum

MAIN RESULTS

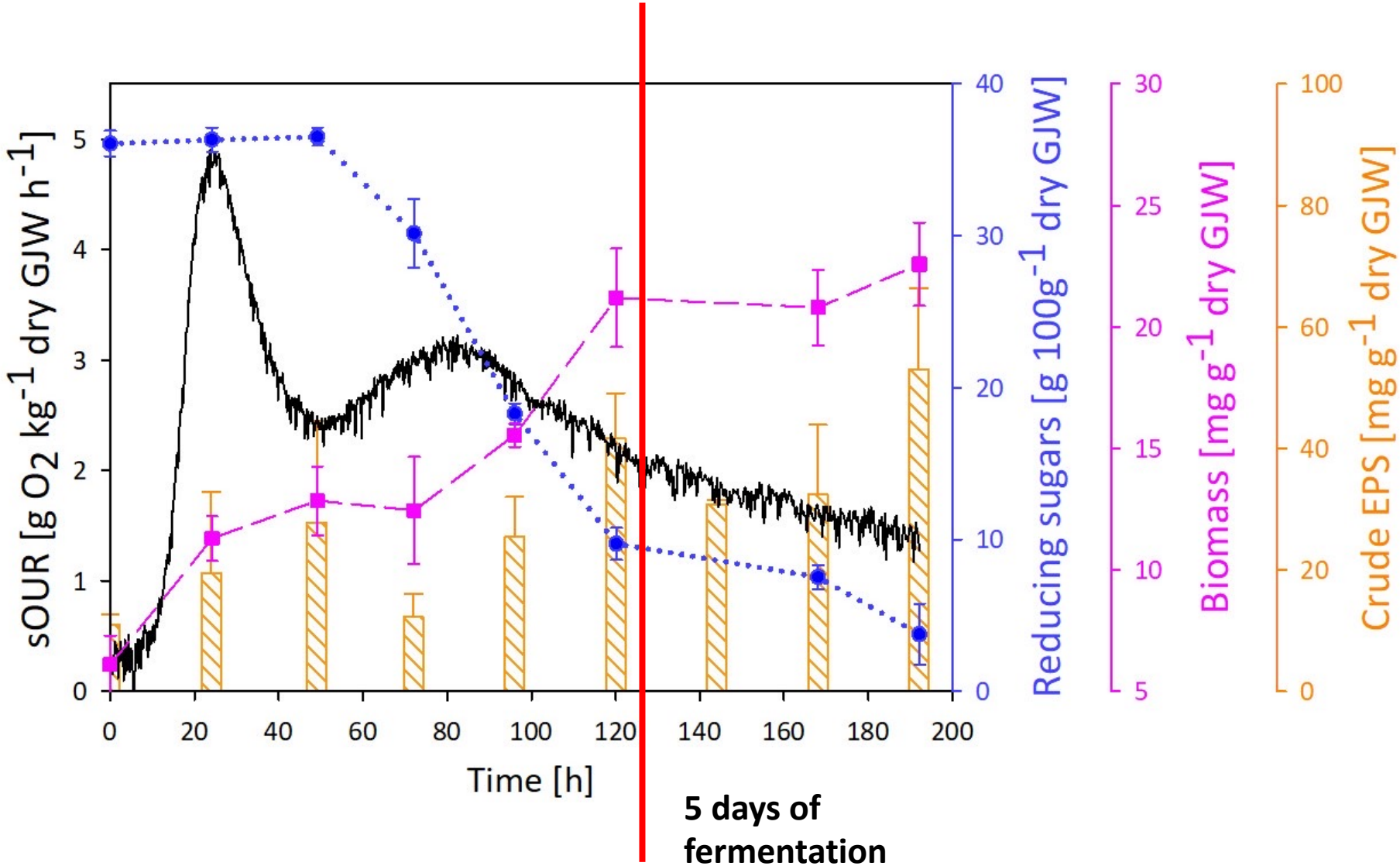
Screening

X

		pH variation	Biomass (mg g^{-1}DM)	Red. Sugars consumed (%)	AT_4 (mg O_2 g^{-1}DM)	Crude EPS (mg g^{-1}DM)
BJW	*B. cepacia*	**2.2**	**15.1**	**71.4**	**301.6**	**44.9**
GJW	*B. cepacia*	**1.5**	**16.6**	**82.2**	**242.5**	**55.4**
AP	*B. cepacia*	0.5	5.0	21.1	**253.6**	0.0
BJW	*A. beijerinckii*	**1.5**	1.6	29.5	103.6	0.0
BJW	*A. acidocaldarius*	0.1	0.0	0.0	33.5	8.1

MAIN RESULTS

Time-course



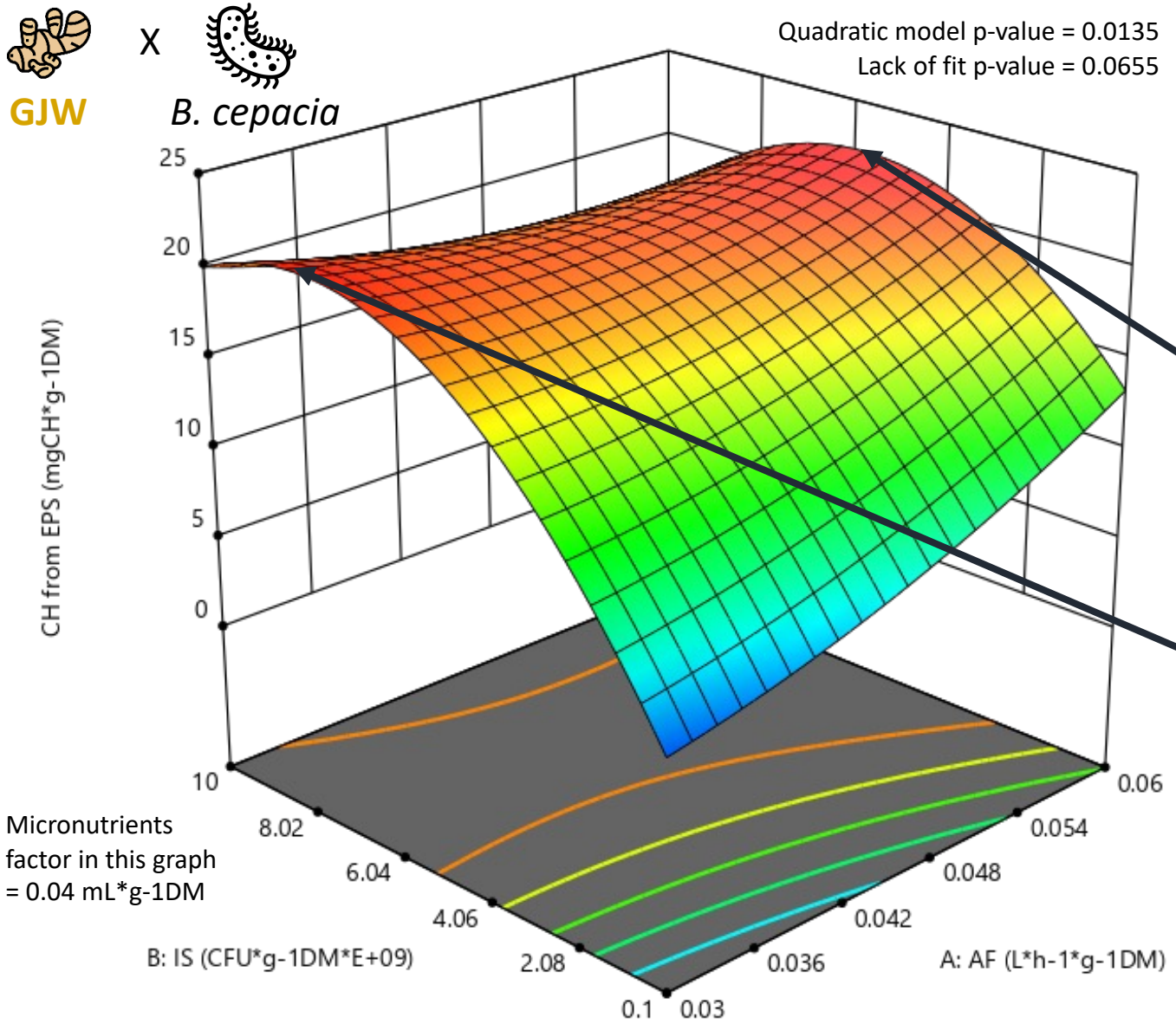
GJW x *B. cepacia*

1. **EPS** production stops in day 5
2. Bacterial **biomass** growth stops in day 5
3. **Reducing sugars** consumption stops in day 5
4. Specific **oxygen** uptake rate shows bacterial growth dynamic

MAIN RESULTS

Optimisation

GJW x *B. cepacia*



Micronutrients factor in this graph = 0.04 mL*g-1DM

Optimised operational parameters:

Airflow rate: 0.03 – 0.06 $L*h^{-1}*g^{-1}DM$

Inoculum size: 1.0E+08 – 1.0E+10 $CFU*g^{-1}DM$

Micronutrients concentration: 0.00 – 0.07 $mL*g^{-1}DM$

Response variable:

Pure EPS (mg $CH*g^{-1}DM$)

Best conditions:

↓	↑
AF = 0.03	AF = 0.06
IS = 7.66E+09	IS = 5.51E+09
MN = 0.037	MN = 0.042

Most significative factor:

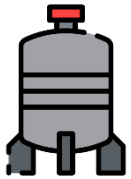
Inoculum size p-value = 0.052

CONCLUSIONS

- **Produce EPS** from agri-food waste through SSF, at **lab-scale.**
- Propose a **valorisation strategy** for agri-food by-products.
- Find the **best combination substrate-strain** and optimize the **SSF time** and some operational parameters (**MN**, **AF** and **IS**).

Produced EPS pellet from 5 g of GJW x *B.cepacia*

FUTURE RESEARCH



- Collect enough information to focus further research in an **scale-up phase**, approaching the industrial perspective.
- Prove the **biostimulant effect** from the obtained EPS on pot-test or field applications.

- **Sustainability assessment** of the production technology.

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Thank you!

enric.garcia.muchart@uvic.cat
www.betatechcenter.com

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