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# Polyhydroxyalkanoates production using the liquid fraction of hydrolysed municipal organic waste

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# LAYOUT OF THE PRESENTATION

- **INTRODUCTION**
  - PHA-BASED BIOPLASTICS
  - PRODUCTION OF BIOPLASTICS FROM RESIDUAL ORGANIC MATTER (ROM)
  - PROPOSED PHA PRODUCTION SYSTEM
- **LAB-SCALE RESULTS AND DISCUSSION**
  - FERMENTATION OF ROM
  - SELECTION OF PHA ACCUMULATING BIOMASS
  - PHA ACCUMULATION
- **CONCLUSIONS**



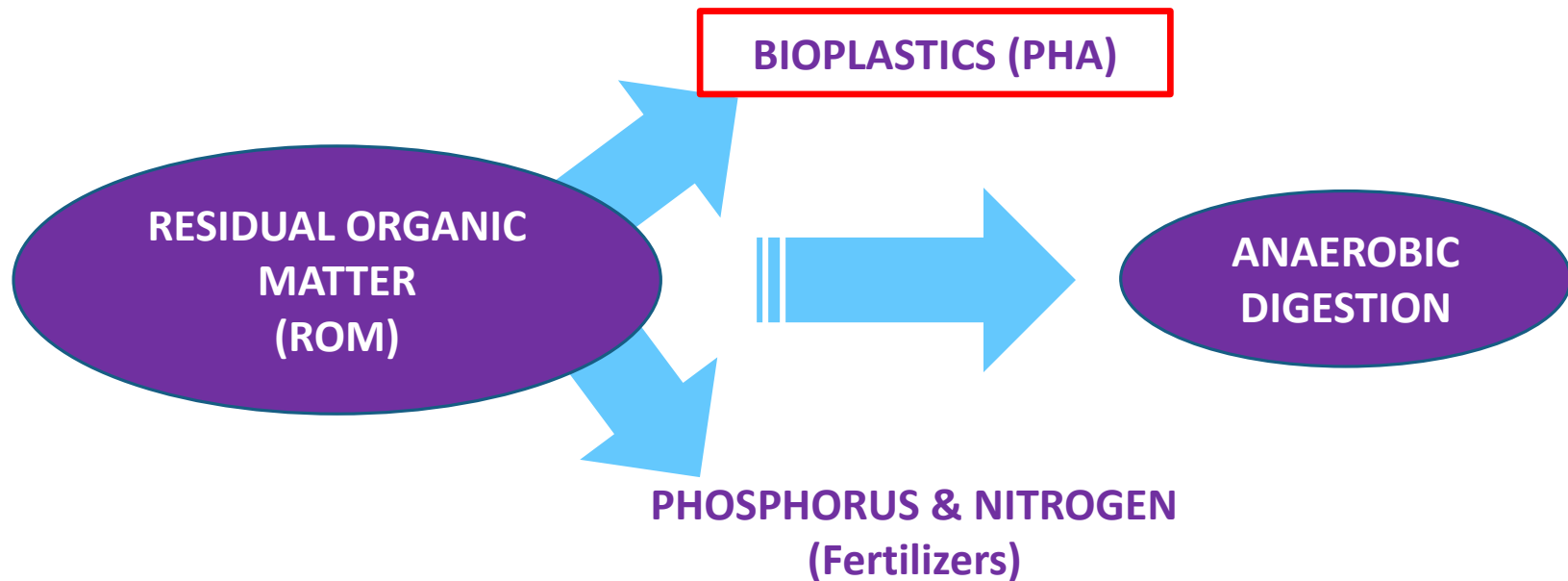
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# Introduction: PHA-based bioplastics

- New trends in waste management: **CIRCULAR ECONOMY**

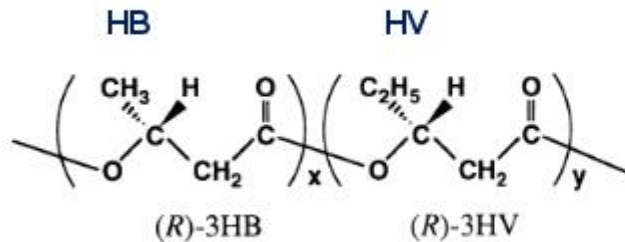


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# Introduction: PHA-based bioplastics: PHA

**Polyesters produced** by bacteria from the **degradation of biodegradable organic matter** as a mechanism to store carbon and energy.



**PHA** have **thermoplastic properties** which are **similar to the ones of conventional polyolefin** (many possible applications) with the advantages of being **biodegradable, biocompatible and renewable**.

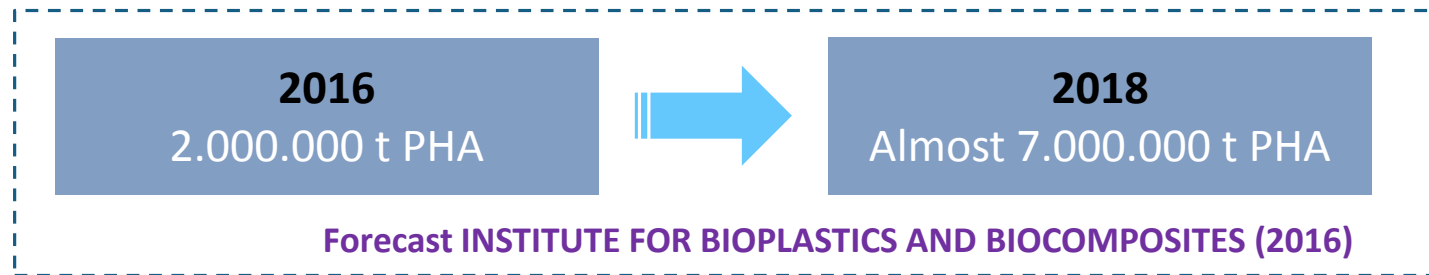


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# Introduction: PHA-based bioplastics: PHA

- PHA can be produced from different sources of biodegradable organic carbon, for example, **Volatile Fatty Acids (VFA)**.
- PHA market is well established and it has a **high expansion potential**.



- Production of bioplastics from ROM:
  - **Alternative** to the production from **dedicated crops** (corn, rice, barley, ...).
  - **Lower production costs** (avoiding raw materials costs and use of pure cultures).



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# Introduction: PHA production from ROM

The production of PHA from wastes needs 3 steps (Reis et al., 2011):

## 1) FERMENTATION OF THE ORGANIC SUBSTRATE

Production of Volatile Fatty Acids (VFA)

## 2) SELECTION OF PHA-ACCUMULATING BIOMASS

Establishing in a bioreactor the appropriate conditions to favour the growth and enrichment of PHA bacteria

## 3) PHA ENRICHMENT

Operate a second bioreactor under the optimum conditions to increase the concentration of PHA in the biomass



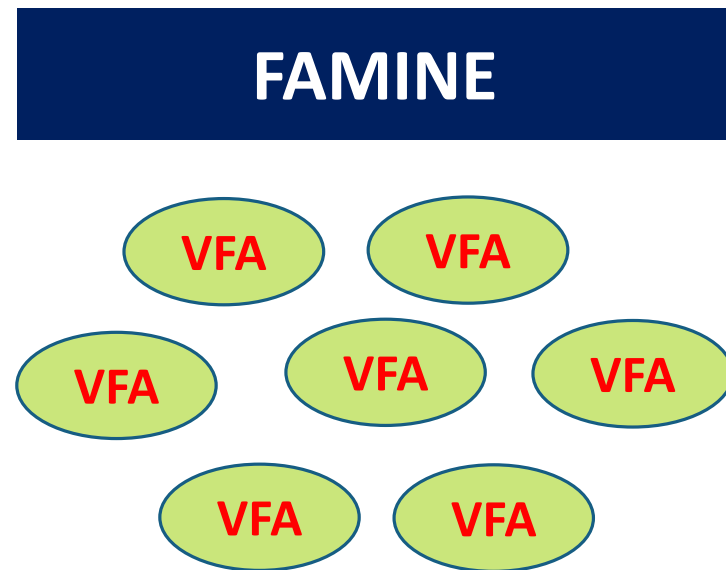
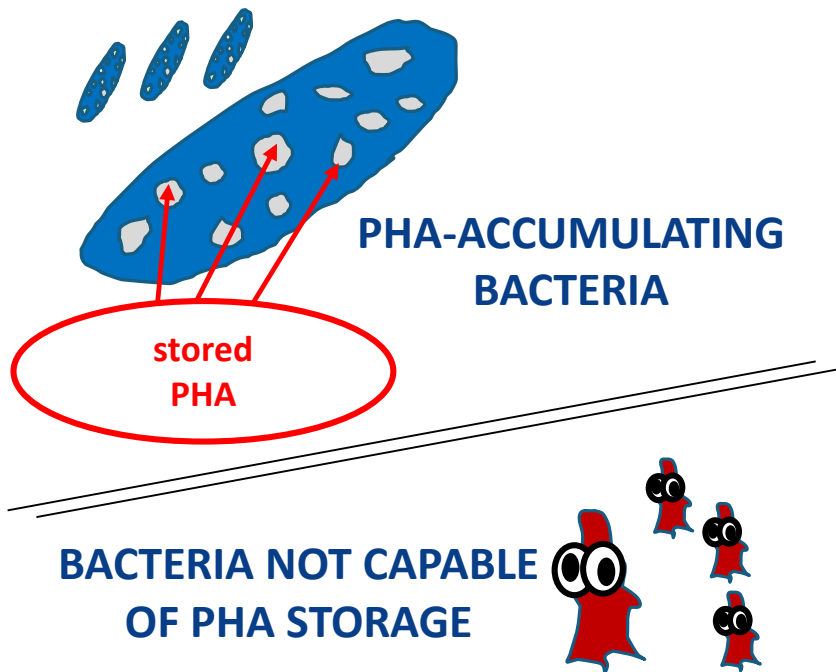
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# Introduction: PHA production from ROM

## 2) SELECTION OF PHA-ACCUMULATING BIOMASS

Use of Feast/Famine cycles to select and enrich the biomass in PHA-accumulating bacteria



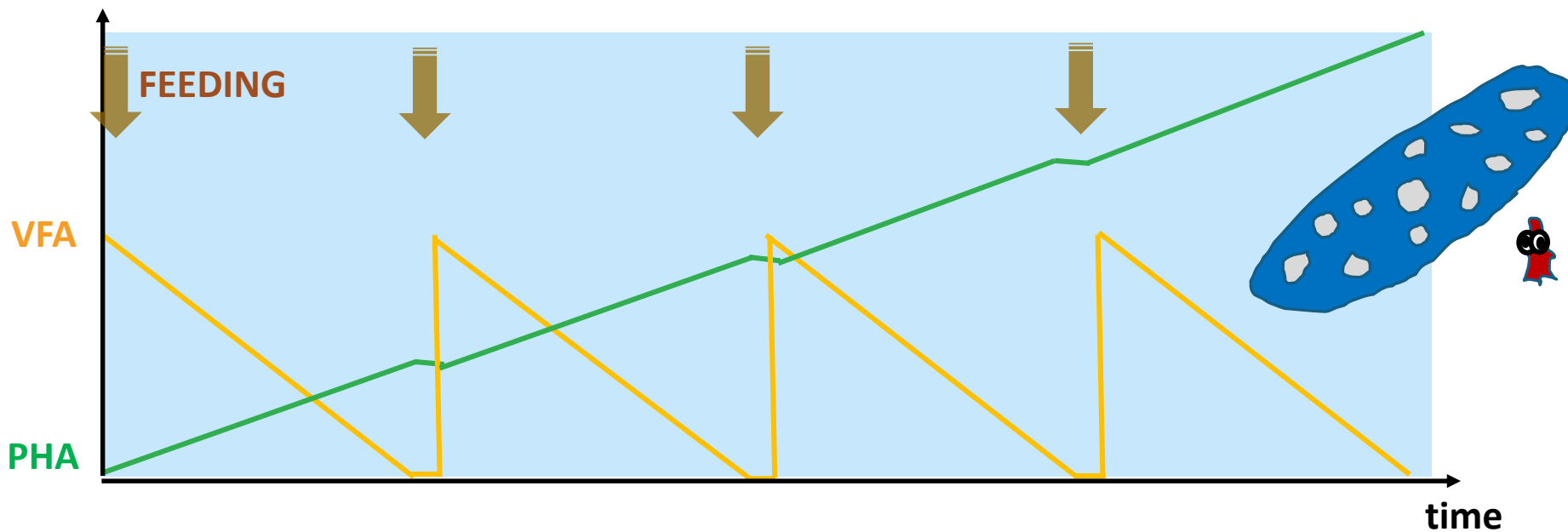
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# Introduction: PHA production from ROM

## 3) PHA ENRICHMENT

PHA storage in the biomass using feed on demand strategies

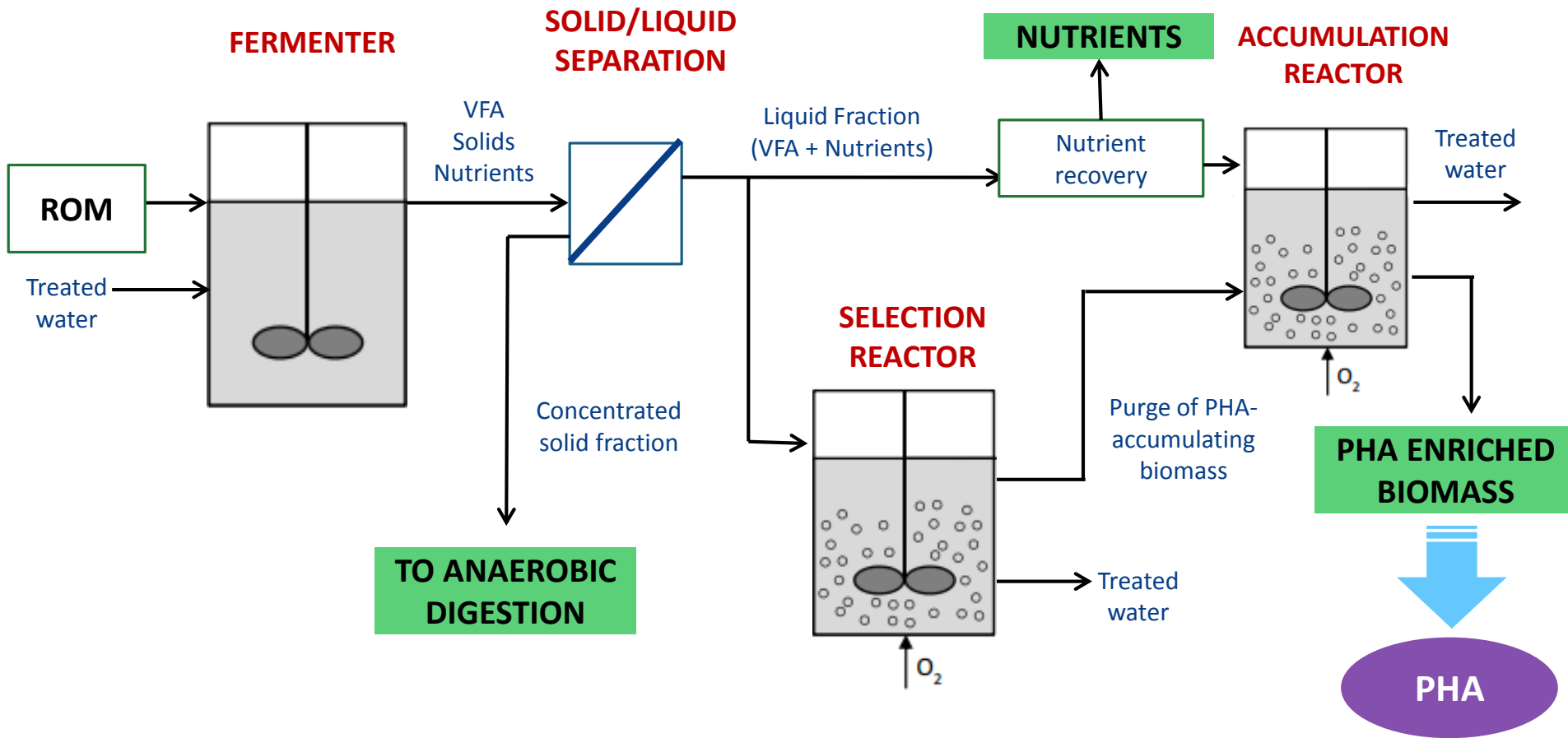


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# Introduction: PHA production from ROM

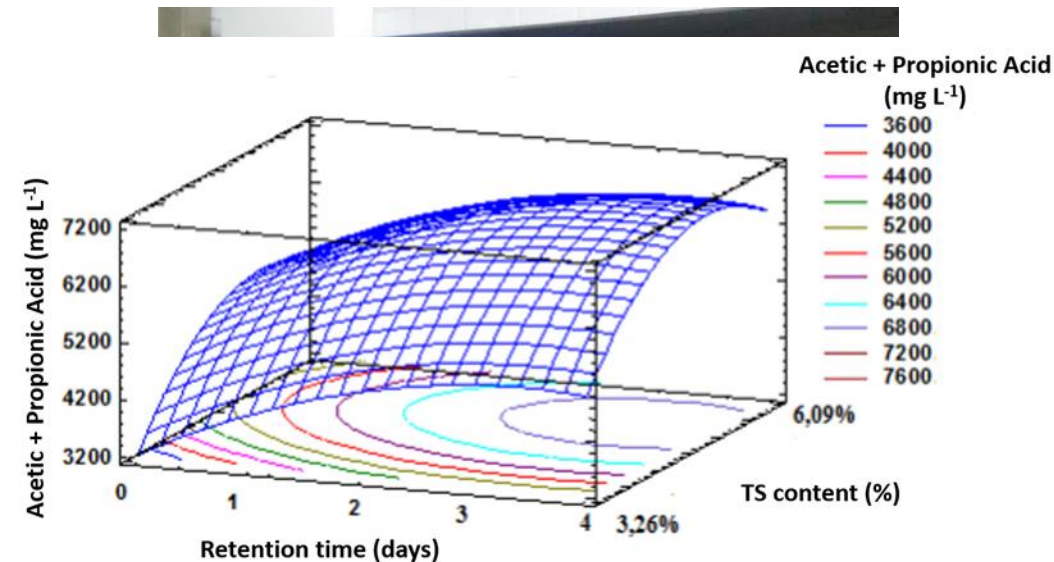


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# Results: Fermentation of ROM

## Batch tests with ROM



**Concentration of Total Solids (TS):**  
3.3 – 4.4 – 5.6 – 6.1 – 8.1 %

**Time of the tests:**  
5 d

**OPTIMUM CONDITIONS:**

**TS: 5.4%**

**Retention time: 3.4 d**

**T: 37 °C**



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# Results: Fermentation of ROM

## Continuous reactor

Volume: 4-5 L

Mechanical stirring

Temperature: 37 °C



Inoculated with anaerobic digester effluent

Initial HRT: 2.5 d  
(wash out of methanogens)

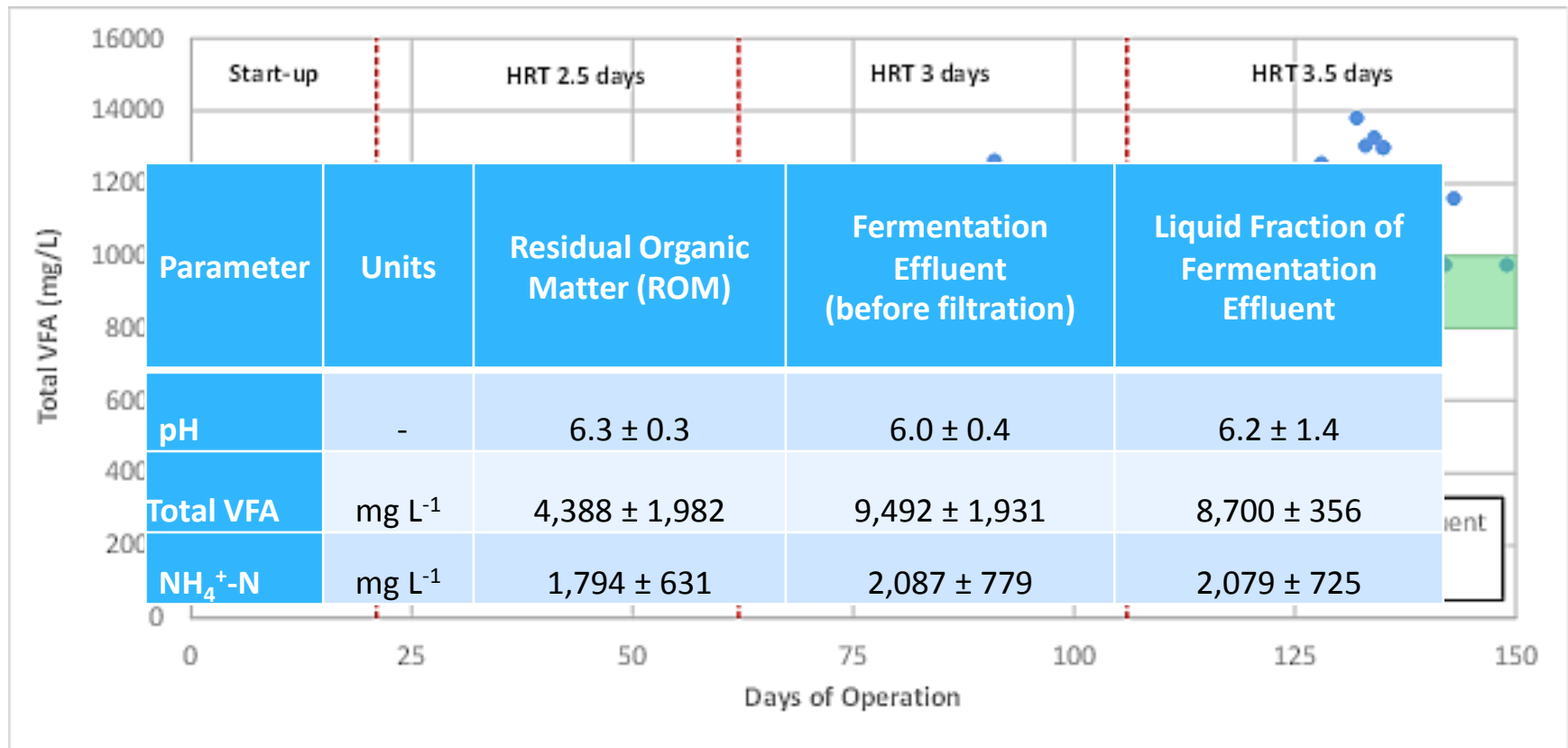


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# Results: Fermentation of ROM

## Fermenter of ROM



HRT 2.5-3.5 d

Temperature 37 °C



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# Results: Selection of PHA-accumulating biomass

## Selection reactor

- Volume 3L
- Mechanical stirring
- Air supply
- Ambient temperature

pH and DO online  
measurement

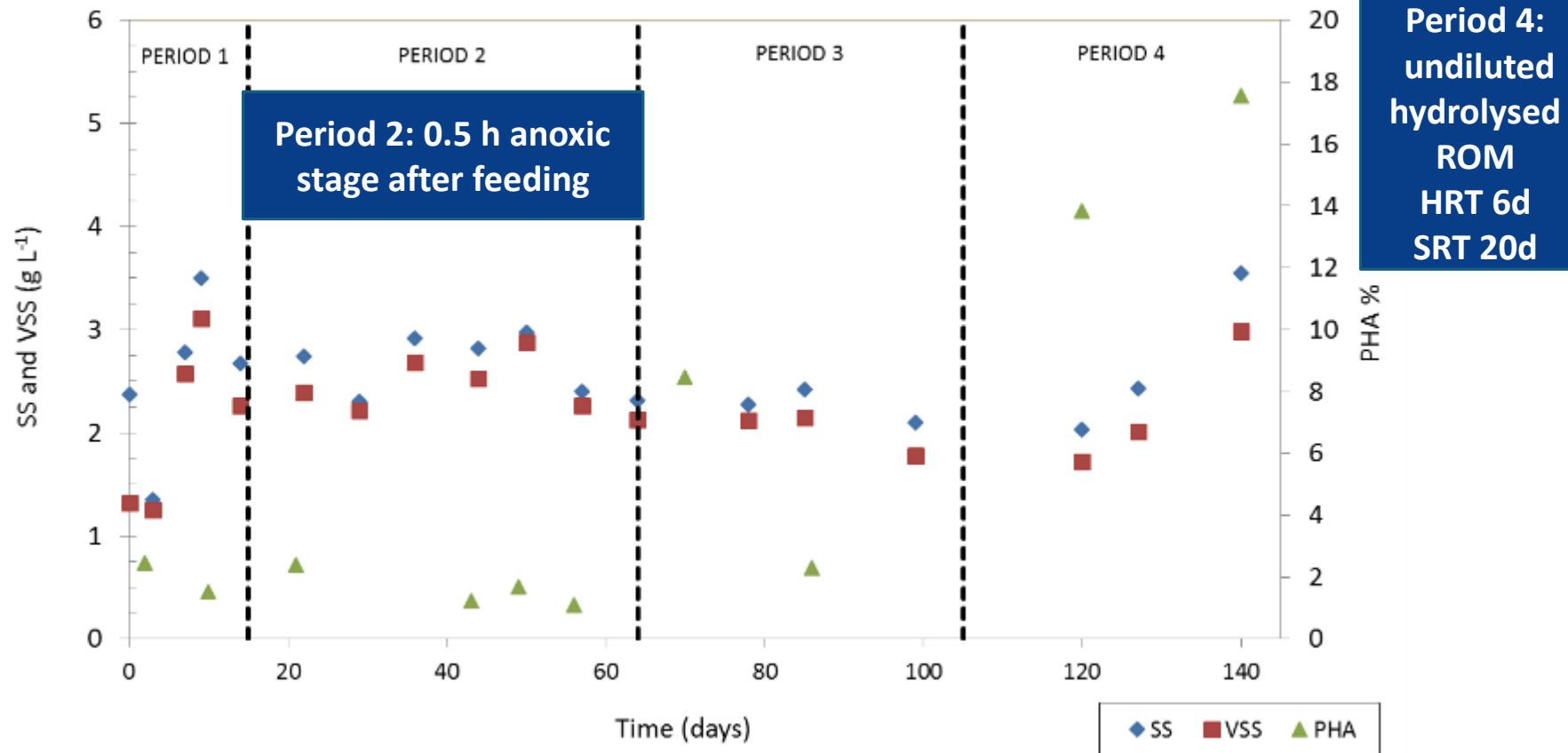
3 peristaltic pumps to  
control: HRT, SRT and  
feeding



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# Results: Selection of PHA-accumulating biomass



**Periods 1 to 3:**  
50% diluted hydrolysed ROM + acetic acid, 6 g VFA/L  
HRT 7.5d, SRT 17d  
Cycle: 7 h air, 0.5 h mixing, 0.5 h settling and effluent withdrawal

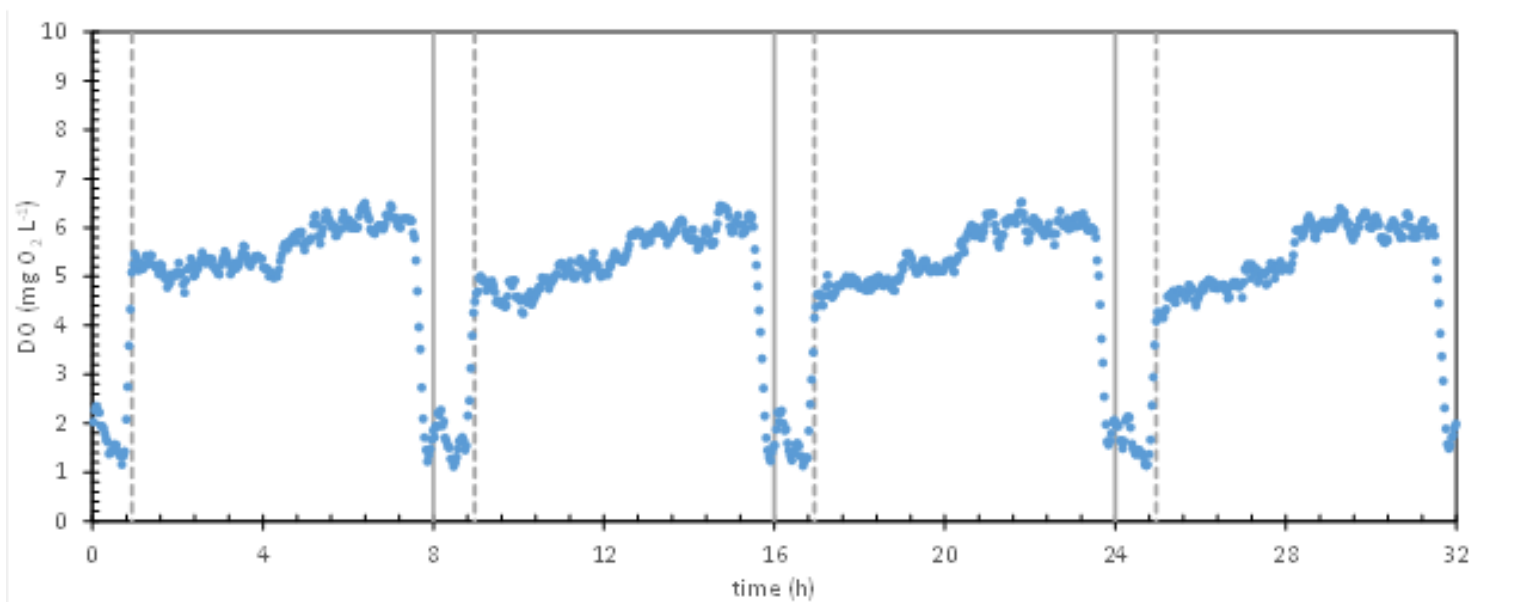
**Feast/Famine 0.15-0.21**  
VFA removal 99%



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# Results: Selection of PHA-accumulating biomass: Period 4, undiluted hydrolysed ROM



Parameter	Average Value	Range Value	Units
Cycle duration	8	-	h
Feeding (with mixing)	2	-	min
Aeration + mixing	432	-	min
Mixing (no aeration)	30	-	min
Settling	15	-	min
Effluent withdrawal	1	-	min
OLR	1.29	0.90-1.67	g VFA (L day) <sup>-1</sup>
% VFA removal	>99	-	%
HRT	6	-	days
SRT	20	-	days
TSS	3.02	2.03-3.54	g SS L <sup>-1</sup>
VSS	2.47	1.72-2.99	g VSS L <sup>-1</sup>
Feast/Famine time ratio	0.15	0.14-0.15	-
% PHA in the purged biomass	15.7	13.8-17.6	% (on VSS basis)

Cycle 8h

OLR  
1.3 kg VFA/(m<sup>3</sup> d)

Feast/Famine 0.15



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# Results: PHA accumulation



## Batch tests

Volume of the reactor: 0.7L

Inoculum: 100 mL biomass purged from the selection reactor per batch (collected mainly during periods 2, 3)

Fermentation Liquid Concentration	10%	33%	100%	
Total VFA	6050 ±705	5839 ±1345	5722 ±1512	mg L <sup>-1</sup>
Acetic	99.2	64.6	32.0	% of Total
Propionic	0.2	14.8	39.1	
Isobutyric	0.0	5.3	11.4	
Butyric	0.2	5.3	13.2	
NH <sub>4</sub> <sup>+</sup> -N	6.9 ±4	725.3*	2,198 ±716	mg N L <sup>-1</sup>
N/COD	0.46	49.8	114.3	mg g <sup>-1</sup>
pH	5.7 ±0.3	5.7 ±0.5	6.2 ±1.4	-
*Calculated based on NH <sub>4</sub> <sup>+</sup> -N concentration in 100% fermentation liquid				



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# Results: PHA accumulation

- Feeding each 4 hours.
- Total time of each batch: 24 h
- PHA yield decreased with the increased concentration of fermentation liquid:  $\text{NH}_4^+$

Highest PHA yield = **38% (VSS basis)**

Fermentation Liquid Concentration		10%	33%	100%		
OLR		1.9 ±0.35	1.86 ±0.56	1.86 ±0.58	kg VFA (m <sup>3</sup> day) <sup>-1</sup>	
Initial F:M		0.63 ±0.18	0.98 ±0.11	0.93 ±0.16	g VFA g <sup>-1</sup> TSS	
VFA removal		58 ±25	50	44 ±13.24	%	
TSS					g L <sup>-1</sup>	
	Initial	0.74 ±0.29	0.46 ±0.10	0.49 ±0.09		
	Final	1.29 ±0.62	1.15 ±0.13	1.54 ±0.09		
VSS					g L <sup>-1</sup>	
	Initial	0.62 ±0.30	0.42 ±0.07	0.47 ±0.09		
Final		1.18 ±0.68	1.07 ±0.16	1.42 ±0.08		
PHA					% (on VSS basis)	
	Initial	2.3 ±0.2	7.5 ±9.0	6.2 ±3.9		
Final		37.5 ±6.3	27.1 ±5.8	18.8 ±5.8		



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# Conclusions

- \* The production of bioplastics from municipal organic waste can shift the management paradigm towards a more circular economy.
- \* About 8-10 g VFA/L have been obtained from the **fermentation** fermentation of ROM under the following conditions 5.4% solids, 37 °C, and 3.4 day HRT.
- \* The **selection reactor** was operated at a feast/famine ratio of 0.15, SRT of 20 days, and HRT of 6 days, achieving PHA-accumulating enriched biomass (up to 18% PHA on VSS basis).
- \* The **accumulation reactor** achieved a maximum PHA content of 38% (on VSS basis).
- \* Using the liquid fraction of fermented ROM as substrate in the PHA-accumulation phase resulted in reduced PHA production likely due to inhibition from high ammonia concentrations.
- \* ROM has been demonstrated as a feasible substrate for PHA production. Further process optimization and incorporation of nutrient recovery should be investigated.



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Polyhydroxyalkanoates production using  
the liquid fraction of hydrolysed  
municipal organic waste

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