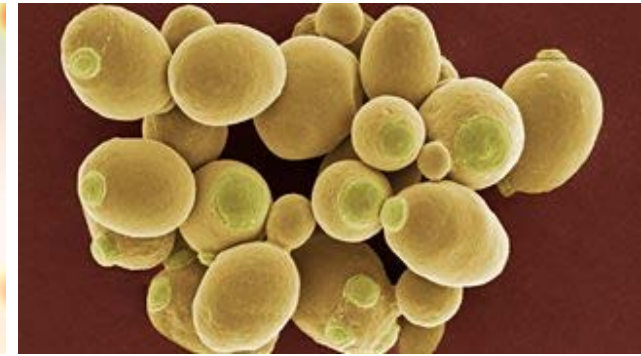


15th of June 2022 – 13:15
Session III

Valorisation of agricultural residues into microbial proteins and protein hydrolysates by PHA-producing bacteria

Giovanna Pesante, Chiara Bastianelli, Anna Zuliani, Riccardo Lo Coco, Stefano Ambrosini, Anita Zamboni, Nicola Frison

**LabCAB – Laboratory of chemical engineering
for the environment and bio-processes**



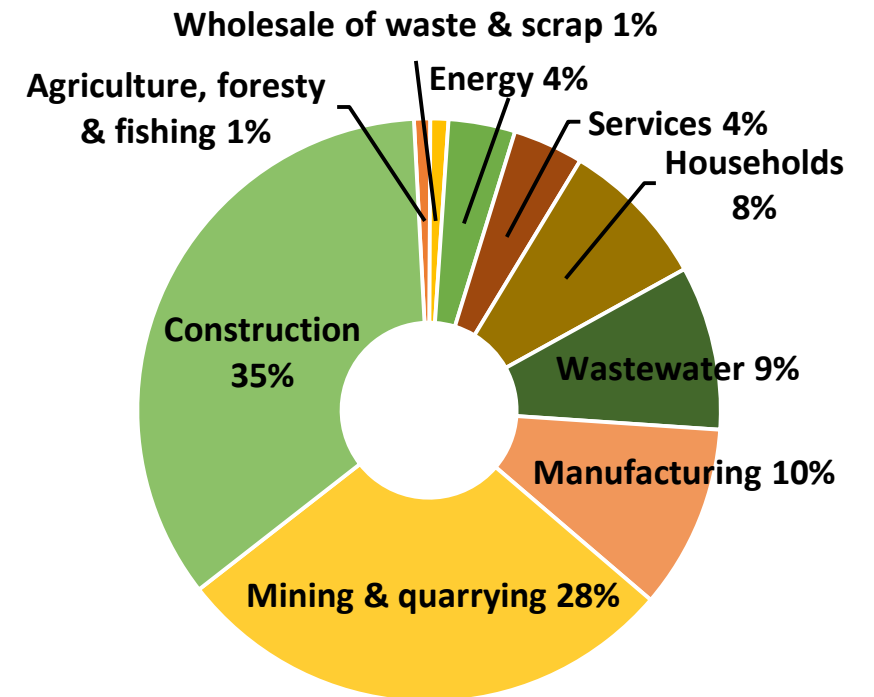
WASTE: a problem...?



Agricultural waste

- 1.3 bt/year globally
- Increasing at 7.5% per year
- 50% of fresh weight of harvest

GLOBAL WASTE PRODUCTION 2014



WASTE: a problem...?

Disposal of agricultural waste

Burned off in the fields



Buried

Left to rot in the fields



Incinerated

PRODUCTION OF:



smoke



toxic gasses (SO_2 , CH_4 , N_2O ...)

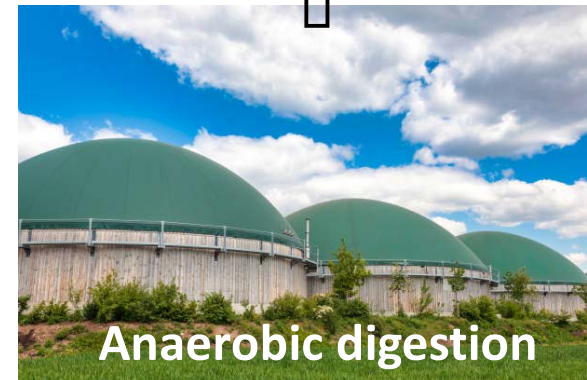


carcinogens (polycyclic aromatic hydrocarbons, furans, dioxins...)



GHGs

Biogas, biomethane



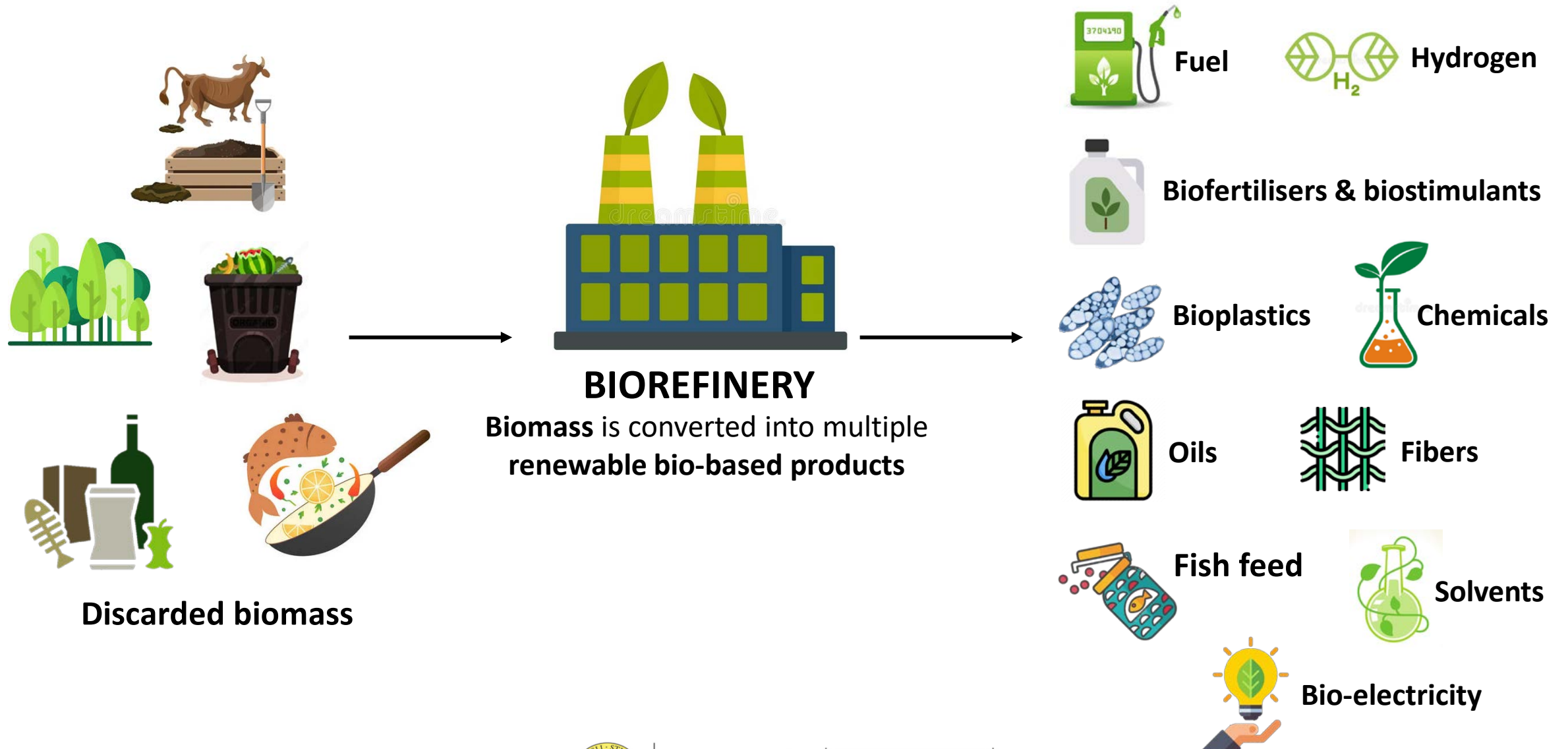
Anaerobic digestion



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WASTE: a problem... or a resource?



The concept

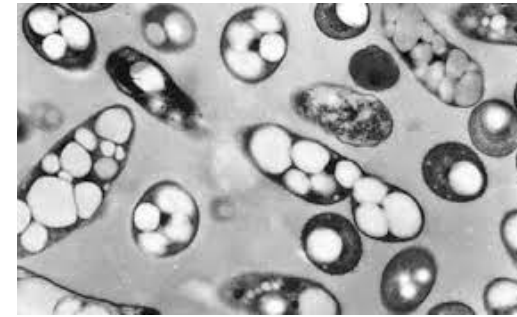
Starting feedstock



Agricultural & animal waste



PHA-producing bacteria



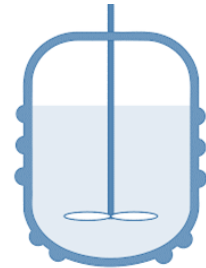
Polyhydroxyalkanoates (PHAs)

biobased and biodegradable plastic synthesised by some bacterial and archaeal species

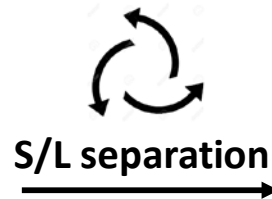


Acidogenic fermentation

VFA-rich fluid



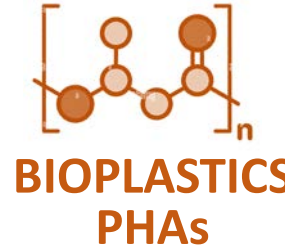
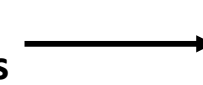
Bioreactor



S/L separation



PHAs-rich bacterial biomass



PHA-based plastic: 1.18-6.12 €/kg (Pagliaro et al., 2021)
2.4-5.5 \$/kg (Crutchik et al., 2020)

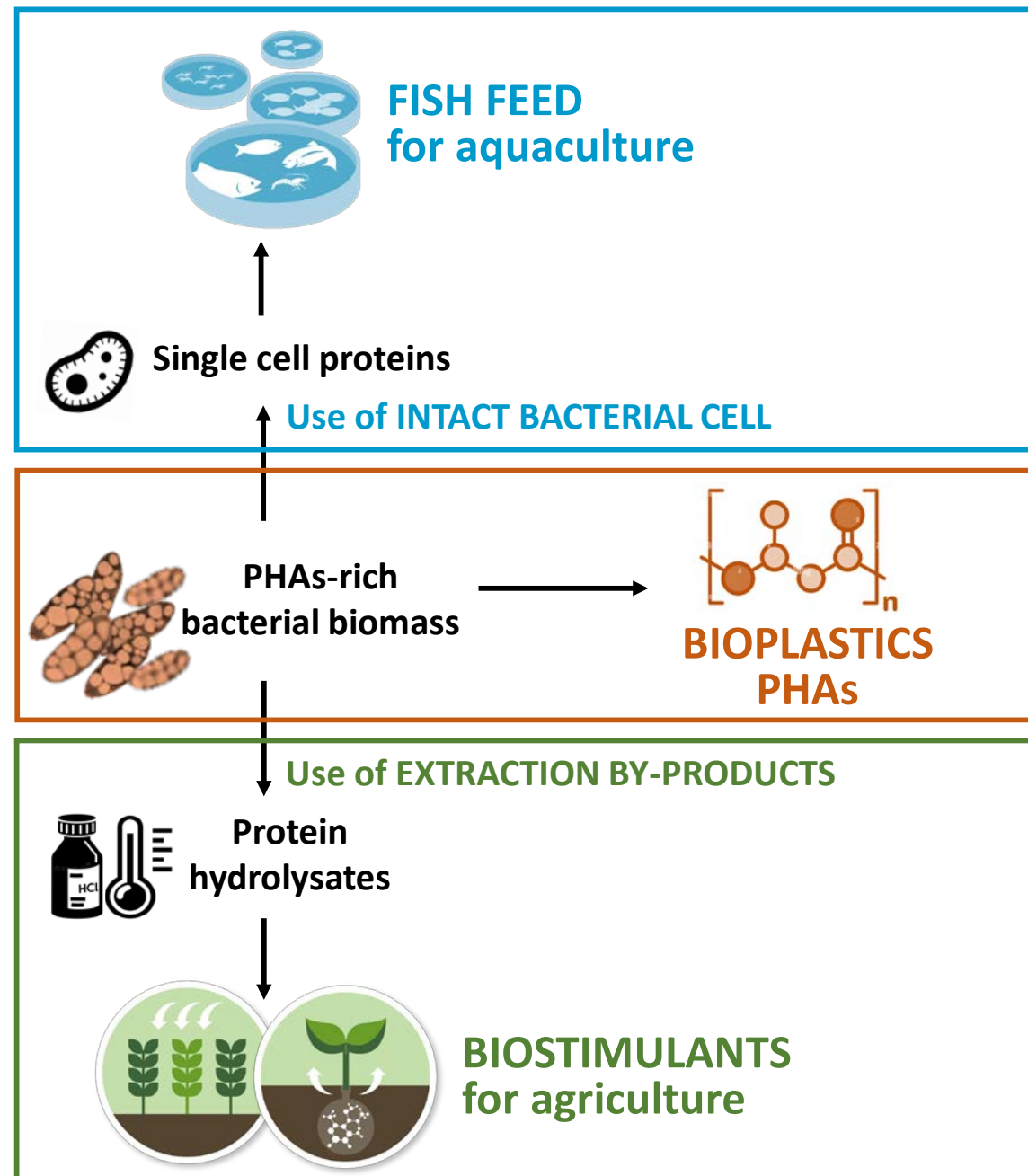
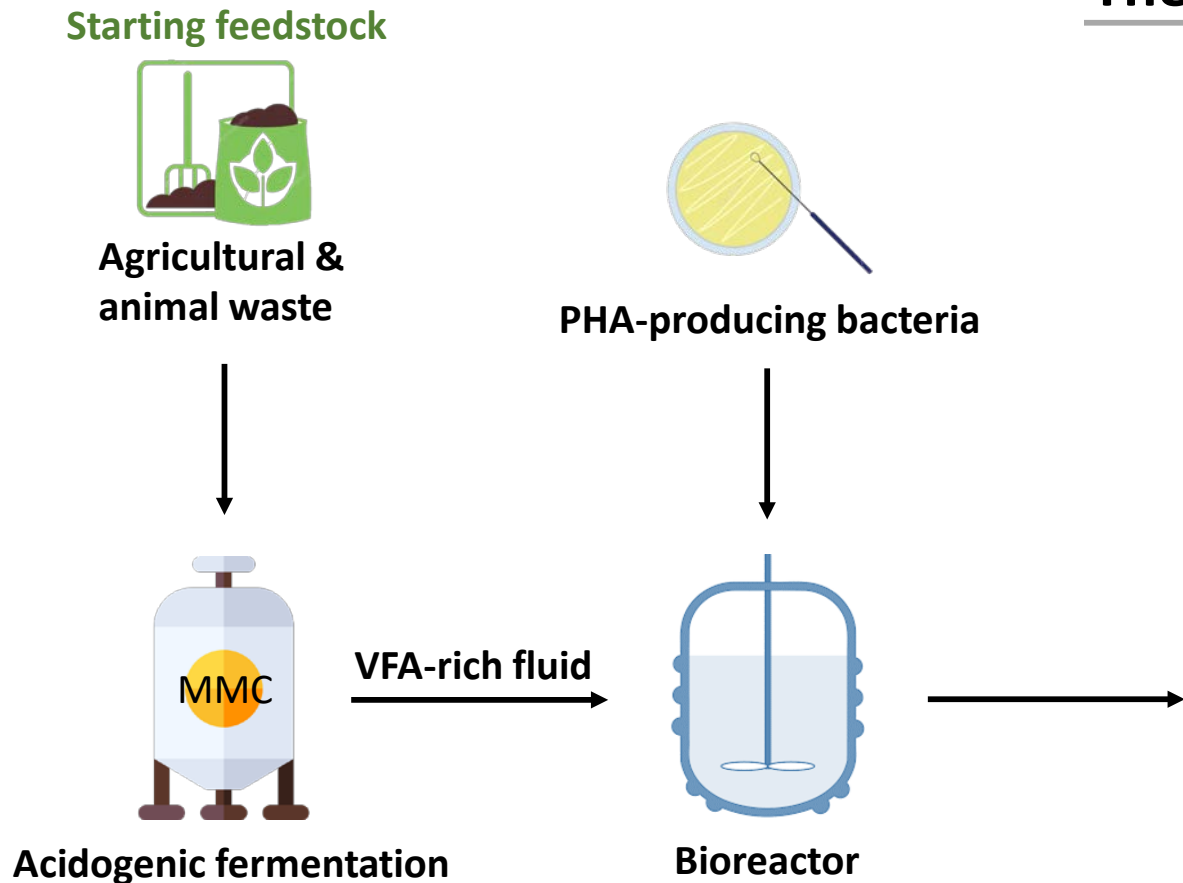
Petroleum-based plastic: <1 €/Kg (Pagliaro et al., 2021)
1.2 \$/kg (Crutchik et al., 2020)



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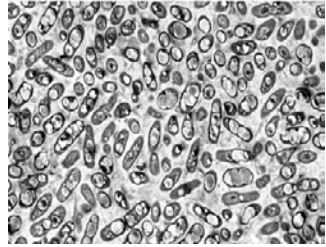
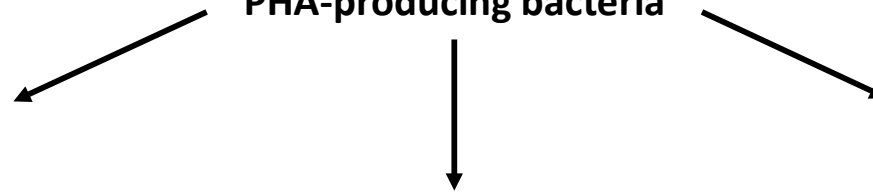
The concept



The project



PHA-producing bacteria



PHAs-producing mixed microbial cultures



***Thauera* sp. Sel9**
(Betaproteobacterium, family Rhodocyclaceae)



Rhodospseudomonas palustris
(Alphaproteobacterium, family Nitrobacteraceae)



Fish feed for aquaculture



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Fish feed for aquaculture

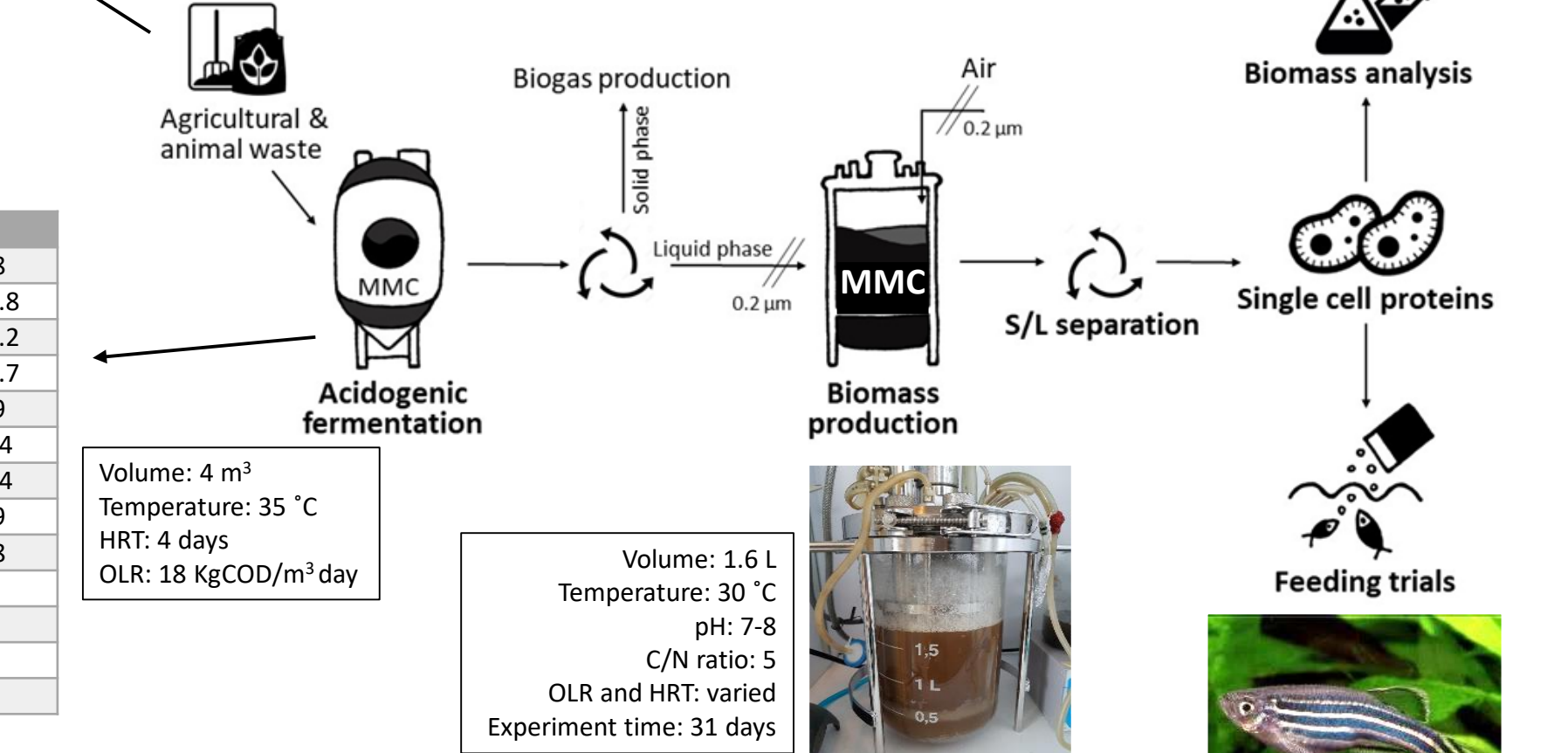
Experimental set-up

Component	Unit	Value	Percentage
Liquid cattle manure	L	0-2000	0-80
Pasta silage	Kg	300-500	13-20
Digestate	L	0-1000	0-56
Water	L	0-1800	0-75

Feedstock

Fermentation fluid

Parameter	Unit	Value
pH	-	3.6-5.8
TSS	g/Kg	48.5-96.8
VSS	g/Kg	40.9-62.2
sCOD	g/L	20.1-53.7
N-NH ₄	g/L	0.4-1.9
VFA tot	g/L	7.5-18.4
Acetic acid	g/L	4.8-14.4
Butyric acid	g/L	0.2-3.9
Propionic acid	g/L	0.5-4.8
Pentanoic acid	g/L	0.3
Isobutyric acid	g/L	0.0
Isopentanoic acid	g/L	0.0
PHAs	%	22.5

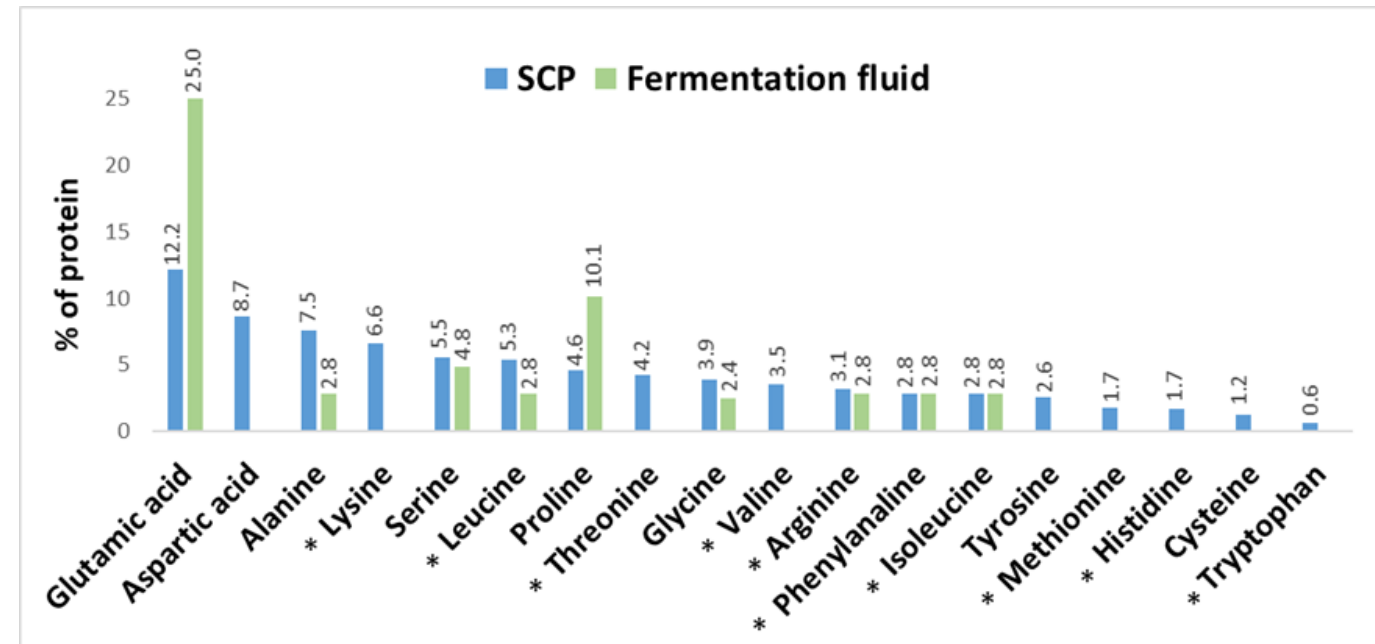


Zebrafish (*Danio rerio*)

Fish feed for aquaculture

Biomass analysis - MMC

Parameter	SCP	Fermentation fluid
Moisture	12.21	98.41*
Ash	16.56	14.46
Crude lipids and oils	<LoQ	<LoQ
Crude protein	61.79	28.94
Carbohydrates	<LoQ	<LoQ
Crude cellulose	<LoQ	<LoQ
Nitrogen-free extracts	21.65	56.60
Proximate composition		
PHAs	22.54	0.00



Amino acid profile

PROTEIN CONTENT

On TS = 61.8%

On TVS = 74.0%

On active biomass (excluding PHAs) = 95.6%



Fish feed for aquaculture

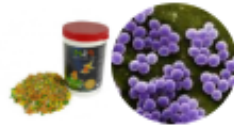
Fish feeding trials - MMC

GROUP A



100% CF

GROUP B



50% CF+ 50% SCP

GROUP C



50% CF + 50% SCP
+ 14% oils

GROUP D

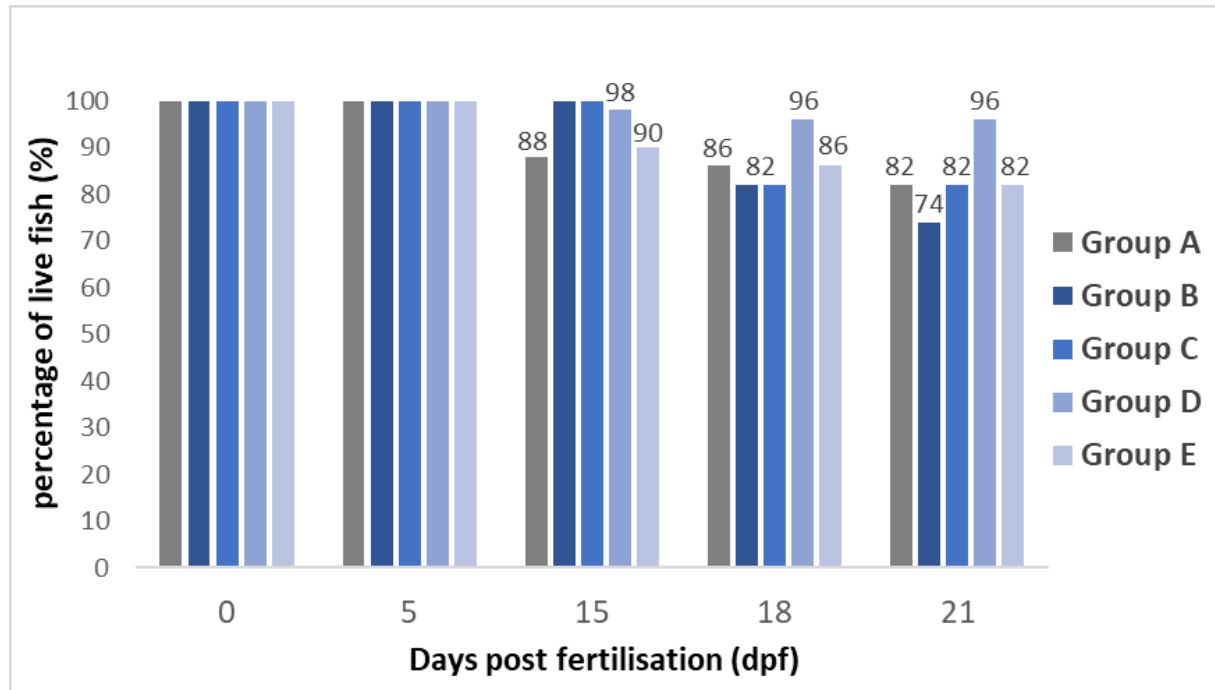


50% CF + 50% SCP +
14% oils + 10% PHA

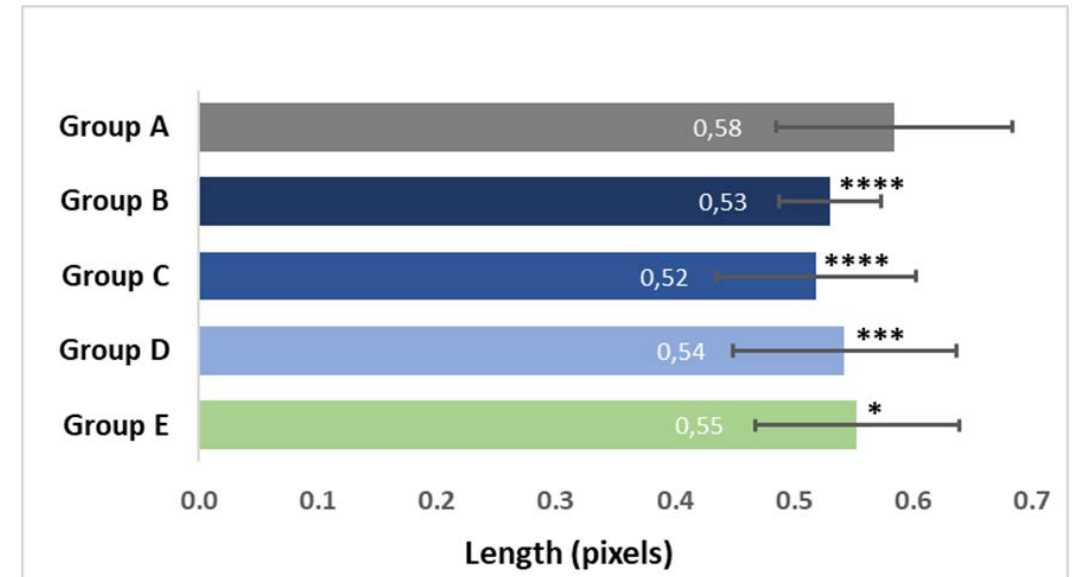
GROUP E



50% CF + 50% SCP +
10% PHA



Survival rates

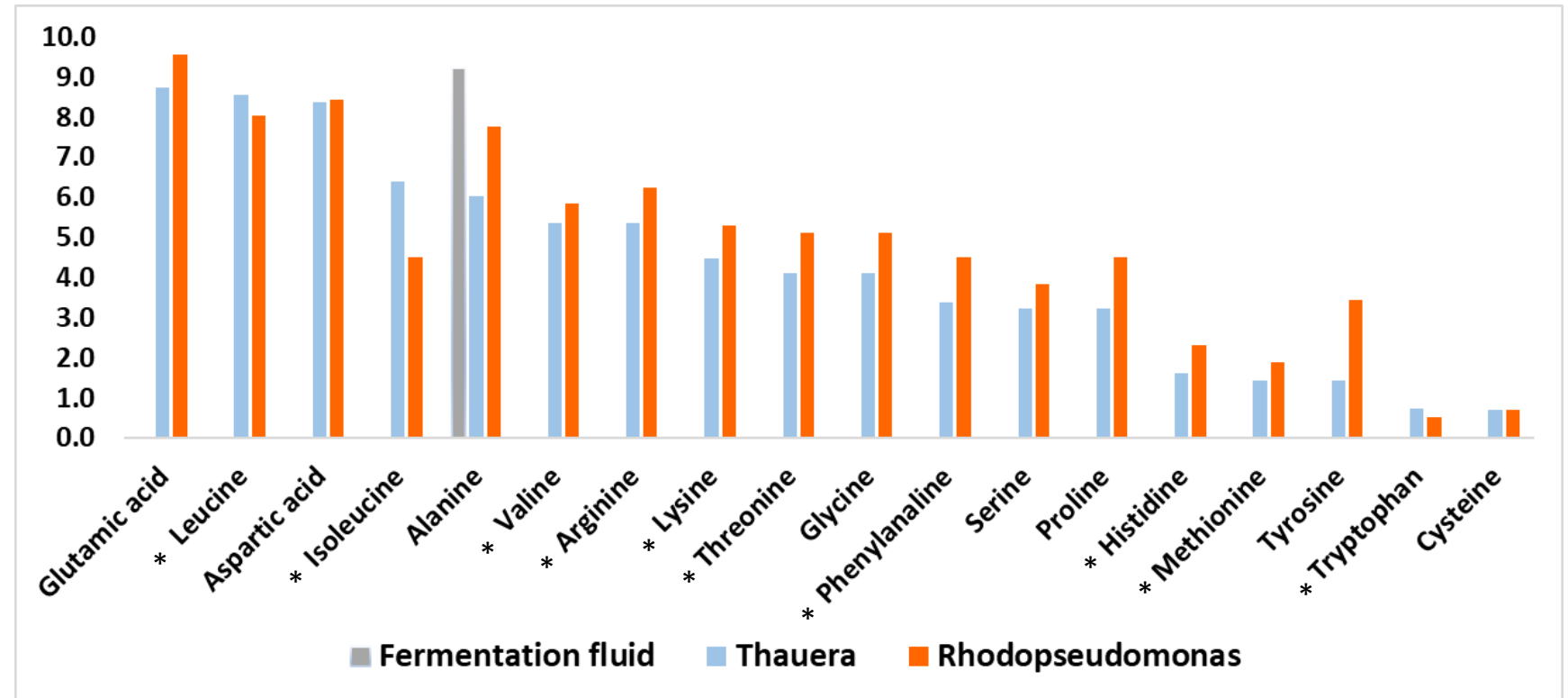
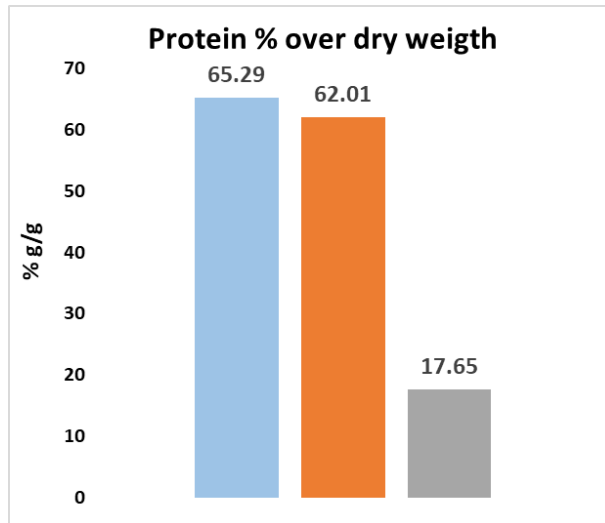


Total body length



Fish feed for aquaculture

Biomass analysis – *Thauera* & *Rhodopseudomonas*



Biostimulants for agriculture



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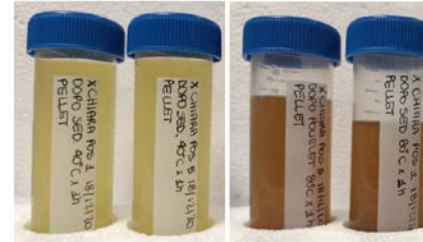
Biostimulants for agriculture

Experimental set-up



Cucumber - *Cucumis sativus*

Tomato - *Solanum lycopersicum*



TKN
N-NH₄
Organic N
Amino acid profile



biostimulant effect tests
with agronomical species

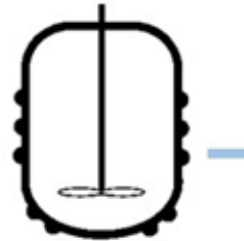


Drench
Foliar spray



Feast & famine regime

selected biomass
(MMC)



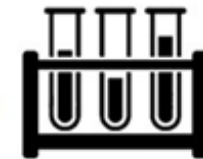
PHAs accumulation



solid phase



chemical-thermal
treatments



protein
hydrolysates

liquid phase

0,45 μm



solid phase



PHA-enriched
biomass

% hydrolysis
% PHA

Multi-spike approach
20 L reactor
27 °C
pH 7.5
60% acetate, 40% propionate

Acid: HCl 6M
Basic: NaOH 4.2M
Thermal (microwave): 40, 60, 80 °C
x 30, 60, 120 min

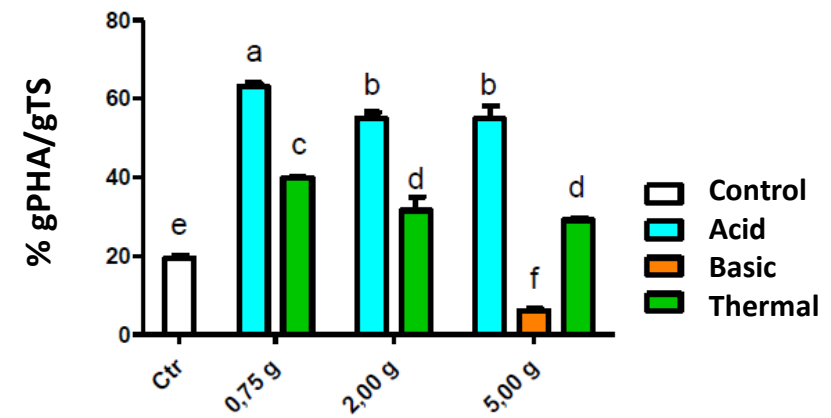
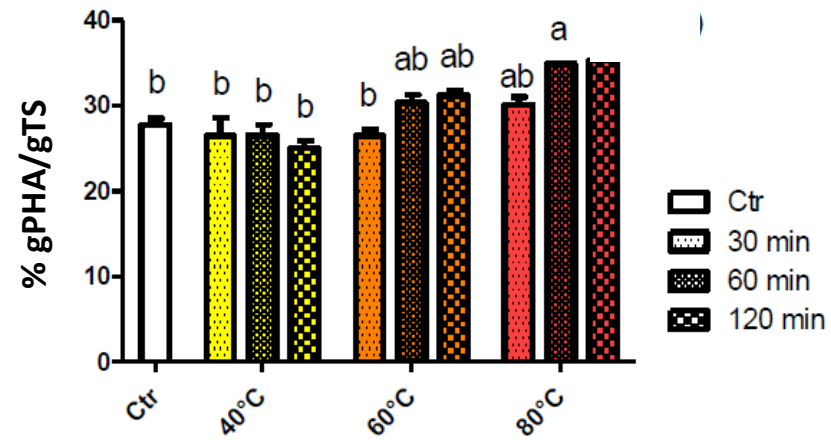
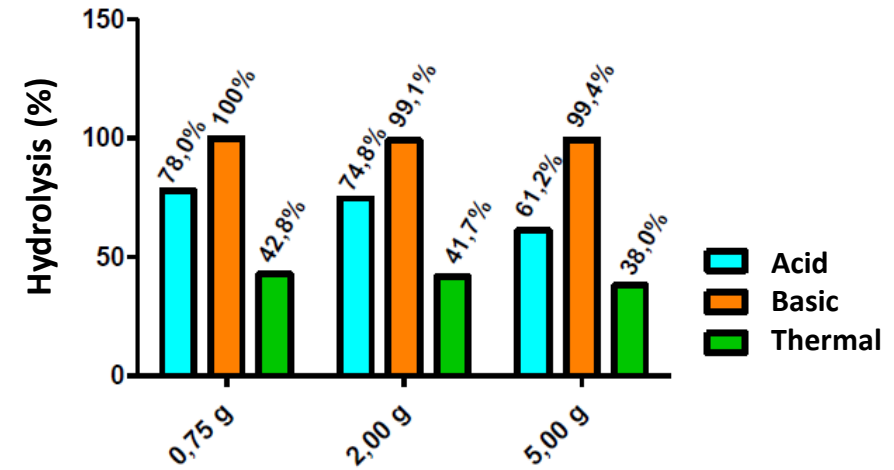
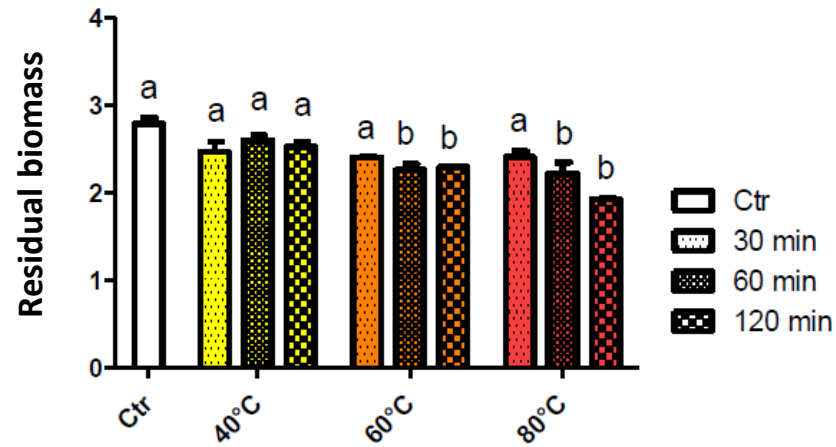


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Biostimulants for agriculture

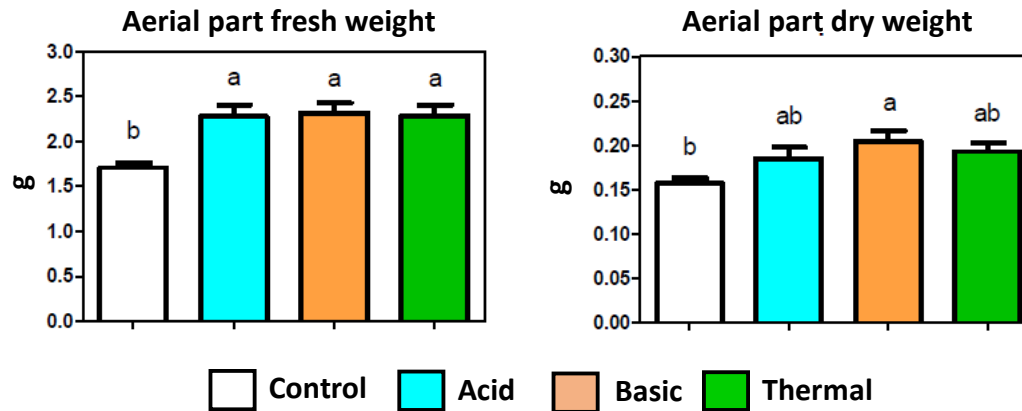
Protein hydrolysates



Biostimulants for agriculture

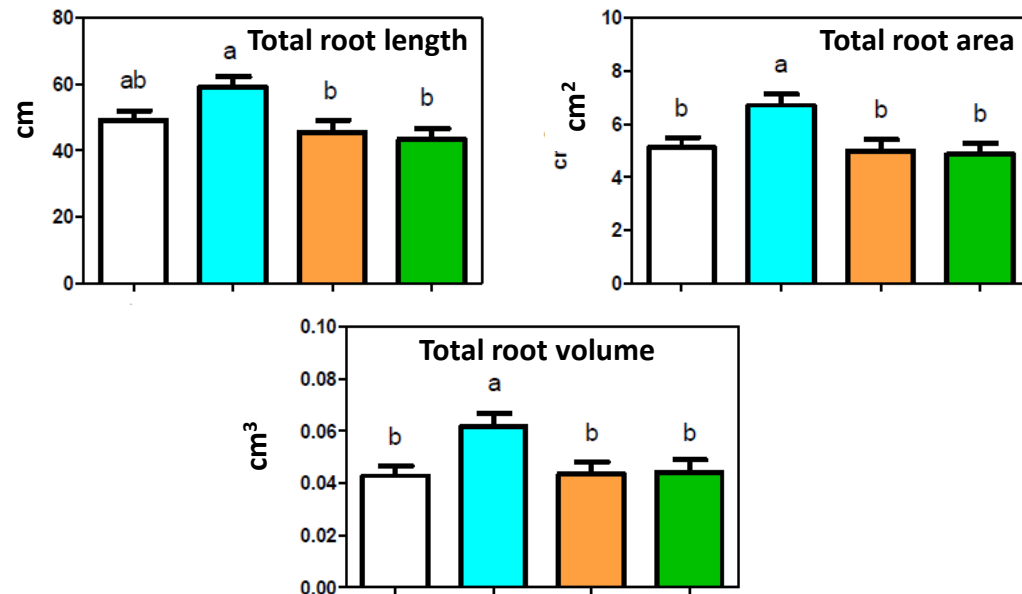
Plant growth trials

Cucumber - hydroponics



No difference in root development

Tomato - hydroponics

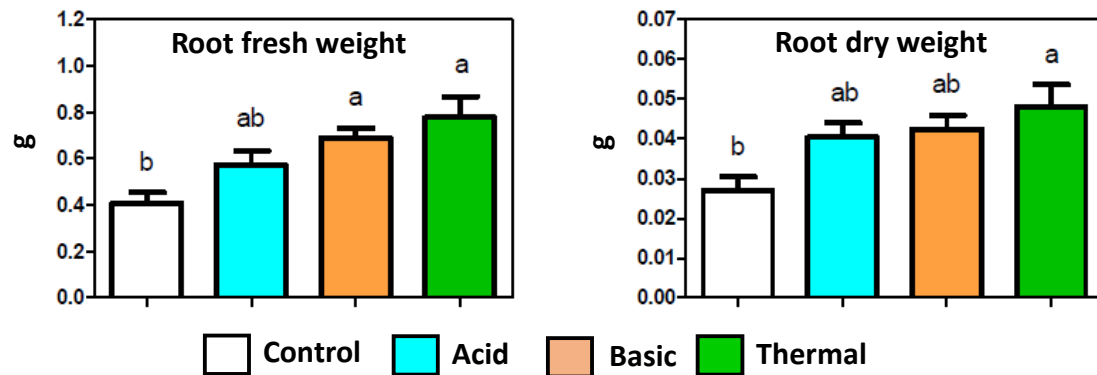


No difference in aerial parts

Biostimulants for agriculture

Plant growth trials

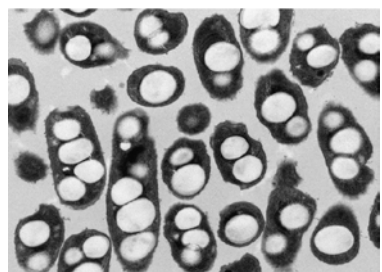
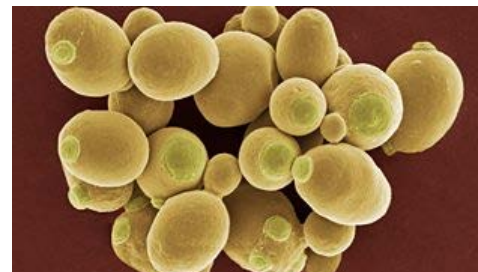
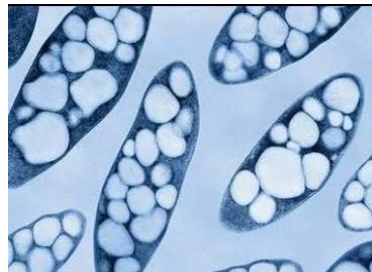
Tomato – foliar spray



No difference in aerial parts

Summary

- **PHA-based** bioplastics are **more expensive** than petroleum-based plastics, need to make the extraction process **more economic**
- SCPs used for fish feed **avoid PHAs extraction** and **use the whole cell as a final product**
- **PHA-containing SCPs** result in **better fish survival rates** and **total body length**
- **By-products** of the PHAs extraction products can be turned into **biostimulants**
- Use of protein hydrolysates result in plants with **more developed roots or foliar system**



Future work

- Fish feeding trials with SCP from *Thauera* & *Rhodopseudomonas*
- Fish feeding trials with **commercial fish species**
- Analysis of **SCP contaminants** (metals, antibiotics, hormones, microplastics...)
- **Repeat** plant growth trials
- **Improve** chemico-thermal treatments for protein hydrolysates (use of enzymes, smaller quantities of chemicals)
- New plant trials with improved biostimulants

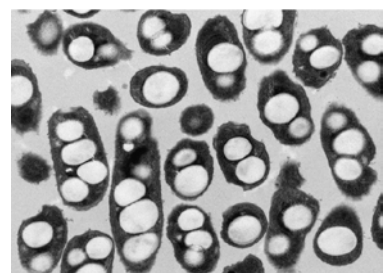
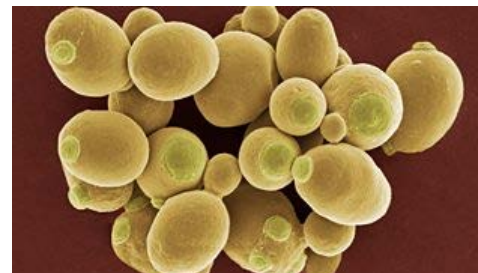
Acknowledgements



Andrea Vettori
Chiara Tesoriero
Francesca Greco
Elena Cannone
Salvatore Fusco
Davide Slaghenaufi

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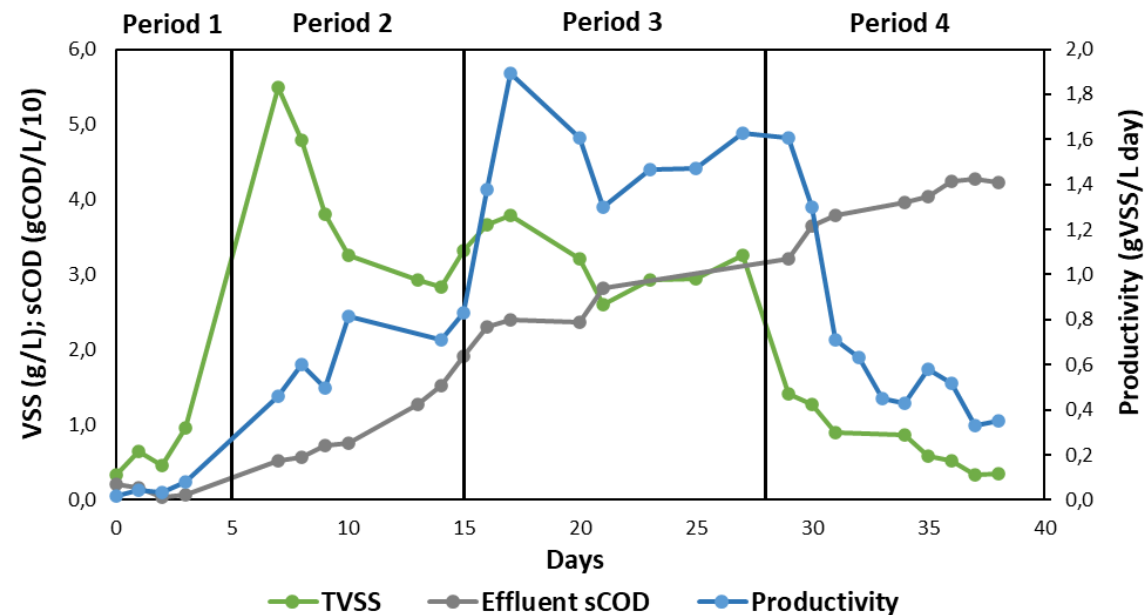
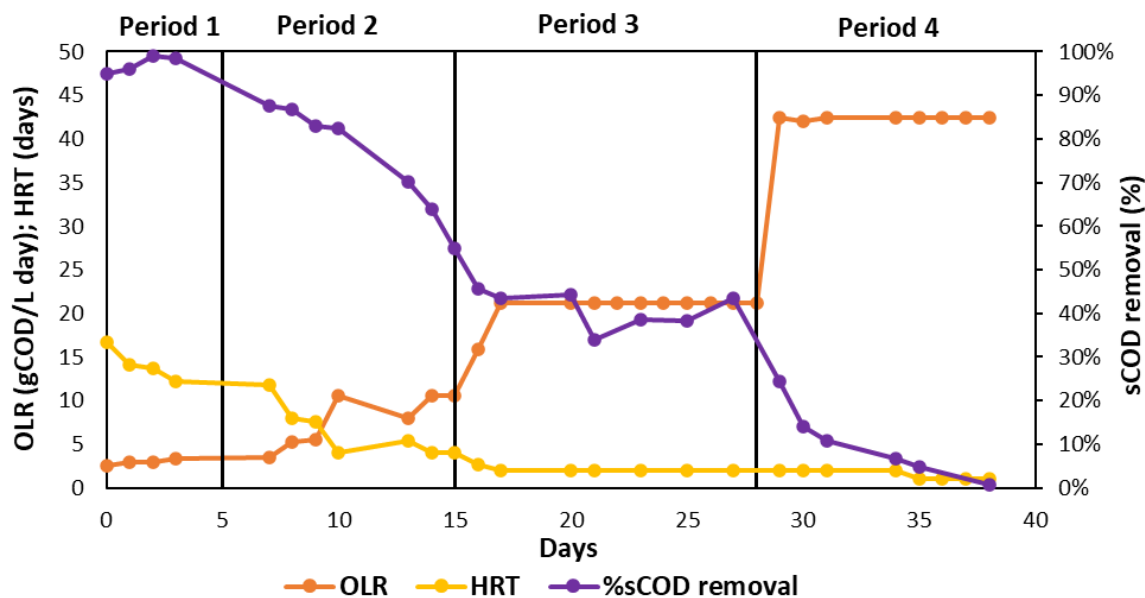
Fish-feed production

CSTR performance

Parameter	Unit	This Study
K	gCOD/gX d	3,01
Y	gX/gCOD	0.29
μ_{max}	d ⁻¹	0.9
K _s	gCOD/L	10.4
K _d	d ⁻¹	0.09

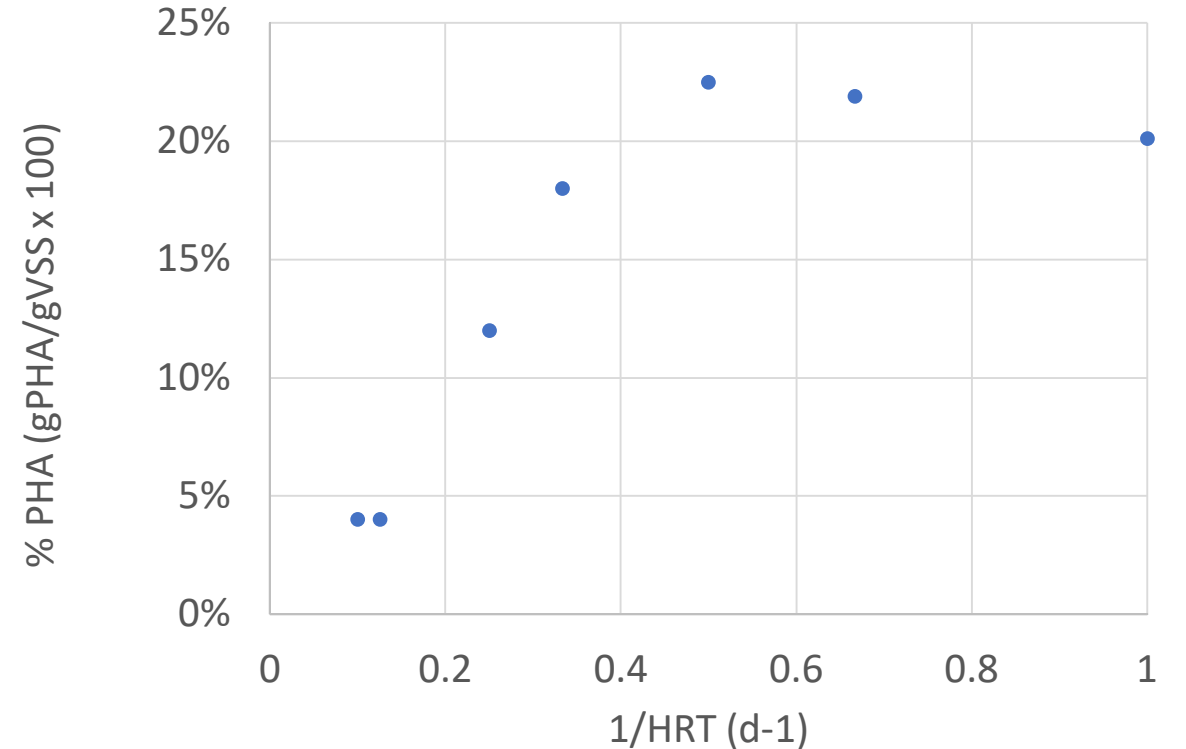
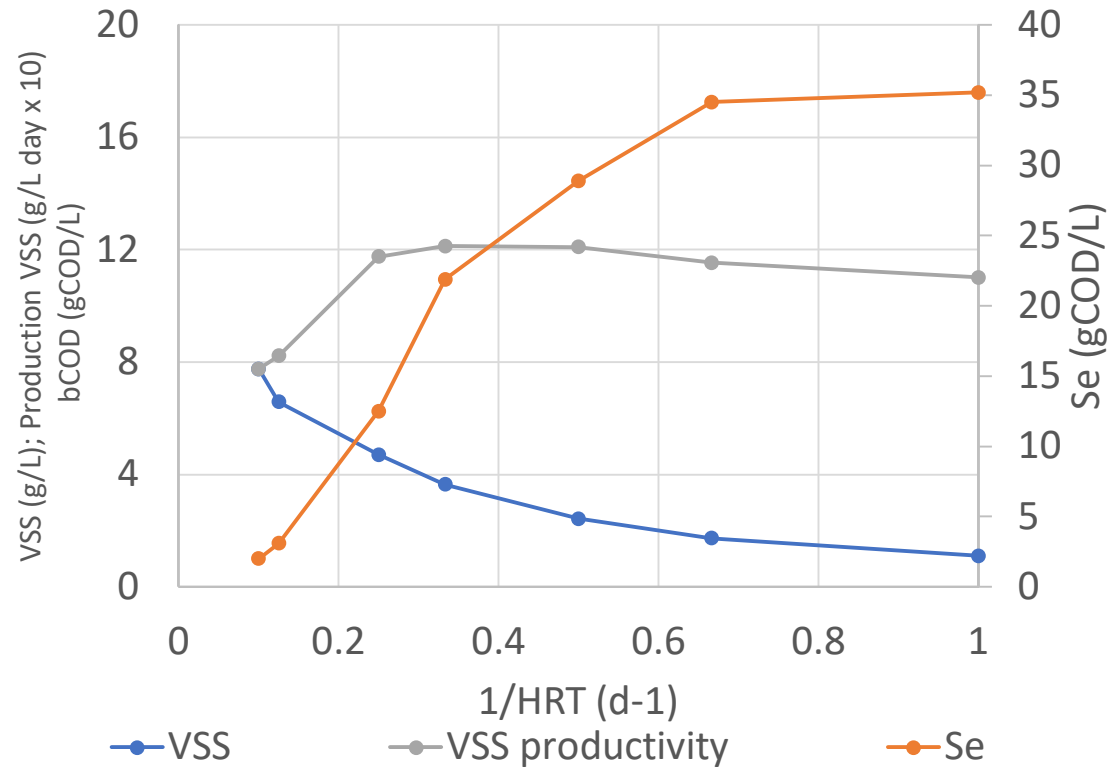
Parameter	Unit	Value							
HRT	day	10	8	4	3	2	1.5	1	
OLR	gCOD/L giorno	4.2	5.3	10.6	14.2	21.2	28.3	42.5	

Each period was evaluated at steady state (duration 3 x HRT)



Fish-feed production

CSTR performance



Max productivity: around 1.2 kgVSS/m³ day
Conversion yield: 0,45-0,50 gCODx/gCOD utilized

Increase the PHA content in the biomass up to 22%



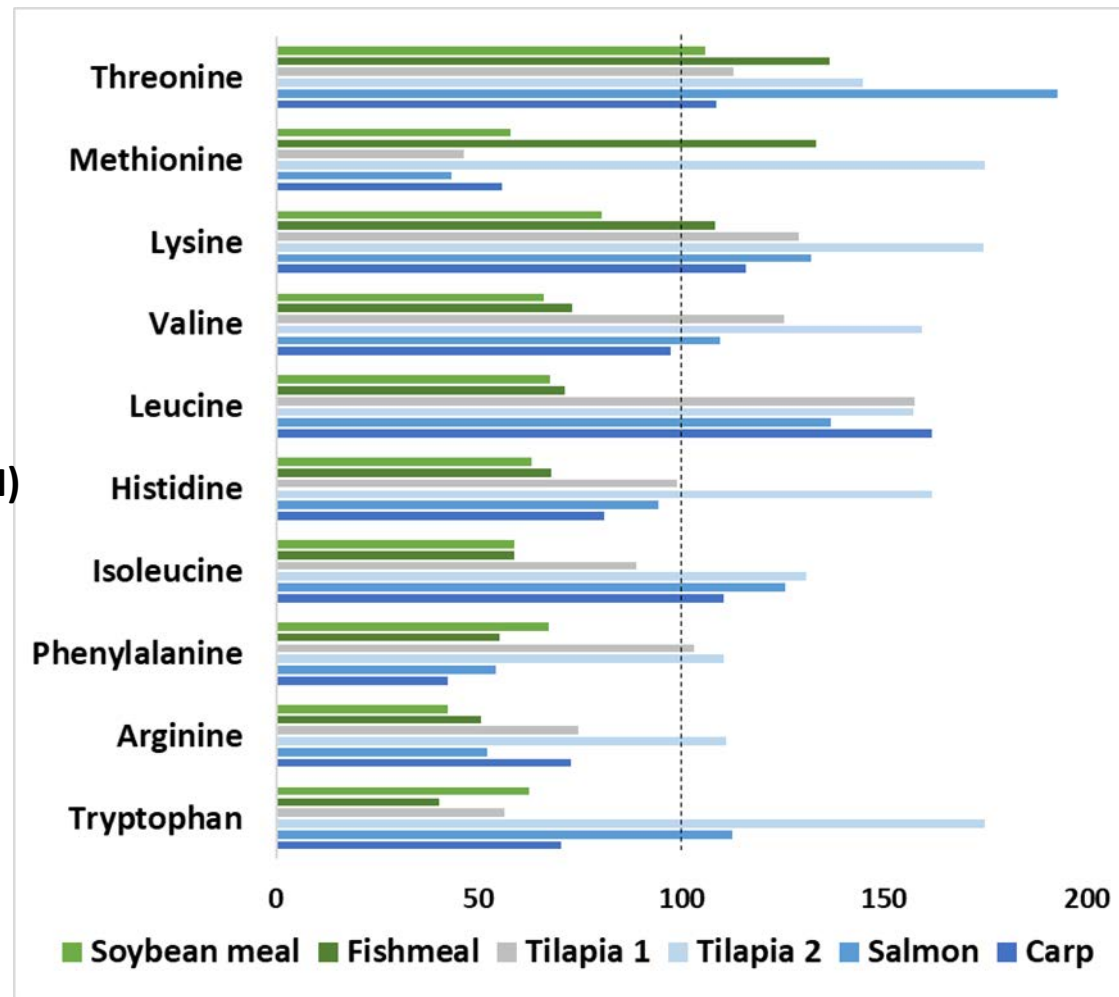
Fish feed production

Biomass analysis - MMC

Parameter	SCP	Fermentation fluid
Moisture	12.21	98.41*
Ash	16.56	14.46
Crude lipids and oils	<LoQ	<LoQ
Crude proteins	61.79	28.94
Carbohydrates	<LoQ	<LoQ
Crude cellulose	<LoQ	<LoQ
Nitrogen-free extracts	21.65	56.60
PHAs	22.54	0.00

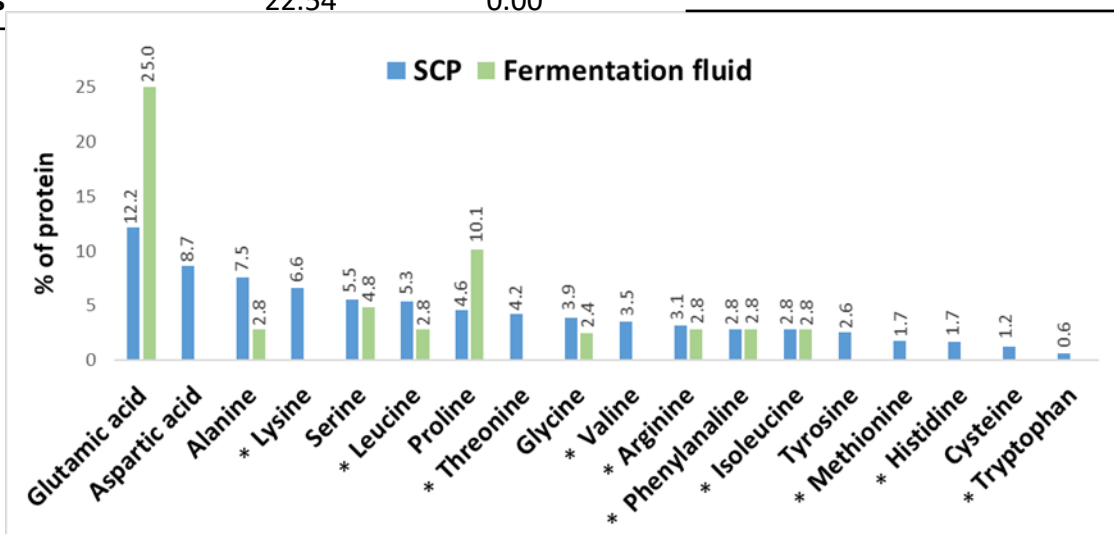
Reference protein	EAAI
Fishmeal	0.73
Soybean meal	0.65
Tilapia 1	0.93
Tilapia 2	1.45
Chinook salmon	0.95

Protein
Over dry weight: 61.8%
Over VVS: 74.0%



Proximate composition

Essential amino acid index (EAAI)



Amino acid profile

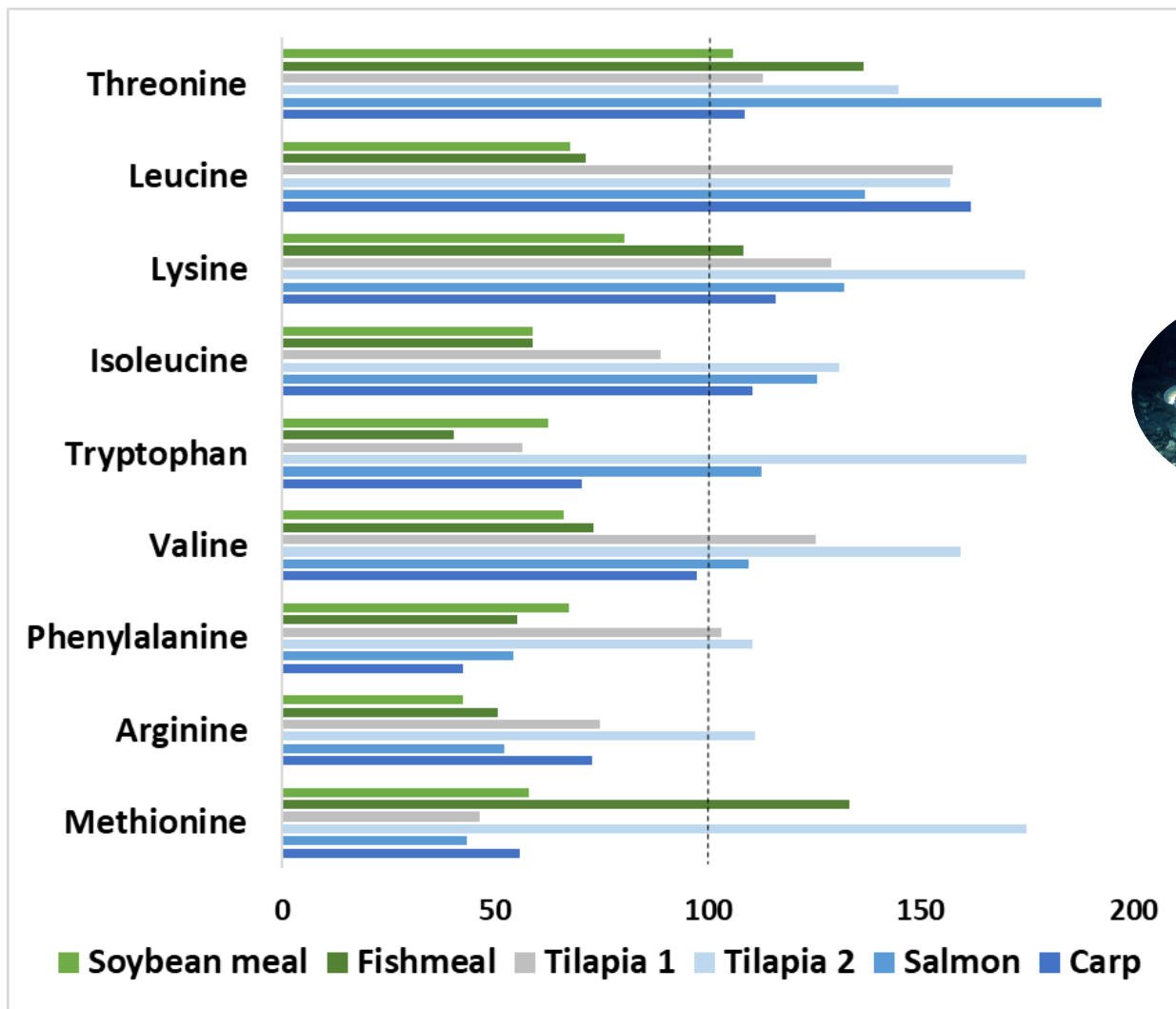


Fish feed production

Biomass analysis - MMC



Chemical score (CS)



Reference proteins



$$EAAI = 10 \log EAA$$



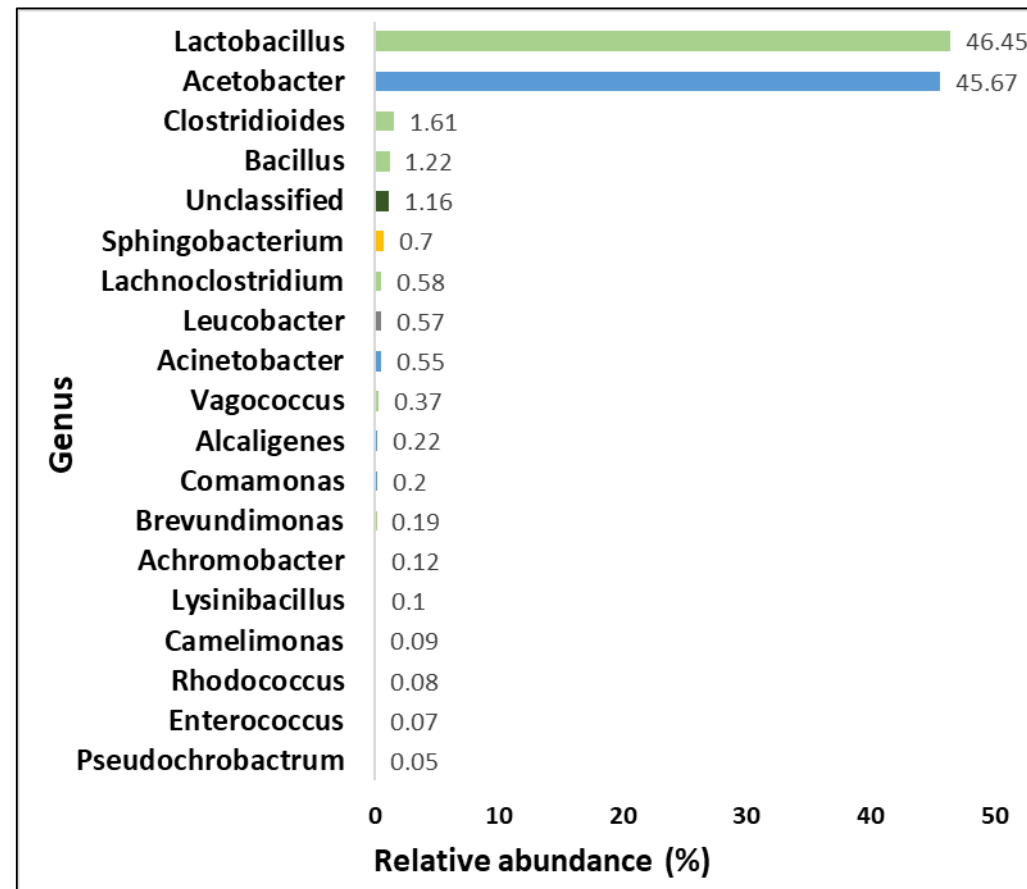
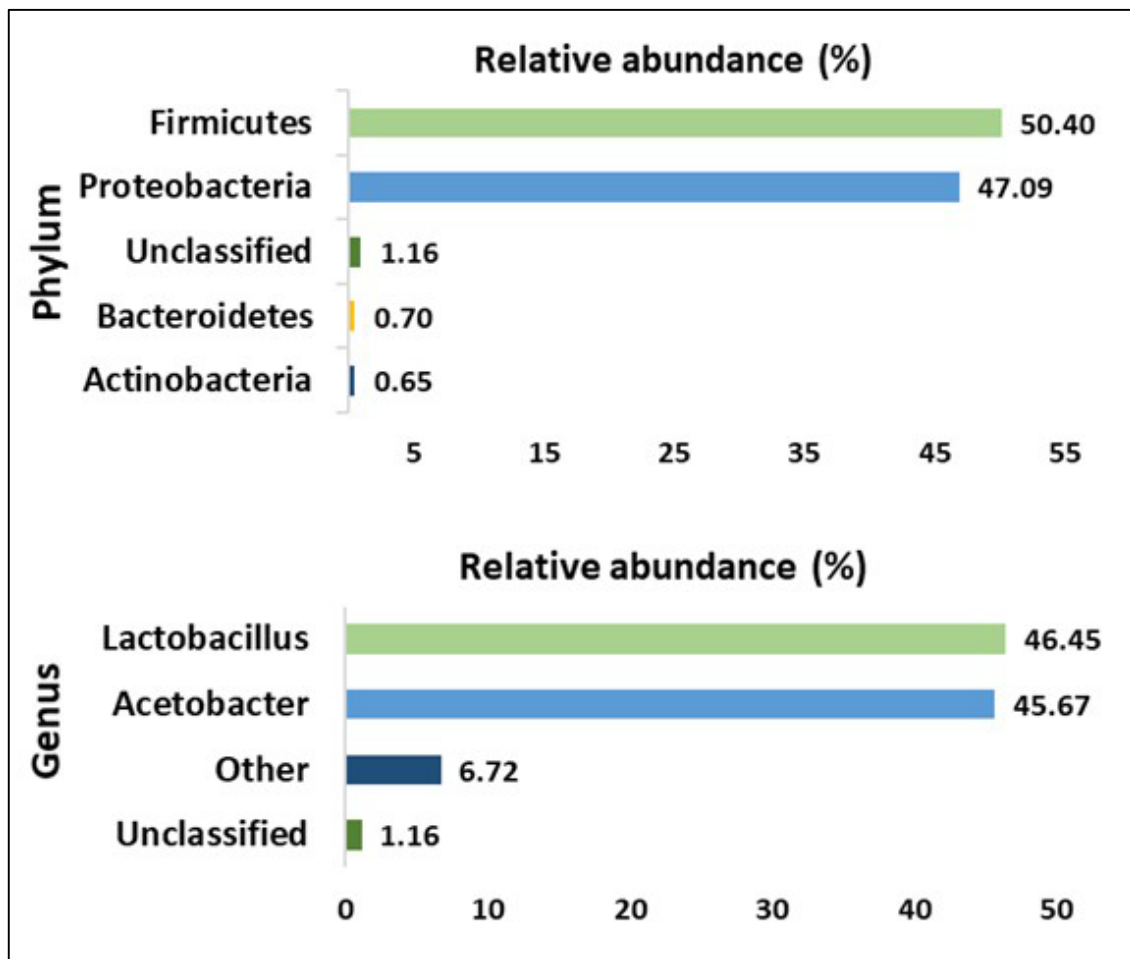
Essential amino acid index (EAAI)

0.48 (0.66) vs fishmeal
0.59 (0.84) vs Tilapia 1

Good quality protein = 0.90
useful protein = 0.80
incomplete protein = 0.70

Fish feed production

Bacterial community analysis



16S rRNA next generation sequencing



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Fish-feed production

Feeding trials



DAY 0: fertilisation

DAY 15: dead/alive count & imaging

DAY 6: feeding starts

DAY 18: dead/alive count

GROUP A



100% CF

GROUP B



50% CF+ 50% SCP
(finely ground)

GROUP C



50% CF + 50% SCP
+ 14% oils

GROUP D



50% CF + 50% SCP +
14% oils + 10% PHA

GROUP E



50% CF + 50% SCP +
10% PHA



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