Exploiting the agri-food waste and by-products potential for bioplastic production through *Haloferax mediterranei* fermentation

Bari, 19/09/2024

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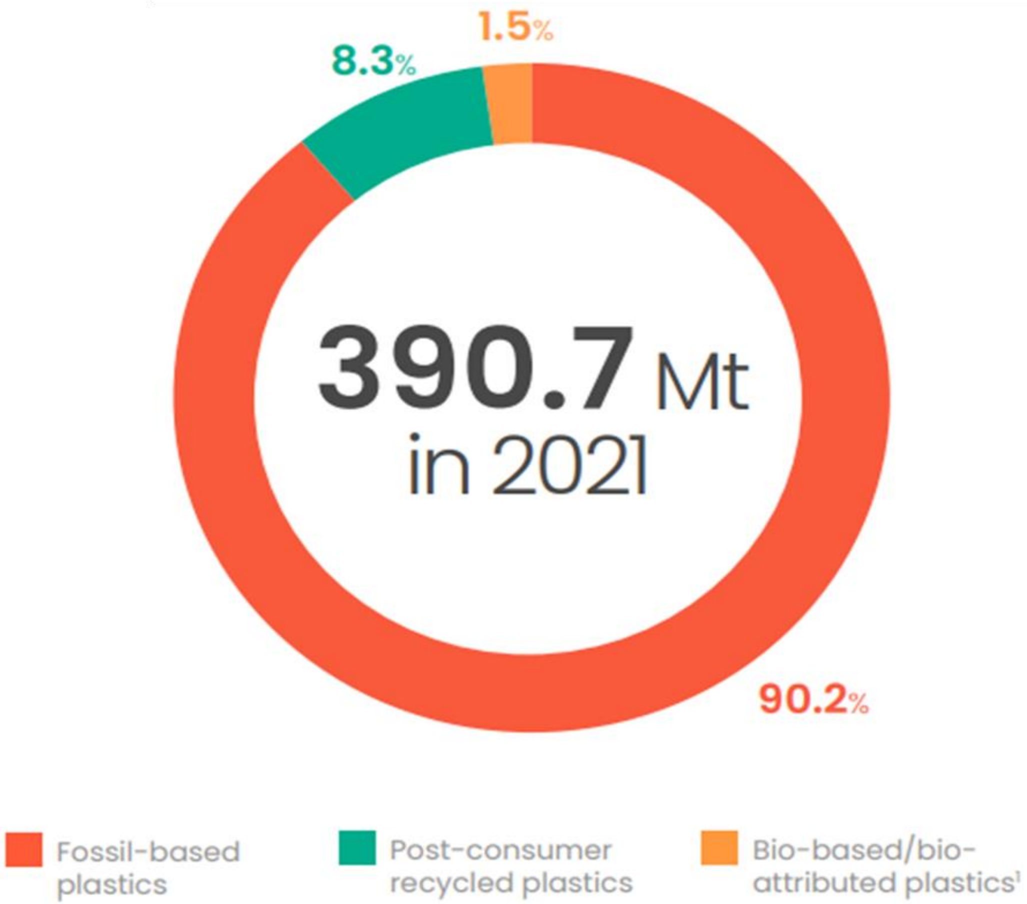
CNR - ISPA

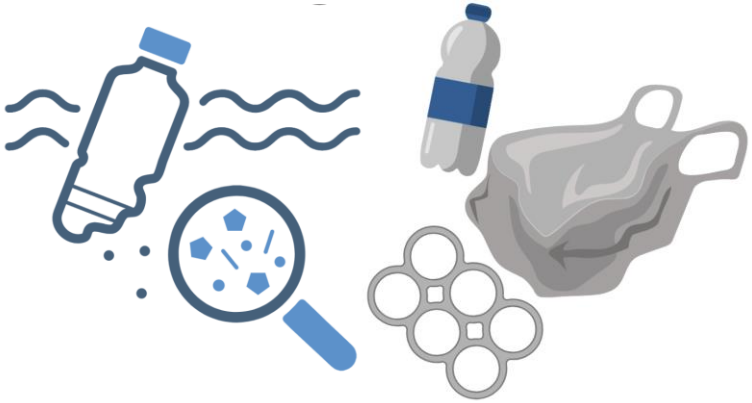
Plastic...

Global plastic production



9% recycled

22% Mismanaged or uncollected



50% landfilled

19% incinerated

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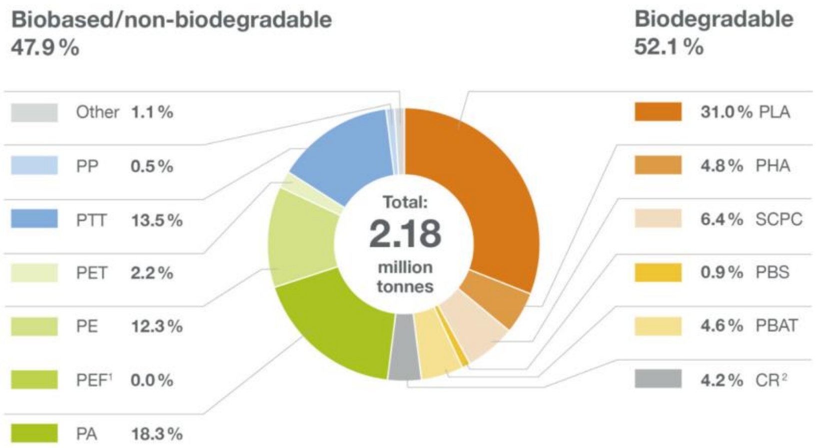
…and bioplastic

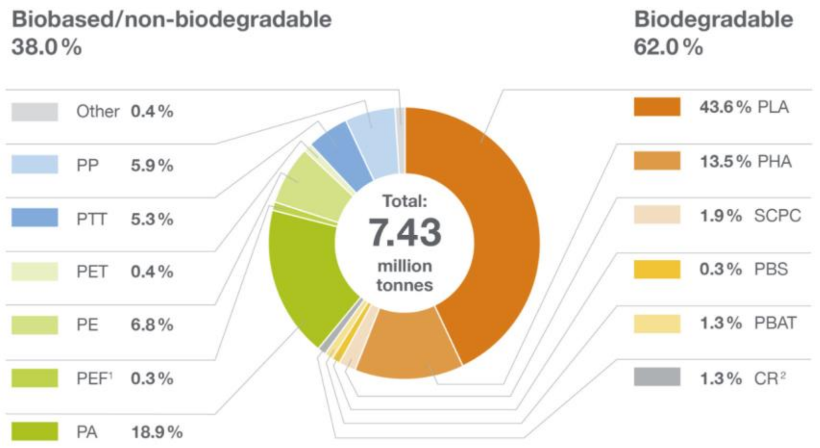
Bioplastics comprise a family of materials with different properties and applications.

A plastic material is defined as a “bioplastic” if it is biobased, biodegradable in specific conditions, or has both properties.

Global production capacities of bioplastics 2023

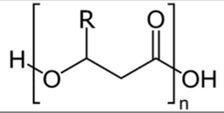


Global production capacities of bioplastics 2028



The bioplastic market is constantly growing. In 2010, 0.7 million tons of bioplastics were produced, versus 1.7 million tons in 2015, and it has been estimated that annual bioplastic production will reach 3 million tons in 2025

The bioplastic producers and agrifood waste

PHA producing microbes 	***Organic waste*** 	***PHAs*** 
Micrococcus aloeverae	Oily wastewater	P (3HB-co-3HV)
Azotobacter beijerinickii	Coconut fiber waste	PHB
Rhodococcus equi	Crude palm kernel oil	P(3HB)
Pseudomonas aeruginosa	Corn oil	PHB
Ralstonia eutropha	Coffee waste oil	PHB
Bacillus megaterium	Milk whey Sugarcane molasses	P(3HB-co-3HV) PHB
Bacillus wiedmannii	Fruit Peel Waste	PHB
Cupriavidus necator	Wine waste; Used sunflower oil; Cheese whey Used cooking oil; Apple pomace	PHB P(3HB-co-3HV) P(3HB)
Haloferax mediterranei	Enzymatic extruded starch Extruded rice bran; Olive mill wastewater Macroalgal hydrolyzate; Date palm sugars	PHBV

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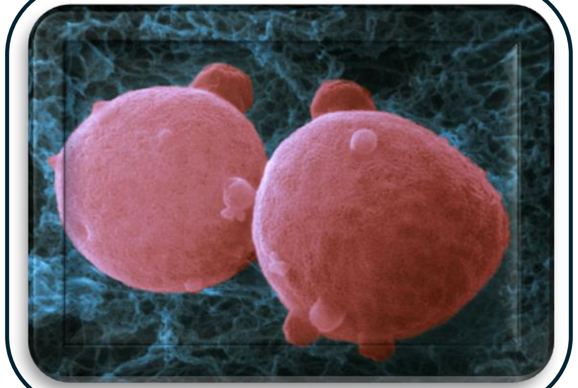
Haloferax mediterranei: the identity card



DOMAIN OF ARCHAEA

Name *Haloferax mediterranei*

Family name *Haloferacaceae*

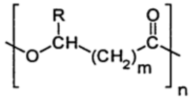
Isolation source *solar salt pond (Spain)*

Type strain *DSM 1411, ATCC 33500, CCM 3361*

Important metabolites Exopolysaccharide (EPS), polyhydroxyalkanoates (PHA)

Growth requirements *Haloferax mediterranei* can growht in 372 HALOBACTERIA MEDIUM which includes sources of amino acids, different minerals and 20% of NaCl)

Signature



Released by Rodriguez-Valera et al. 1983

Interesting fact *Haloferax mediterranei* was the microorganism studied by Francis Mojica finding Clustered Regularly Interspaced Short Palindromic Repeats, then recognized as "the memory of the bacteria".

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Haloferax mediterranei: why?

Haloferax mediterranei, an archaeon that thrives in highly saline conditions, can metabolize different types of waste feedstocks.

The growth in hypersaline substrates makes halophiles the most promising candidates for industrial applications for two reasons:

1) The hypersaline substrates avoid contaminations, preventing the growth of non-halophilic microorganisms and, thus, reducing the costs of the energy input for substrates and bioreactors' sterilization.

2) *Haloferax mediterranei* cells can be lysed in water due to the high intracellular osmotic pressure, reducing PHA recovery costs.

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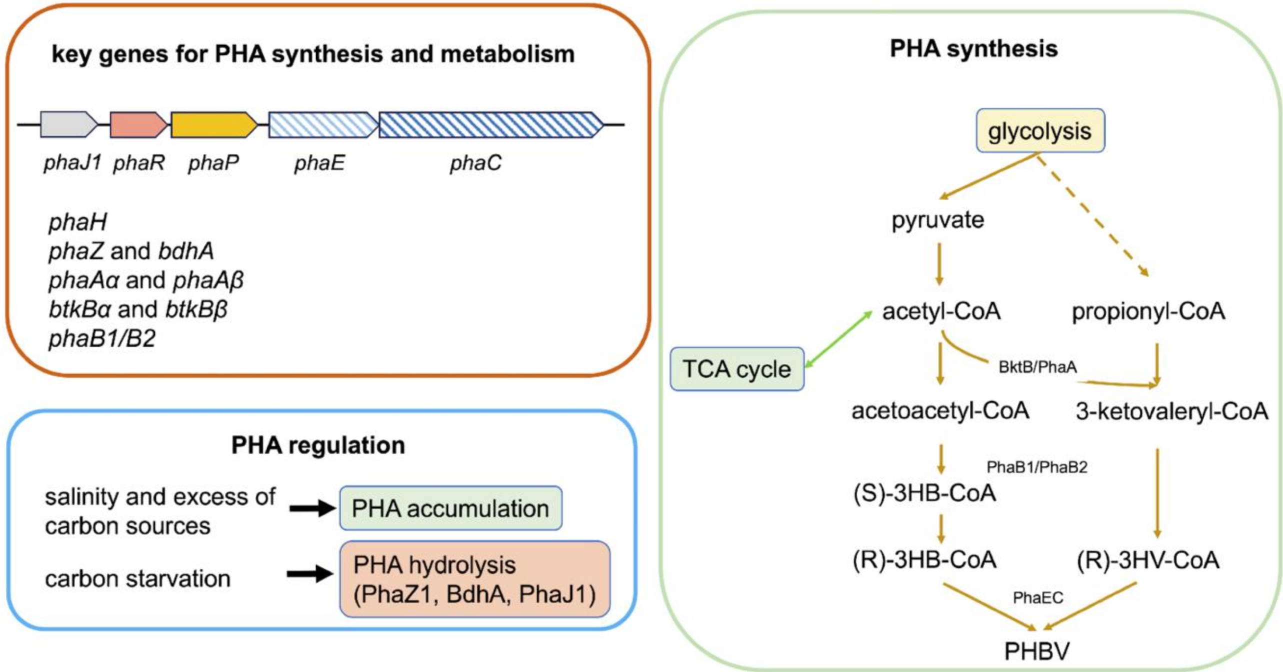
Genes and pathways for bioplastic

PHA biosynthetic cluster: PHA synthase (type III PHA synthase) which is composed of the PhaE and PhaC subunits

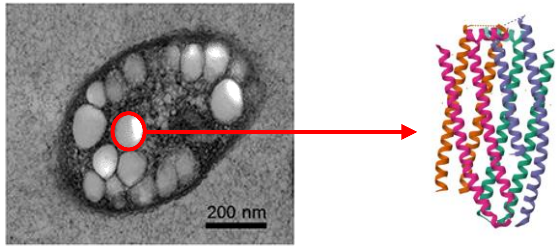
H. mediterranei does not require any 3HV precursor for PHBV synthesis.



phaH → PHA Binding Protein PhaP (Phasin)
major structural protein in PHA granules

PhaR → promote PHA synthesis and granule formation independently

PhaJ1 → PHA mobilization linking PHA degradation to the β-oxidation



Phasin

Use of agrifood waste as a carbon source

Carbon Source	CDW (g/L)	PHA (%)	References
Enzymatic extruded starch	39.4	50.8	Chen et al., 2006
Extruded rice bran	140.0	55.6	Huang et al., 2006
Hydrolyzed whey permeate	13.6	66.0	Koller, 2015
Cheese whey hydrolysate	7.5	53.0	Pais J. et al., 2016
Ricotta Cheese Whey	18.3	7.0	Raho et al., 2020
Olive mill wastewater	0.2	43.0	Alsafadi and Al-Mashaqbeh, 2017
Macroalgal hydrolyzate	3.8	57.9	Ghosh et al., 2019
Defatted Chlorella biomass	3.8	55.5	Khomlaem et al., 2021
Date palm sugars	18.0	25.0	Alsafadi et al., 2020
Fermented food waste	2.99	52.5	Wang et al., 2021
Sesame seed wastewater + glucose	50.0	75.0	Alsafadi et al., 2023
Vinasse	19.7	70.0	Bhattacharyya et al., 2012
Wasted bread	6.7	24.0	Montemurro et al., 2022

Longo, A. et al. (2024). Bioplastic Production from Agri-Food Waste through the Use of Haloferax mediterranei: A Comprehensive Initial Overview. *Microorganisms*, 12(6), 1038.

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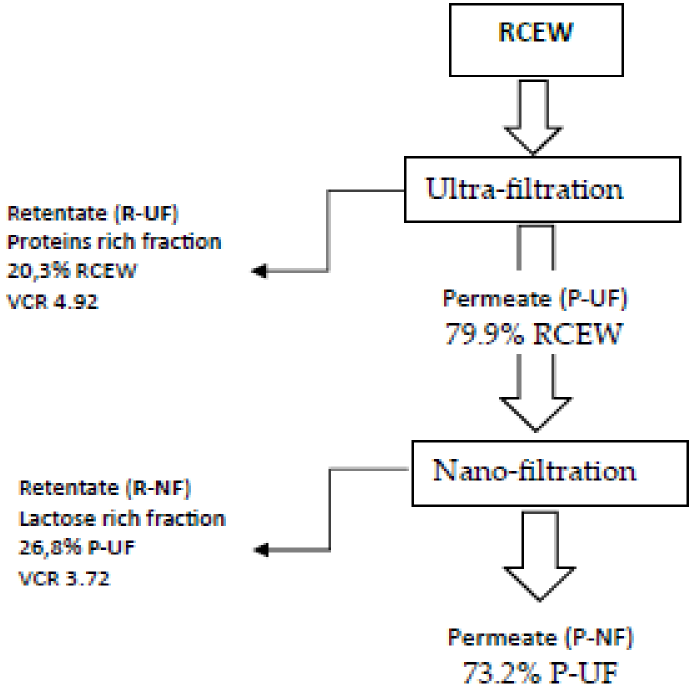
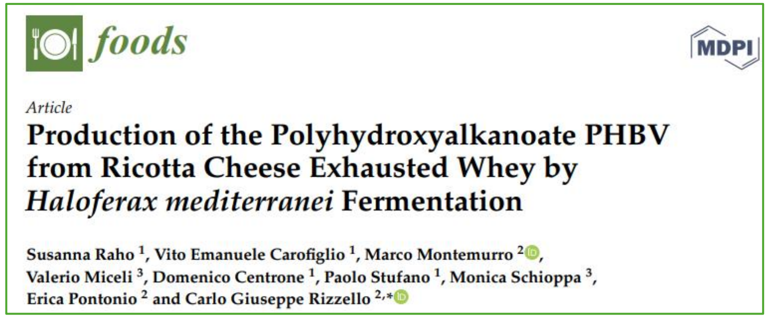
Case study I: Cheese way




foods

MDPI

Article

Production of the Polyhydroxyalkanoate PHBV from Ricotta Cheese Exhausted Whey by *Haloferax mediterranei* Fermentation

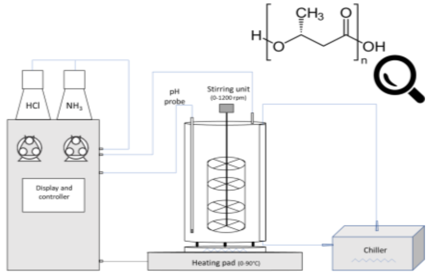
Susanna Raho [1], Vito Emanuele Carofiglio [1], Marco Montemurro [2], Valerio Miceli [3], Domenico Centrone [1], Paolo Stufano [1], Monica Schioppa [3], Erica Pontonio [2] and Carlo Giuseppe Rizzello [2,*]

R-NF was used for growing *Haloferax mediterranei* DSM1411 evaluating different variables:

- Lactose hydrolisis
- Concentration of NaCl
- Mineral supplementation
- Addition of nitrogen sources (e.g. yeast extract)
- Temperature
- Time

NaCl 10% (w/v)
Trace Element Solution (SL6) 1% (v/v)
pH 7.2
β-galactosidase treatment

1.27 ± 0.09 g/L of PHBV comparable to commercial PHBV



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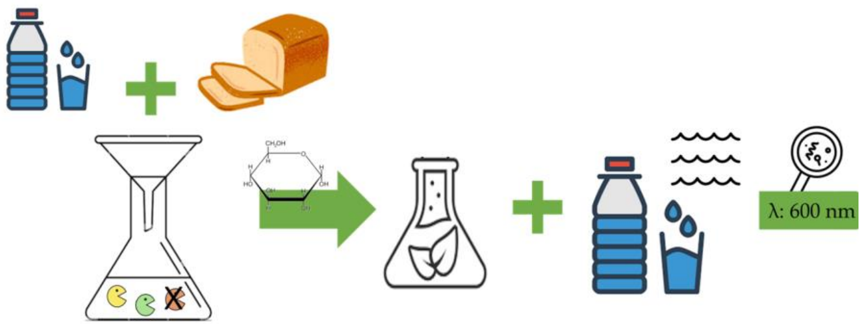
Case study II: Wasted bread

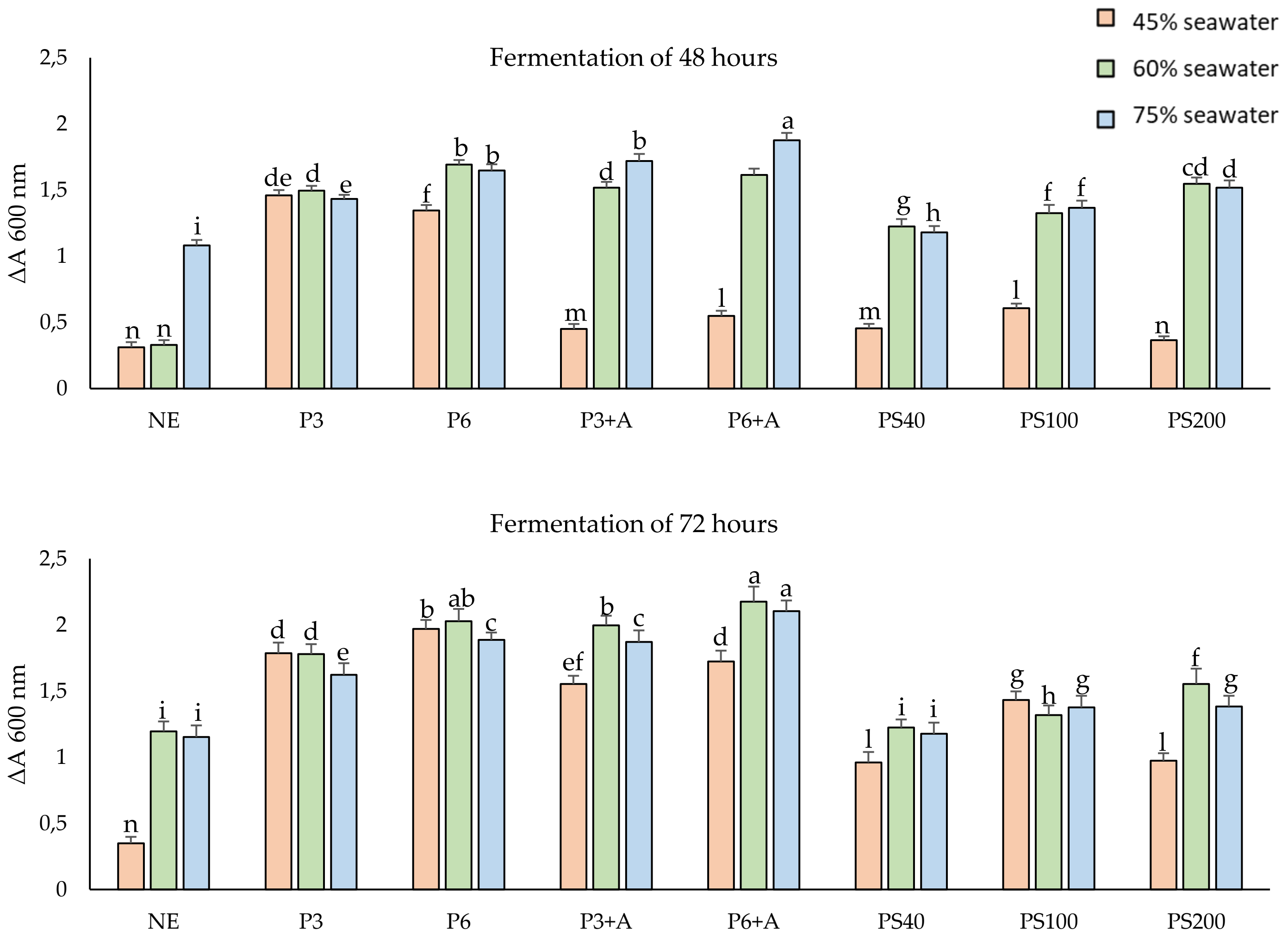
Selection of best bread/water ratio: 150 g/L

Effect of protease (P) and/or amylase(A)

Substitution of seawater to wasted bread extract

Monitorin of optical density after 24, 48, and 72 hours





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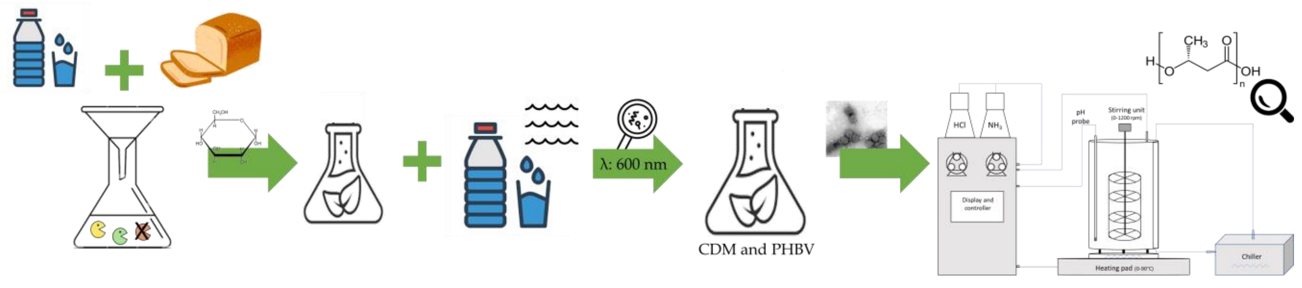
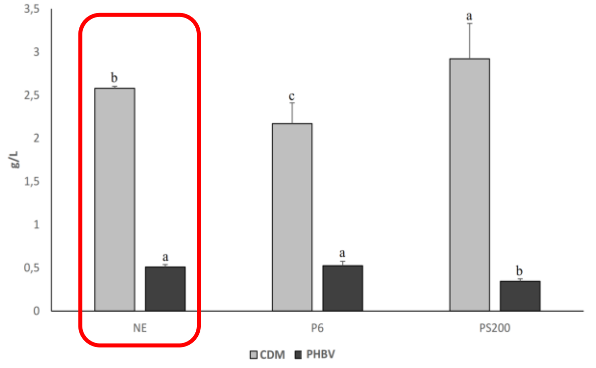
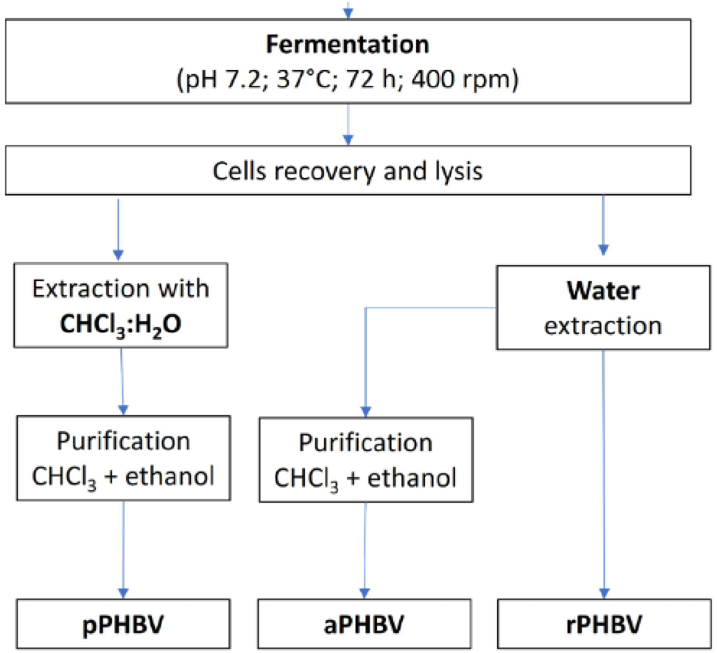
Case study II: Wasted bread





- fermentation of the substrate obtained with 6 h-pepsin treatment (P6)
- fermentation of the substrate treated with 200 g/100 kg of Veron PS (PS200)
- fermentation of the substrate obtained without enzymatic treatment (NE), as control.



1 kg of PHBV characterized by high purity grade and a HV content can be produced from 50 kg of wasted bread

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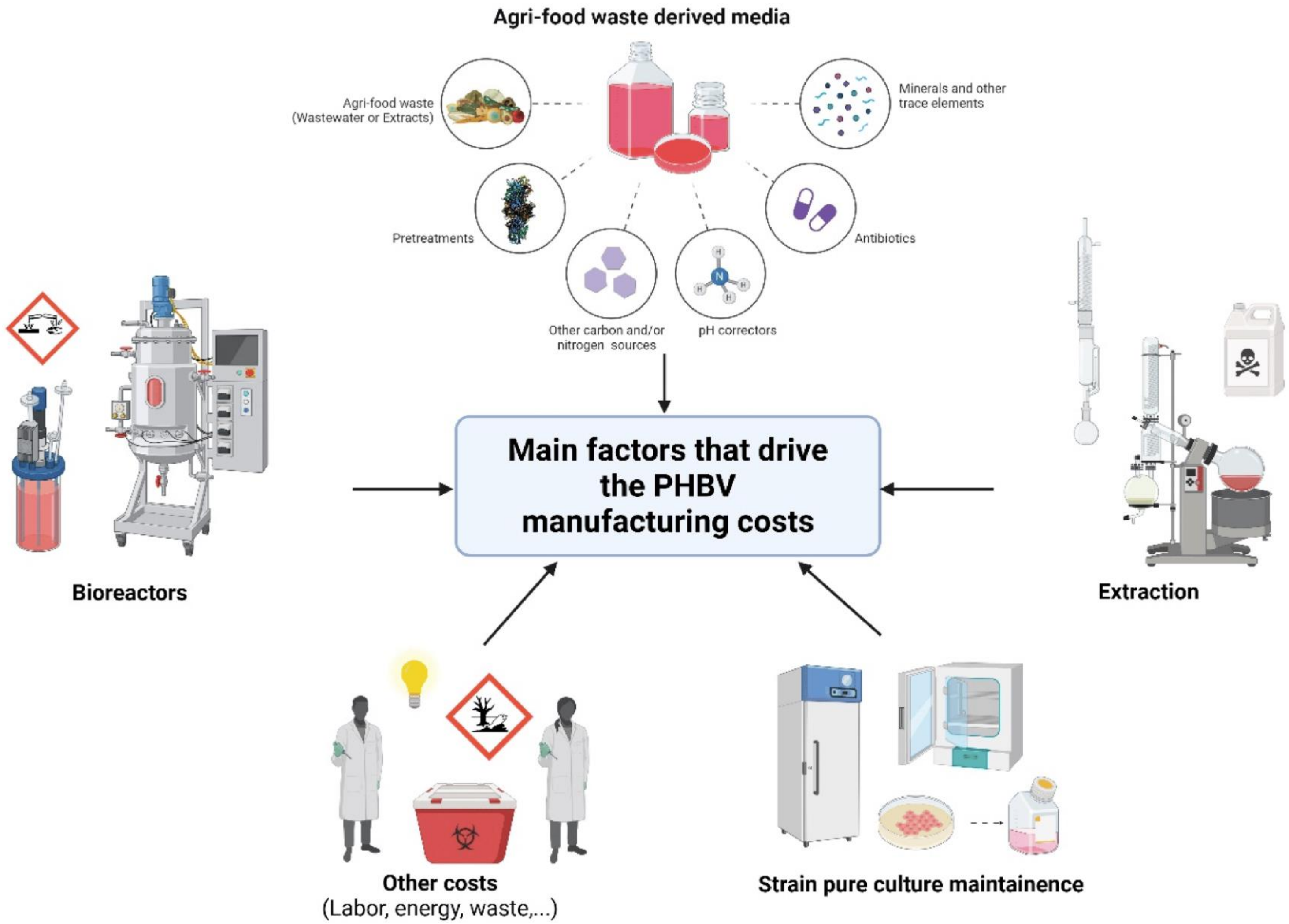
Possibility to scale-up the process?

Points to be addressed:

- Variability of food waste and by-products composition
- Possibility to set-up fed-batch processes
- Recover and reuse exhausted salt-rich media
- Solvent free extraction



THANK YOU FOR THE ATTENTION!

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microorganisms

MDPI

Review

Bioplastic Production from Agri-Food Waste through the Use of *Haloferax mediterranei*: A Comprehensive Initial Overview

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