

Production of PHA with mixed cultures from fermented food waste rich in ammonia

Fernando Silva - Mariana Matos - Gilda Carvalho - Maria Reis

**7TH INTERNATIONAL CONFERENCE ON SUSTAINABLE SOLID
WASTE MANAGEMENT
26 - 29 JUNE 2019**

Why use food waste as feedstock?

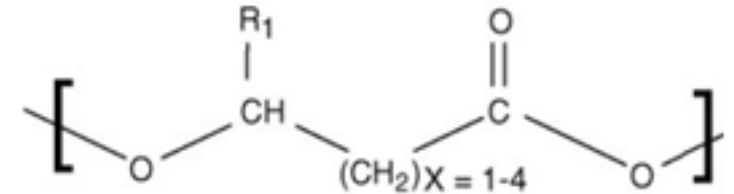
- Food waste is food that was lost or discarded uneaten
- Around 1/3 of food is wasted annually; in EU alone, it amounts to 88 million tonnes alone.
- A significant portion of the food waste ends in landfills, which result in unwanted CH₄ emissions.



Solutions?

Why produce polyhydroxyalkanoates (PHA)?

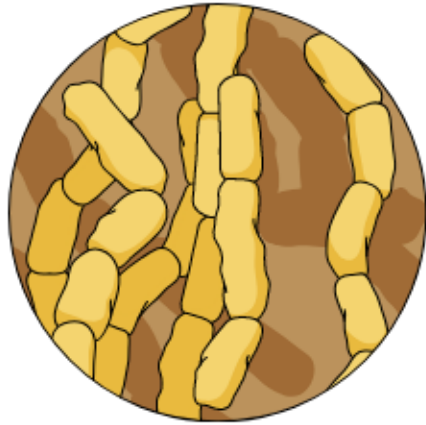
- Biologically synthesized polyester
- Biocompatible and completely biodegradable into CO₂ and H₂O
- Wide range of structural, mechanical and thermal properties



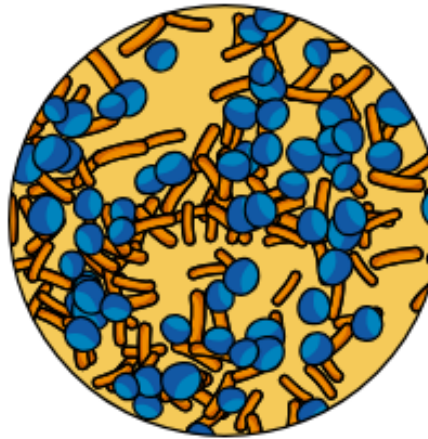
Sustainable alternative to conventional plastics



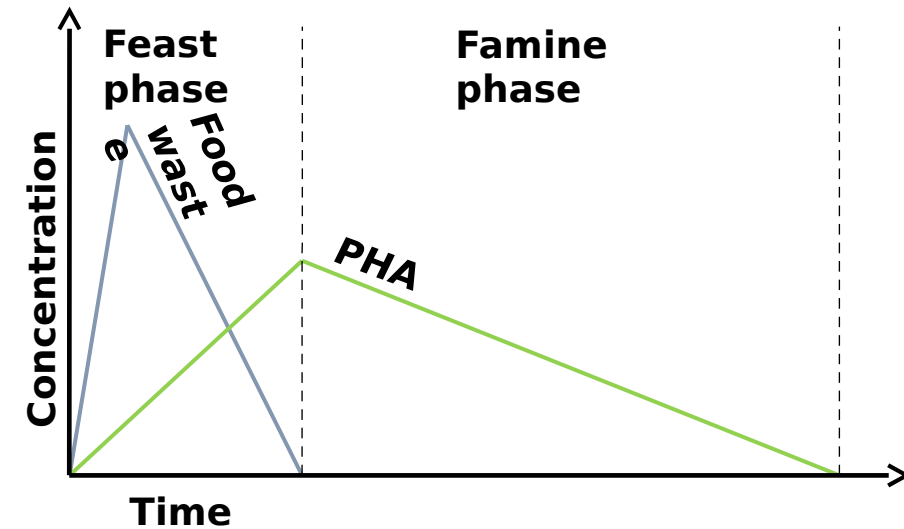
Why use mixed microbial cultures?



Volumetric productivity
Investment and operational costs
Sterile conditions

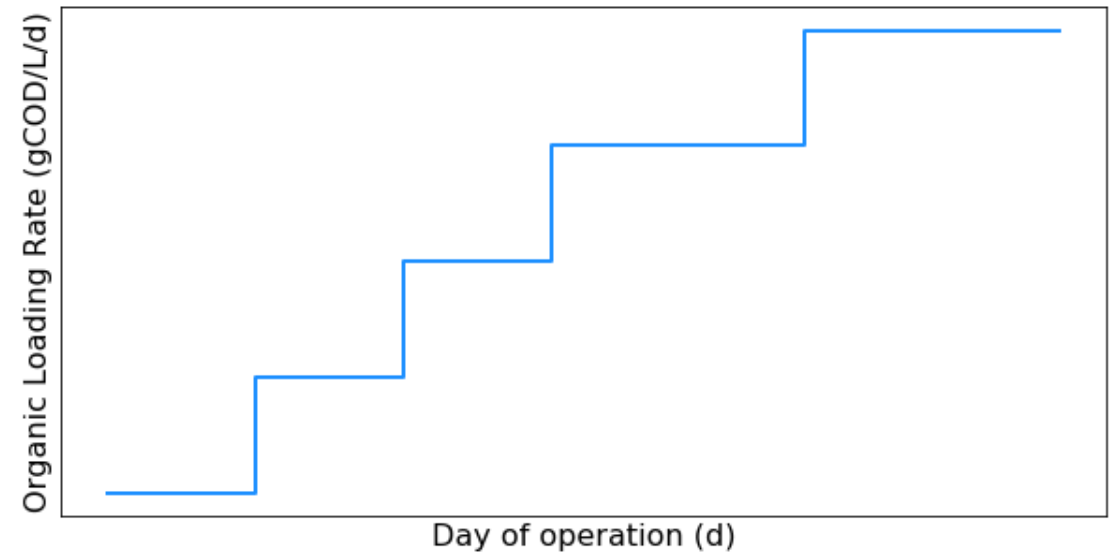


Investment costs
Sterile conditions
Volumetric productivity

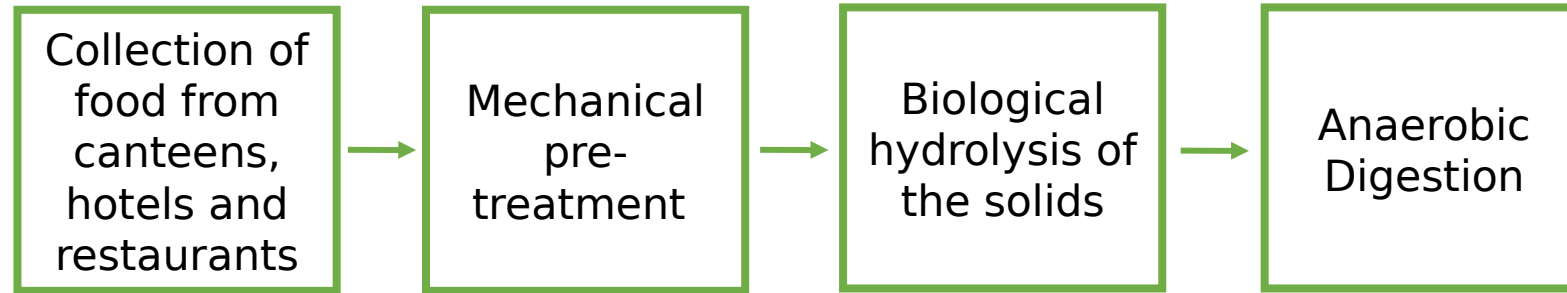


Approach

- 01** Characterize food waste and evaluate its feasibility as a feedstock for PHA production
- 02** Inoculate a SBR and select a culture with PHA accumulation capacity
- 03** Study the maximum accumulation capacity of the selected culture



Fermented food waste source



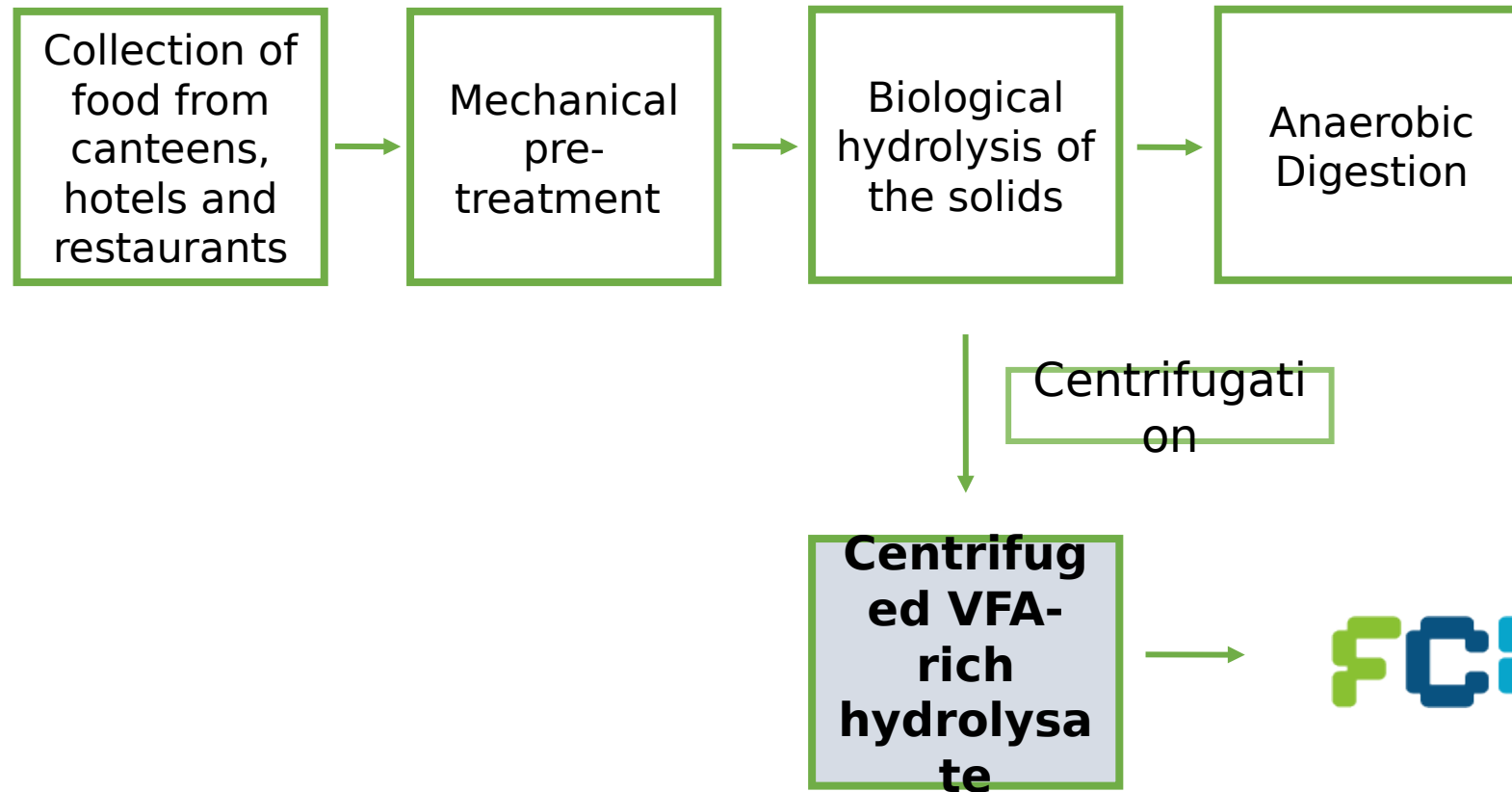
Food waste characterization

	Mean ± SD
COD _{TOT} (g L ⁻¹)	119 ± 0,28
COD _{SOL} (g L ⁻¹)	37,5 ± 1,34
TC (g L ⁻¹)	12,4 ± 0,01
TOC (g L ⁻¹)	12,3 ± 0,02
TOC (Cmmol L ⁻¹)	1026 ± 2,04
IC (mg L ⁻¹)	0,04 ± 0,01
Prot _{TOT} (gCOD L ⁻¹)	14,9 ± 0,73
Prot _{SOL} (gCOD L ⁻¹)	1,85 ± 0,04
CH _{TOT} (gCOD L ⁻¹)	7,53 ± 1,02
CH _{SOL} (gCOD L ⁻¹)	1,17 ± 0,38
FP (gCOD L ⁻¹)	31,9 ± 0,45
FP (Cmmol L ⁻¹)	898 ± 12,7
FP/COD _{SOL}	0,85 ± 0,02

	Mean ± SD
TS (g L ⁻¹)	62,4 ± 3,94
VS (g L ⁻¹)	53,6 ± 3,68
VS/TS (g g ⁻¹)	0,86 ± 0,005
N-NH ₃ (gN L ⁻¹)	1,11 ± 0,01
N-NO ₃ (gN L ⁻¹)	0
N-NO ₂ (gN L ⁻¹)	0
Kejdhal (gN L ⁻¹)	3,2 ± 0,1
N-NH ₃ /Kejdhal	34,2 ± 1,2
P-PO ₄ (mgP L ⁻¹)	331 ± 3,92
Nmol:Pmol	7,39 ± 0,15

- High COD content;
- High solid content, with plenty protein and carbohydrates (HC) content unfermented;
- High fermentation products (FP) to COD_{SOL} ratio;
- Nutrient-rich feedstock

Fermented food waste source



FACULDADE DE
CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

SBR operation

	Mean \pm SD
$\text{gCOD}_{\text{TOT}} \text{ L}^{-1}$	43.8 ± 5.47
$\text{gCOD}_{\text{SOL}} \text{ L}^{-1}$	31.4 ± 0.94
$\text{gCOD}_{\text{FP}} \text{ L}^{-1}$	25.9 ± 1.56
$\text{COD}_{\text{FP}}/\text{COD}_{\text{SOL}}, \%$	82.7 ± 3.59
Cmmol L^{-1}	663 ± 39.7
Nmmol L^{-1}	143 ± 6.15
Pmmol L^{-1}	4.62 ± 0.42
N/C, %	21.3 ± 0.72
P/C, %	0.69 ± 0.05

Operating conditions	
Feedstock	Fermented Food Waste
OLR ($\text{gCOD L}^{-1} \text{ d}^{-1}$)	3.6 - 10.4 (gradual increase)
C:N:P (mol basis)	100:21:0.69
SRT (d)	4
Cycle length (h)	12
HRT (d)	1
T & pH	Uncontrolled
Volume (L)	2

	Lactate	Acetate	Propionate	Ethanol	Butyrate	Iso-Valerate	Valerate	Caproate	Hexanoate	Octanoate	Total
[FP] (gCOD L^{-1})	0	4.8	3.1	0.3	5.8	0.8	2.9	6.1	2.2	0	25.9
% FP	0	18	12	1	22	3	11	24	8	0	100%

SBR operation

01

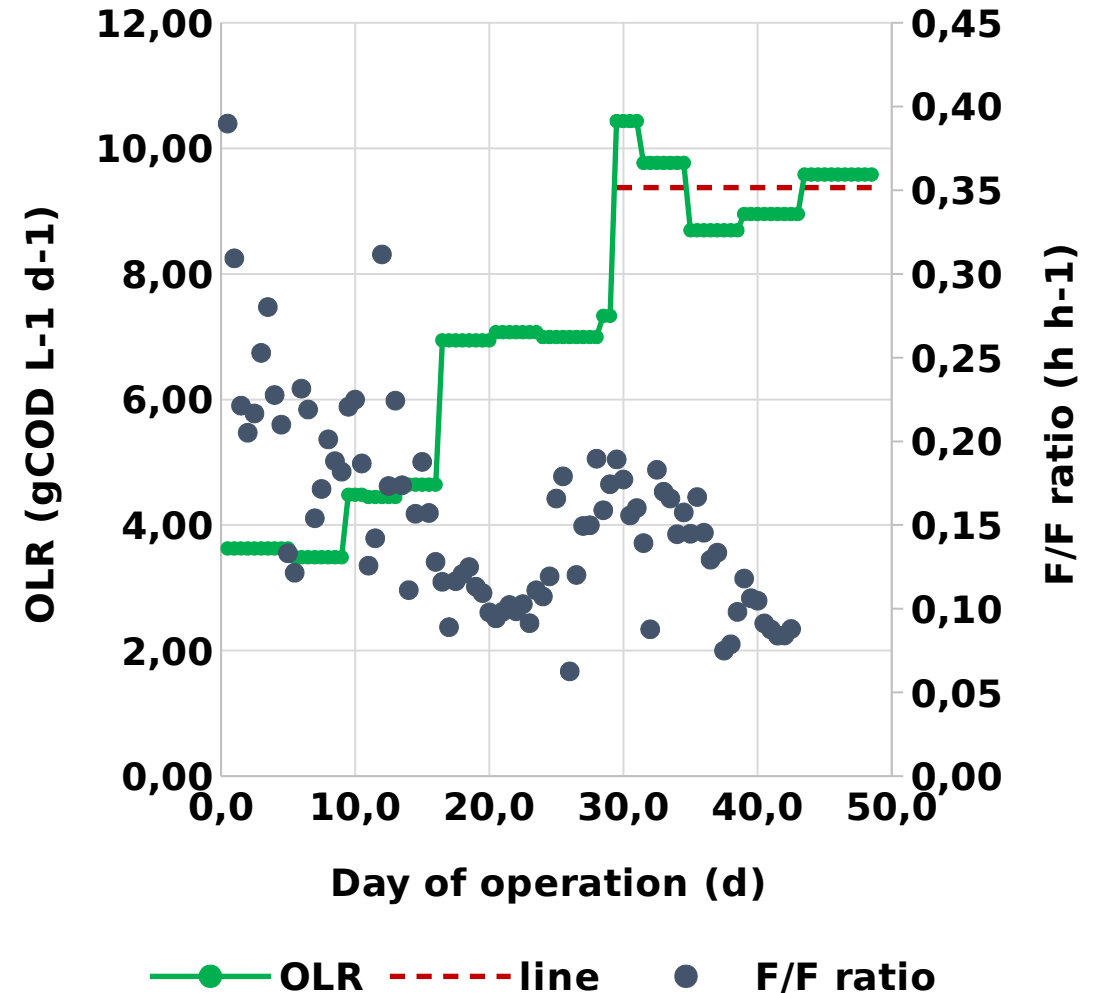
OLR up to around $9.3 \text{ gCOD L}^{-1} \text{ d}^{-1}$ was achieved;

02

F/F ratios as low as 0.1 h h^{-1} can be achieved, hence SBR stability accomplished;

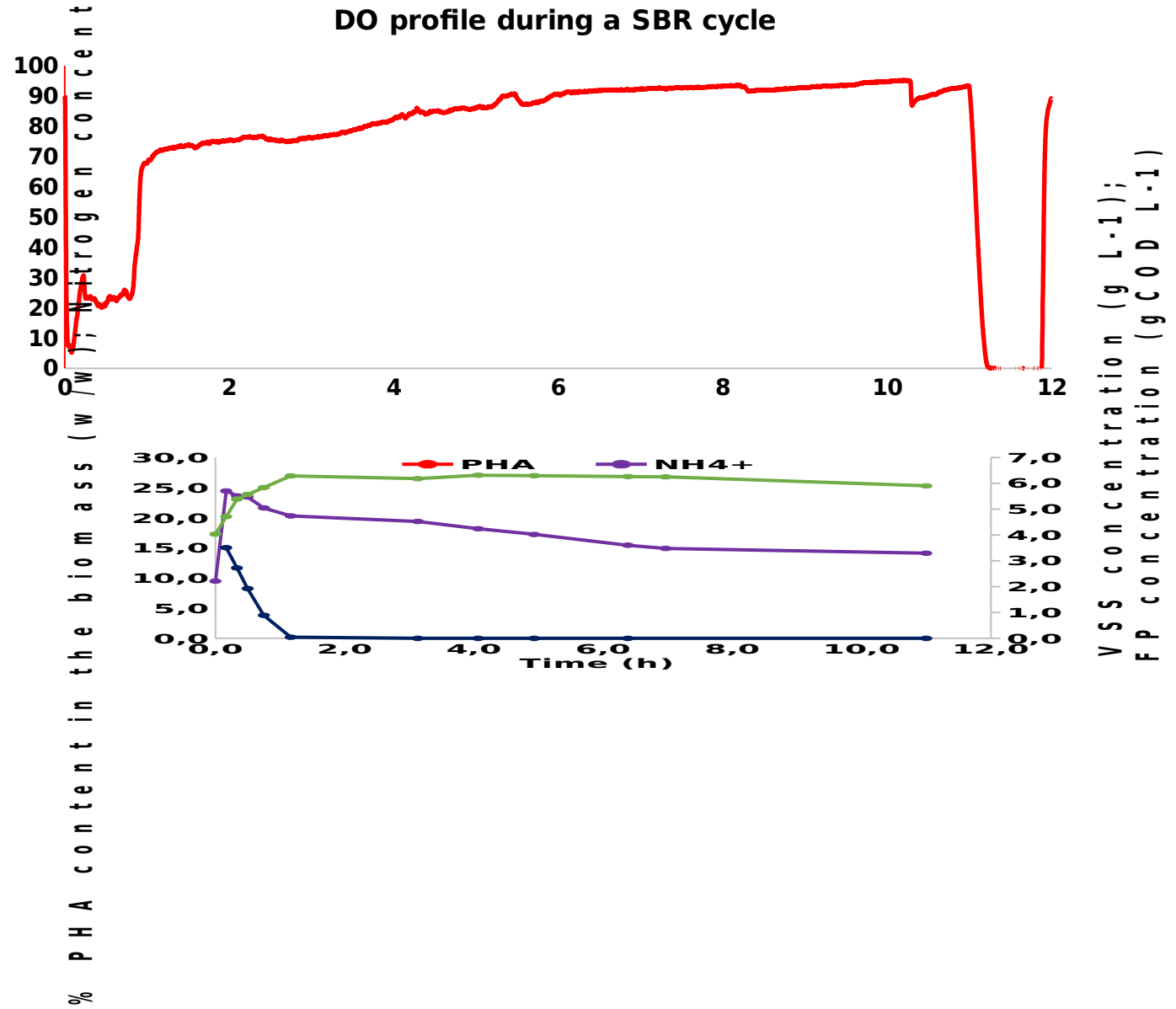
03

Owing to the variability of the FP concentration in the food waste, OLR fluctuated. That variation didn't seem to affect the reactor negatively.



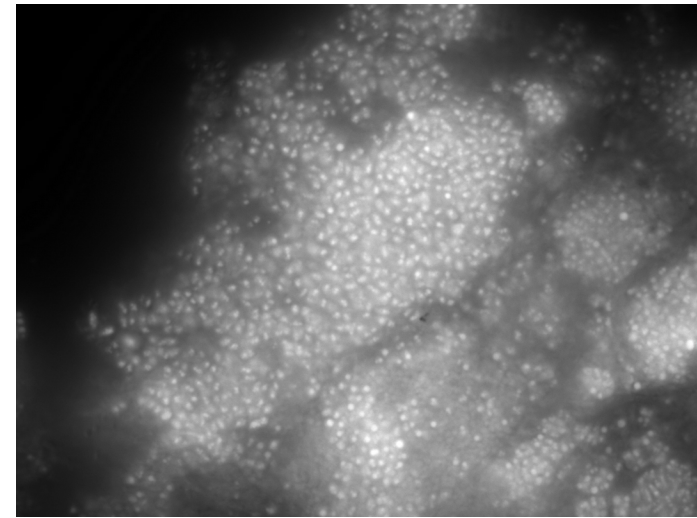
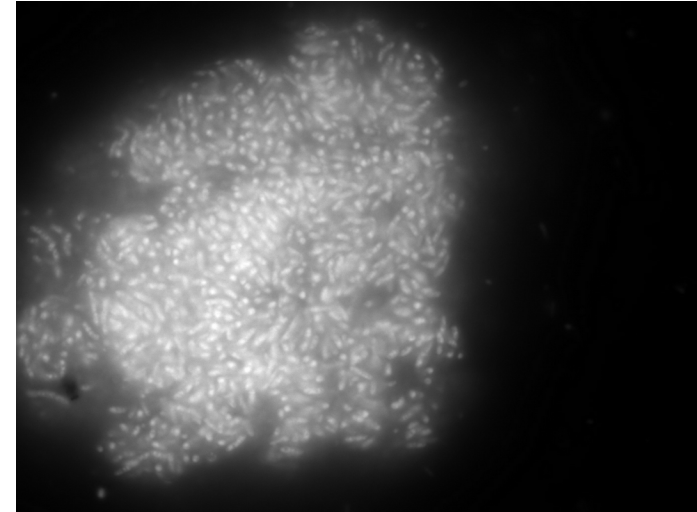
SBR operation

	Phase #1	Phase #2
OLR (gCOD L ⁻¹ d ⁻¹)	7.06 ± 0.12	9.42 ± 0.29
Feast/Famine (h h ⁻¹)	0.11 ± 0.01	0.10 ± 0.03
r _{FP} (gCOD L ⁻¹ h ⁻¹)	2.78 ± 0.73	4.08 ± 1.15
r _N (Nmmol L ⁻¹ h ⁻¹)	1.67 ± 0.52	2.32 ± 0.81
r _{PHA} (g L ⁻¹ h ⁻¹)	1.18	1.93
Maximum PHA concentration (g L ⁻¹)	2.70	2.95
PHA content at feast phase (gPHA gTS ⁻¹)	24.9	27.4
HV content (gHV gPHA ⁻¹)	37.0	37.0
[VSS] at feast phase (g L ⁻¹)	6.49 ± 0.28	10.2 ± 0.49
N removal in the cycle (Nmol Nmol ⁻¹ , %)	50.8 ± 12.4	60.4 ± 10.3
Y _{P/S} (gCOD gCOD _{FP} ⁻¹)	61.4	69.9
Y _{X/S} ^{FEAST} (gCOD gCOD _{FP} ⁻¹)	21.5 ± 3.5	17.5 ± 13.4
Y _{X/P} ^{FAMINE} (gCOD gCOD _{PHA} ⁻¹)	30.8	38.8



SBR operation

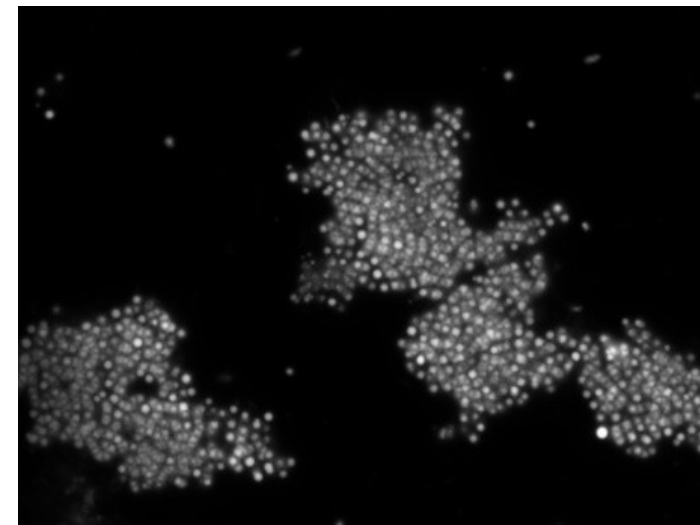
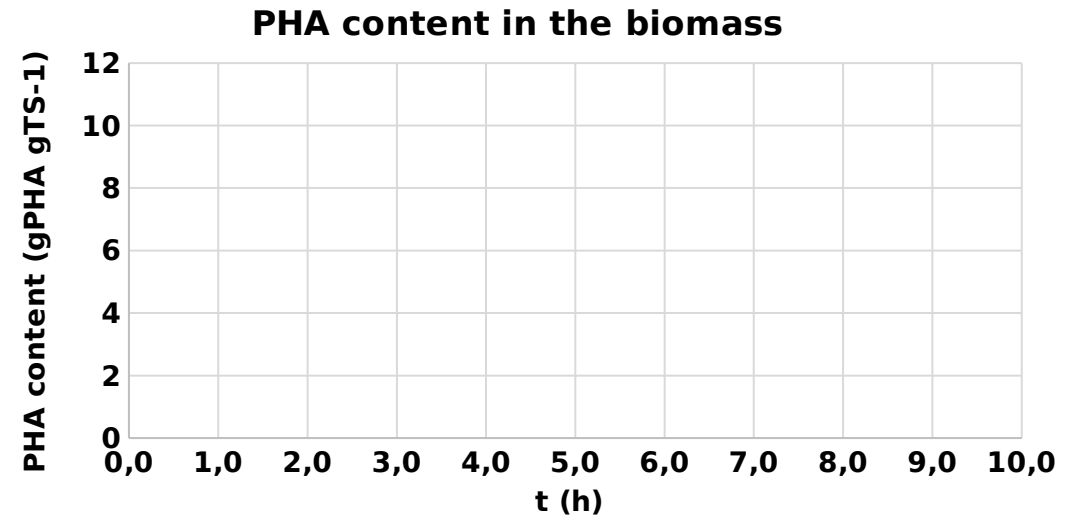
- 01** PHA data confirmed that selection of PHA-accumulating occurred under these conditions
- 02** Low COD:N ratio led to ammonia accumulation; However, a rather high VSS concentration was obtained and considerable N removal was achieved
- 03** No NO_2^- and NO_3^- means no nitrification (no thiourea was fed).



Nile blue staining for PHA granules, day 43

Accumulation reactor operation

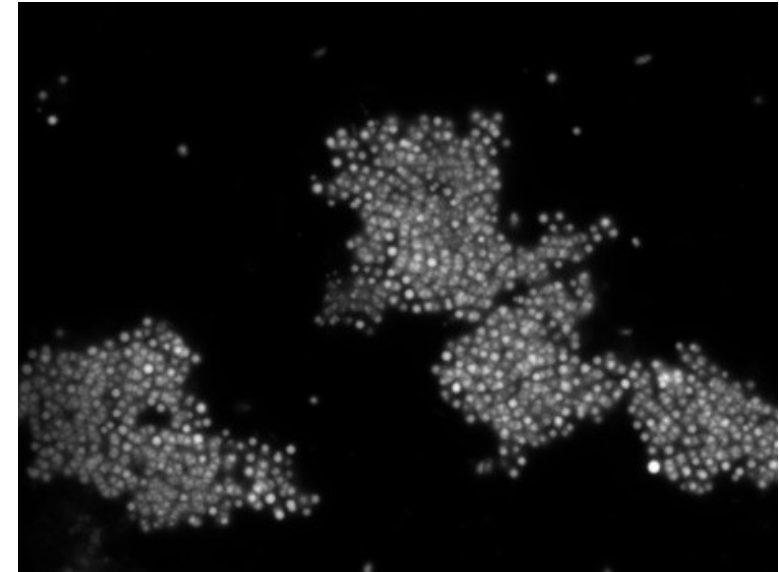
	Mean \pm SD
PHA content at the end (gPHA gTS ⁻¹)	43.9 \pm 3.49
HV content (gHV gPHA ⁻¹)	33.3 \pm 1.53
r_{FP}^{AVE} (gCOD L ⁻¹ h ⁻¹)	1.92 \pm 0.20
r_{PHA}^{AVE} (g L ⁻¹ h ⁻¹)	0.72 \pm 0.17
r_N^{AVE} (Nmmol L ⁻¹ h ⁻¹)	3.85 \pm 0.47
PHA concentration at the end (g L ⁻¹)	5.62 \pm 0.32
Storage yield (gCOD _{PHA} gCOD _{FP} ⁻¹)	0.56 \pm 0.07
Storage yield (gCOD _{PHA} COD _{SOL} ⁻¹)	0.45 \pm 0.06
Storage yield (gCOD _{XA} gCOD _{SOL} ⁻¹)	0.37 \pm 0.06
Global Productivity (g L ⁻¹ h ⁻¹)	0.61 \pm 0.09



Nile blue staining for PHA granules, day 28

Accumulation reactor operation

- 01** PHA content above 40 gPHA gTS⁻¹ in all assays
- 02** Butyrate and Valerate were preferable than longer chain FP; medium-chain FP were preferred to acetate and propionate
- 03** Nitrogen uptake increased along accumulation assay
- 04** Some residual glucose was consumed along with FP at the beginning of the pulse



Nile blue staining for PHA granules, day 28

33 % HV
content
 $M_w = 5.48 \times 10^5$
 $M_n = 2.67 \times 10^5$
PDI = 2.05

Conclusions and future perspectives

- 01** The SBR was stable for the period of operation, thus allowing the selection of PHA-accumulating culture
- 02** Despite the fact that the fermented food waste was rich in ammonia, the selection occurred at a high OLR regardless.
- 03** PHA content was high enough to be considered economically viable for the PHA-rich biomass be extracted.
- 04** Demonstration at pilot scale for a longer period of operation that it is technically feasible to produce PHA using this feedstock
- 05** Owing to the variability of this feedstock, potentially variable parameters (COD/N ratio, unfermented glucose/proteins, variable FP profile, etc...) should be studied on their impact on the stability of the SBR in the long-term

Acknowledgments

- European project REsources from URban BlowaSte (H2020-CIRC05-2016-730-349)
- Fundação para a Ciência e Tecnologia (Portugal) for funding through PD/BD/126626/2016
- UCIBIO financed by national funds from FCT/MCTES (UID/Multi/04378/2019)
- Biochemical Engineering Group (BIOENG)



Contact info:

Fernando Silva, PhD
student

Faculty of Sciences and
Technology of NOVA
University of Lisbon,
Portugal

fra.silva@campus.fct.unl.
pt