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Evaluation of the optimal sewage sludge pre-treatment technology through continuous reactor operation

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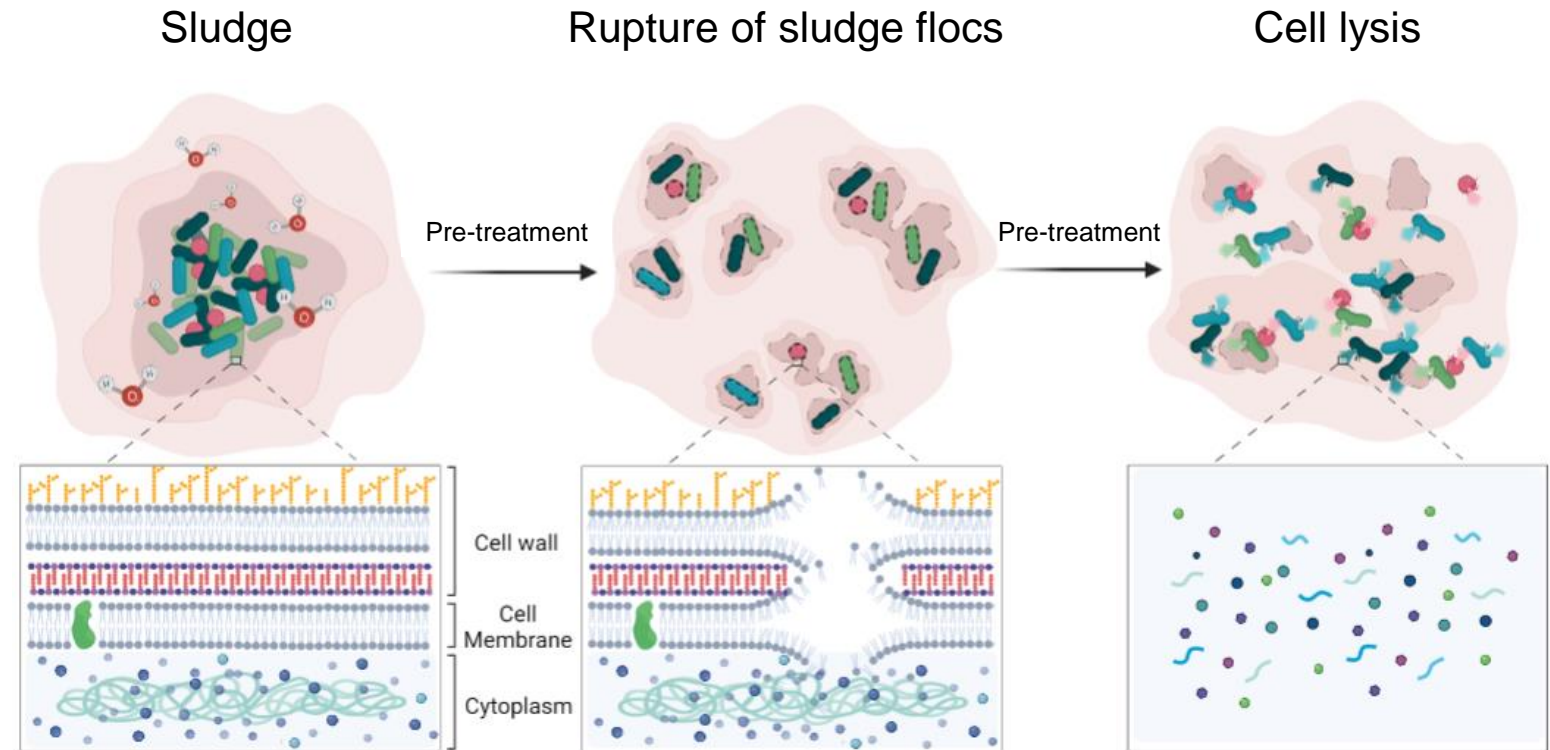
Why is sludge's pre-treatment important?

Pre-treatment's beneficial aspects

- Rupture of sludge flocs
 - Particle size reduction
 - Increased surface area
 - Enhanced hydrolysis
- Rupture of EPS and cell walls
 - Release of intracellular organic substances
 - Increased bioavailability



- ✓ Enhanced Anaerobic Digestion efficiency
- ✓ Increased Biogas Production



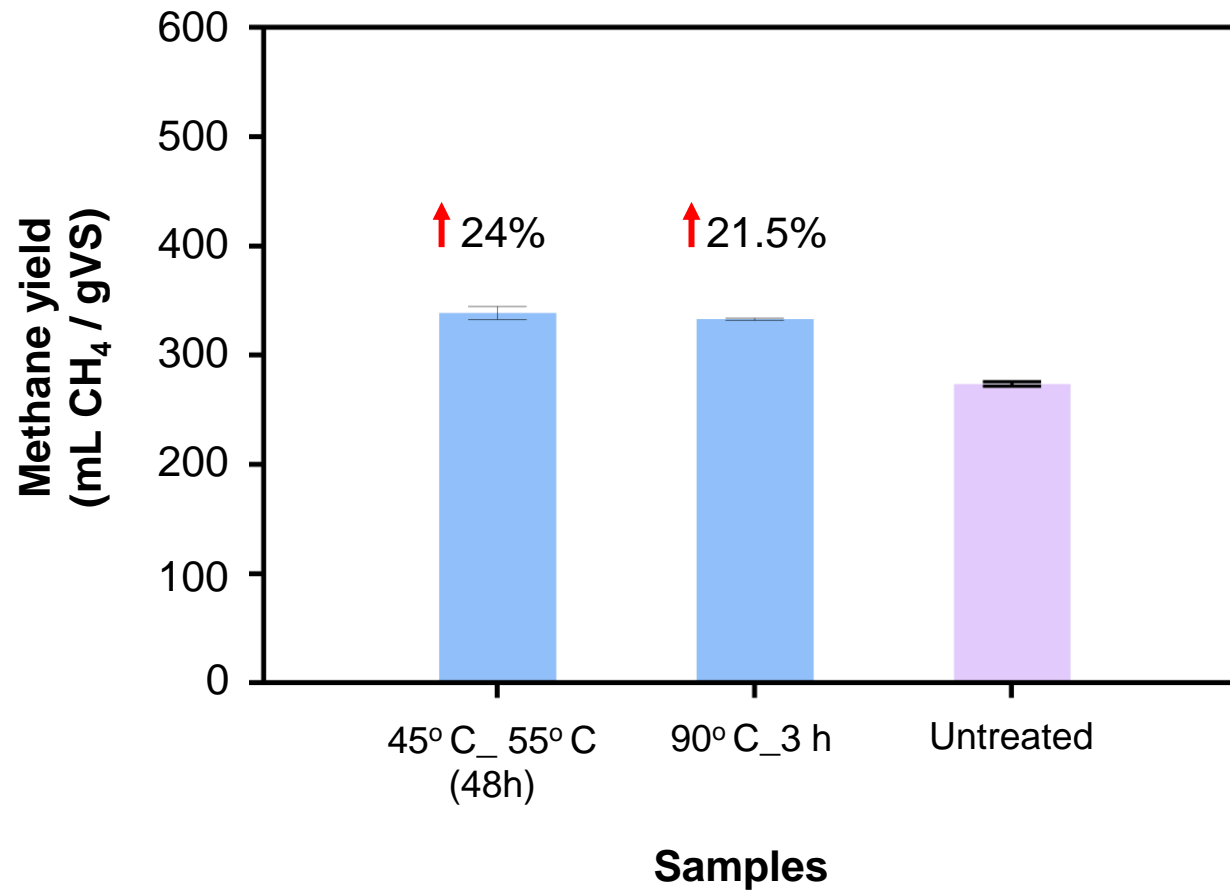
Evaluation of different pre-treatments



Thermal Hydrolysis	
1	45° C for 48 h and then 55° C for extra 48 h
2	45° C for 72 h and then 55° C for extra 72 h
3	45° C for 72 h and then 55° C for extra 72 h under 0.5 bar of CO ₂ pressure
4	45° C for 72 h and then 55° C for extra 72 h under 1.0 bar of CO ₂ pressure
5	90° C for 3 h
6	90° C for 3 h under 0.5 bar of CO ₂ pressure
7	90° C for 3 h under 1 bar of CO ₂ pressure
Alkaline Hydrolysis	
8	2% v/v NaOH
9	4% v/v NaOH
Thermochemical pre-treatments	
10	4% v/v NaOH, 45° C for 72 h and then 55° C for extra 72 h
11	4% v/v NaOH, 45° C for 72 h and then 55° C for extra 72 h, under 0.5 bar of CO ₂ pressure
12	4% v/v NaOH, 45° C for 72 h and then 55° C for extra 72 h, under 1 bar of CO ₂ pressure
13	4% v/v NaOH and 90° C for 3 h, under 0.5 bar of CO ₂ pressure
14	4% v/v NaOH and 90° C for 3 h, under 1 bar of CO ₂ pressure

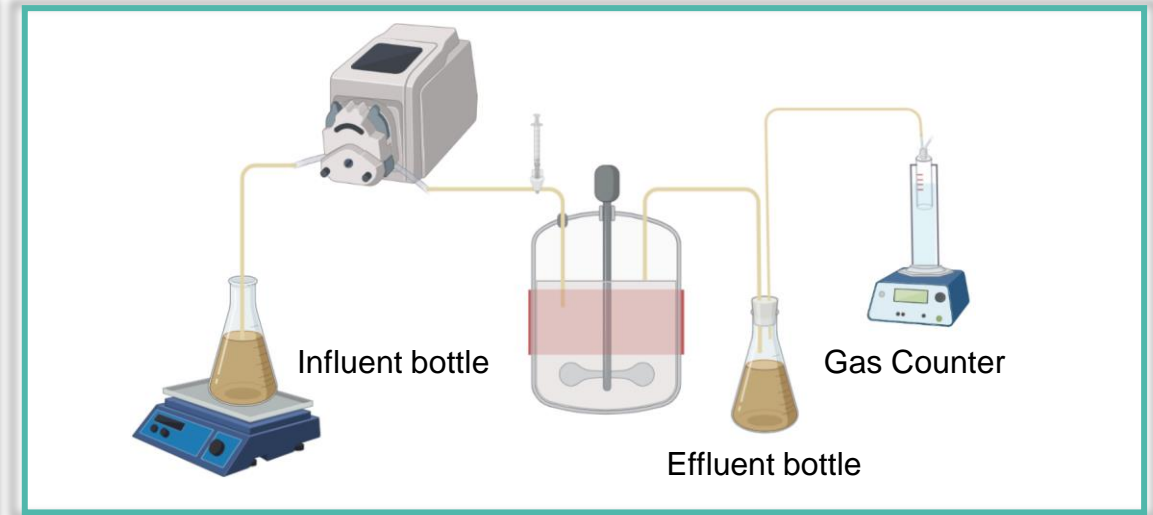
Aim & Objectives

Optimum pre-treatments



Further evaluation
through continuous reactor operation

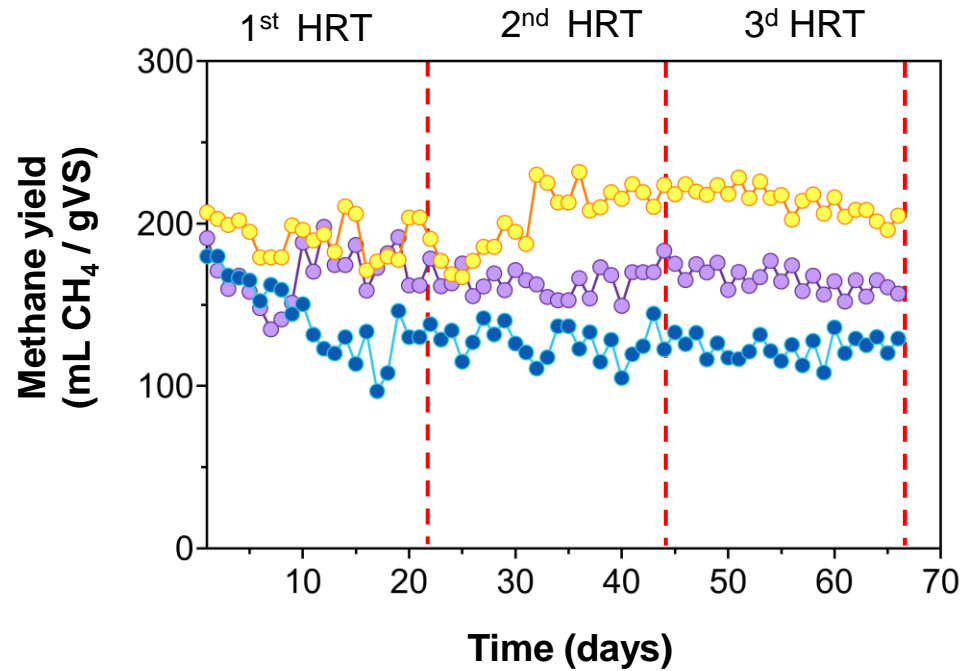
Experimental set-up



- 3 CSTRs
 - Reactor 1: **Un**-treated sludge
 - Reactor 2: **Pre**-treated sludge (45°C for 48 h & 55°C for 48 h)
 - Reactor 3: **Pre**-treated sludge (90°C for 3 h)
- Mesophilic Conditions ($37 \pm 2^\circ \text{C}$)
- 3 periods \rightarrow Increasing Organic Loading Rate (OLR)
- Biochemical parameters x2/week
- DNA extraction (Period 1 and Period 2) \rightarrow Effect of sludge thickening on microbial community

1st Experimental Period

- Hydraulic Retention Time: HRT = 22 days
- OLR = 1 (g VS/(LReactor*day))

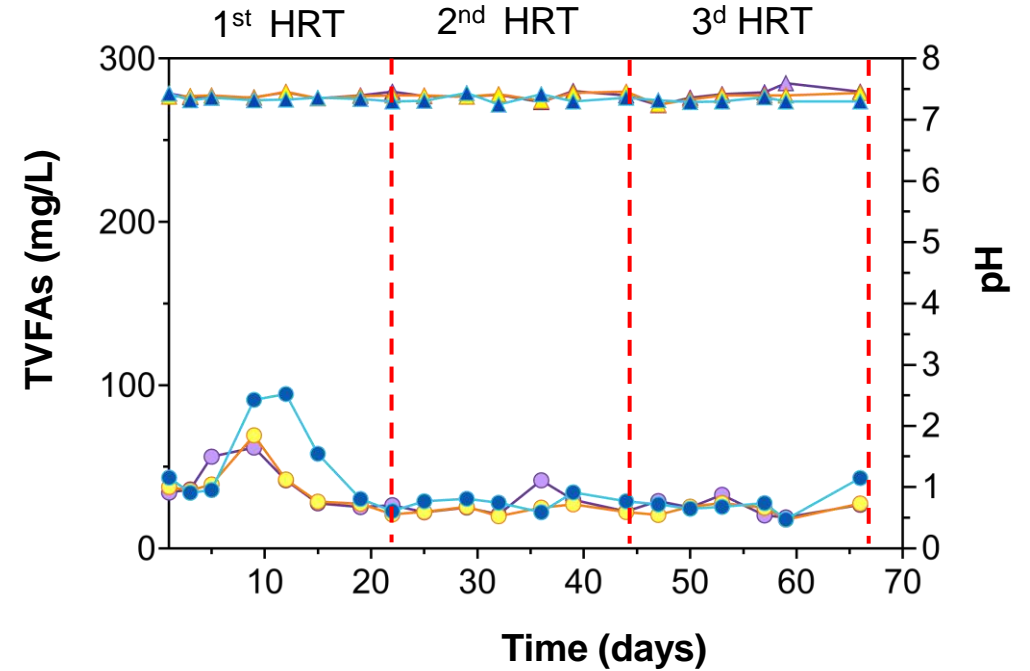


Reactor 1 Reactor 2 Reactor 3

126 ± 9.69
mL CH₄/g VS

207 ± 6.78
mL CH₄/g VS

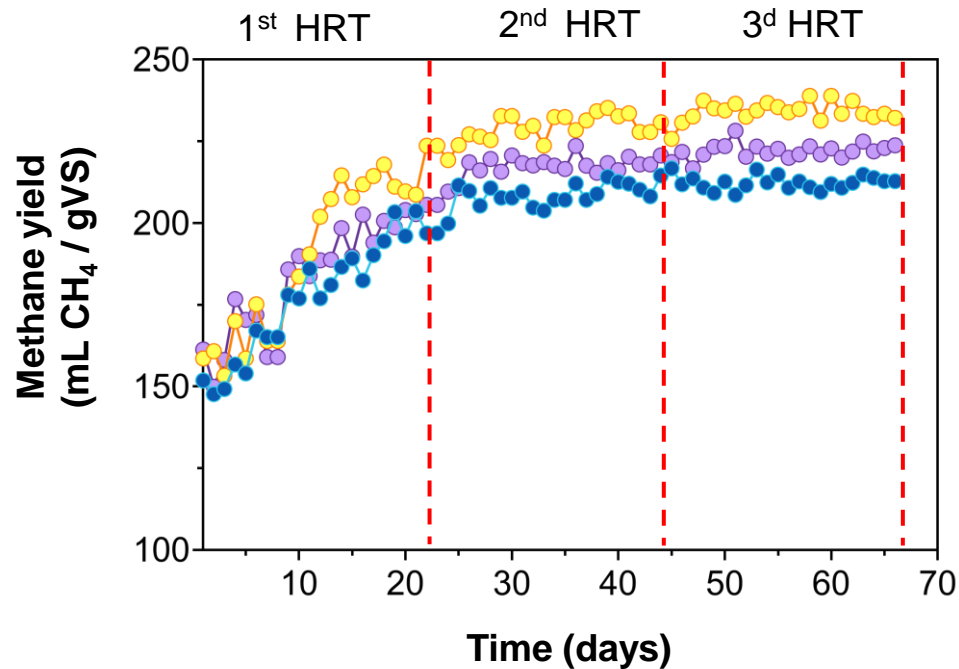
161 ± 7.47
mL CH₄/g VS



T-VFA Reactor 1 T-VFA Reactor 2 T-VFA Reactor 3
pH Reactor 1 pH Reactor 2 pH Reactor 3

2nd Experimental Period

- Feeding: **Thickened** Sludge
- Hydraulic Retention Time: HRT = 22 days
- OLR = 2 (g VS/(L_{Reactor} * day))

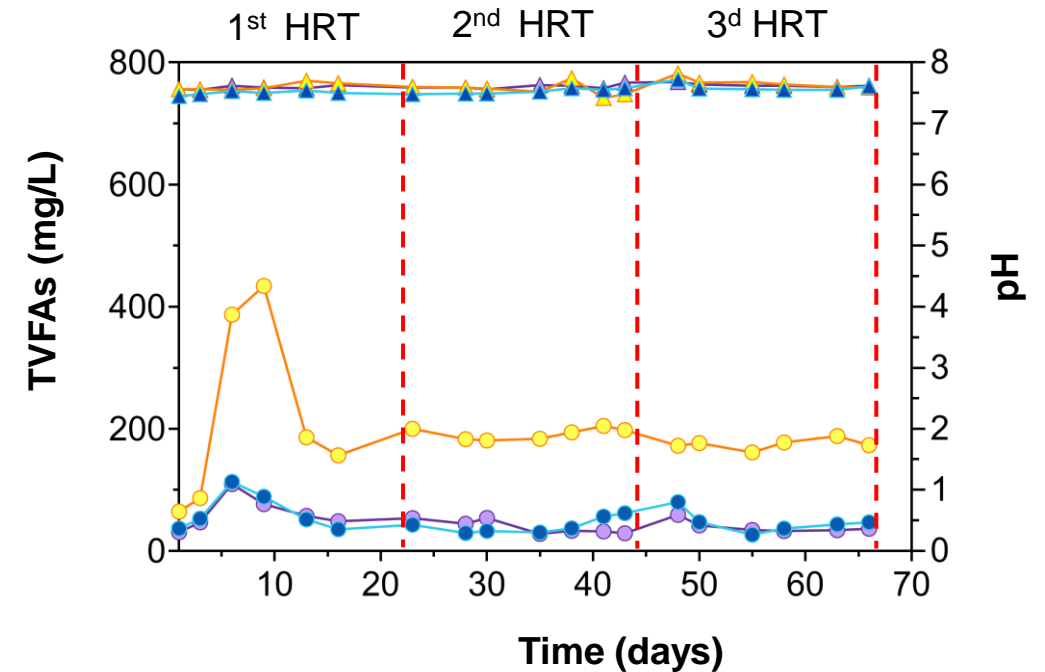


Reactor 1 Reactor 2 Reactor 3

212 ± 1.47
mL CH₄/g VS

234 ± 2.52
mL CH₄/g VS

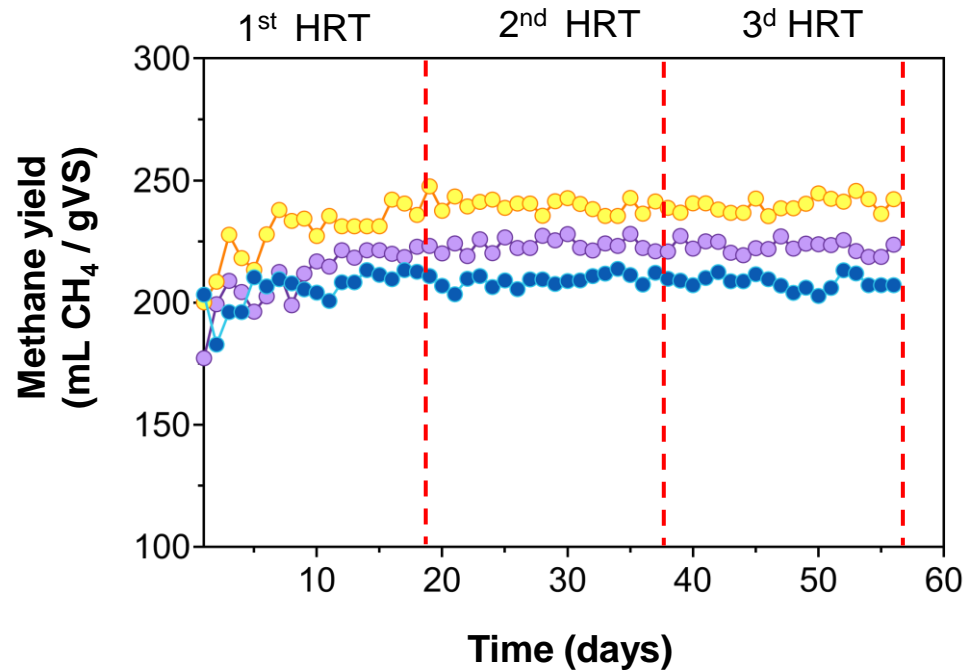
222 ± 1.52
mL CH₄/g VS



T-VFA Reactor 1 T-VFA Reactor 2 T-VFA Reactor 3
pH Reactor 1 pH Reactor 2 pH Reactor 3

3rd Experimental Period

- Feeding: **Thickened** Sludge
- Hydraulic Retention Time: HRT = 18 days
- OLR = 2.5 (g VS/(L_{Reactor} * day))

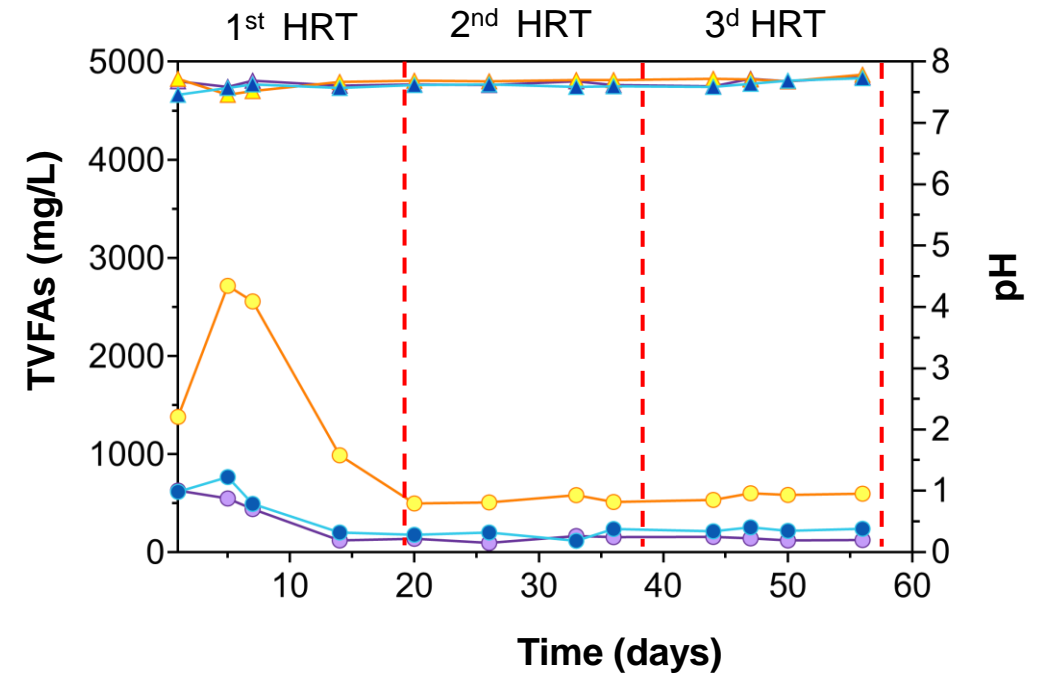


● Reactor 1 ● Reactor 2 ● Reactor 3

207 ± 2.96
mL CH₄/g VS

240 ± 3.09
mL CH₄/g VS

224 ± 2.49
mL CH₄/g VS



● T-VFA Reactor 1 ● T-VFA Reactor 2 ● T-VFA Reactor 3
▲ pH Reactor 1 ▲ pH Reactor 2 ▲ pH Reactor 3

Microbial composition changes

Most abundant

Synergistota:

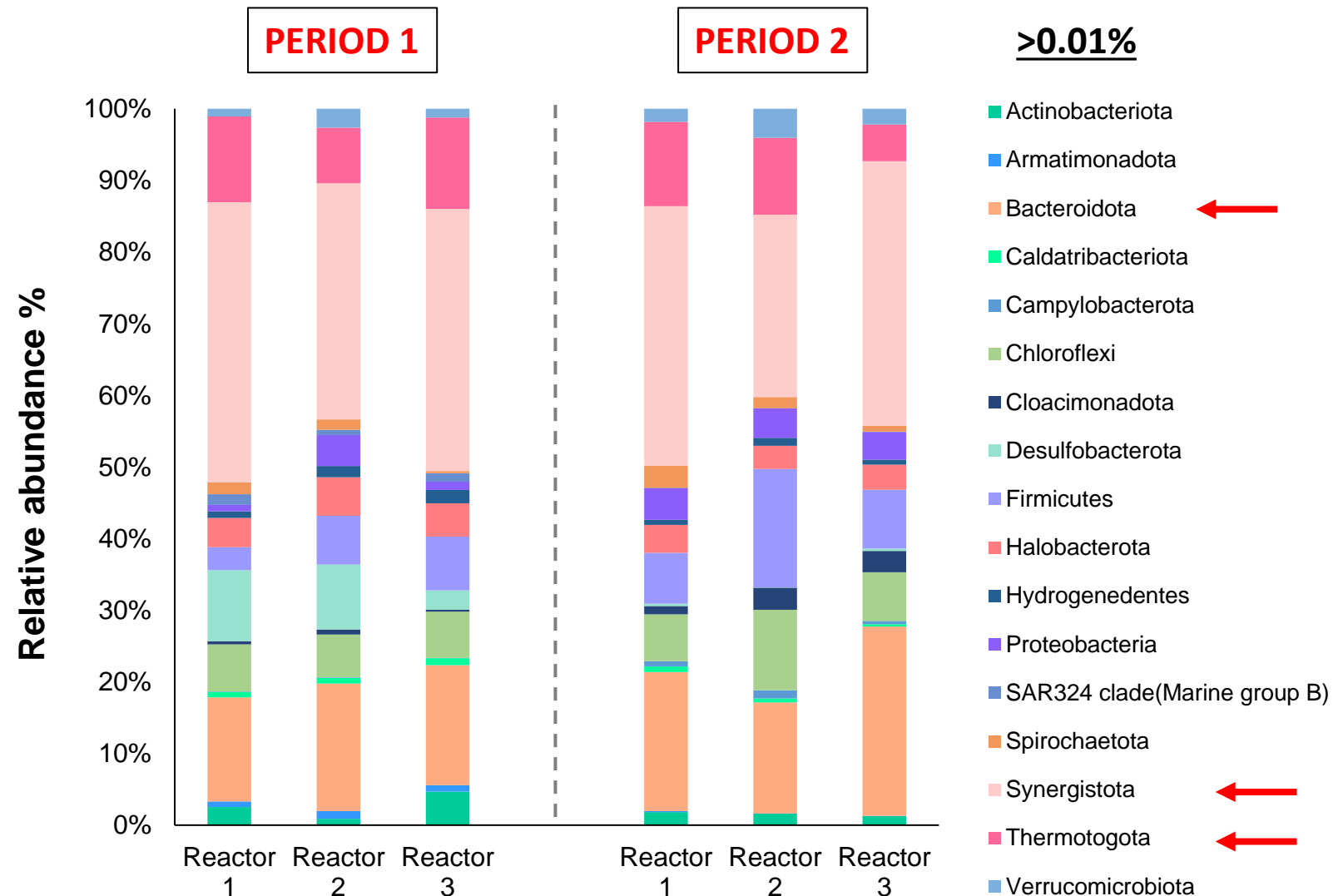
- ❖ *Thermovirga* sp.: can degrade amino acids, but also reported to participate in the direct interspecies electron transfer (DIET) of microorganisms.

Thermotogota:

- ❖ *Mesotoga* sp.: linked to the syntrophic acetate oxidation.

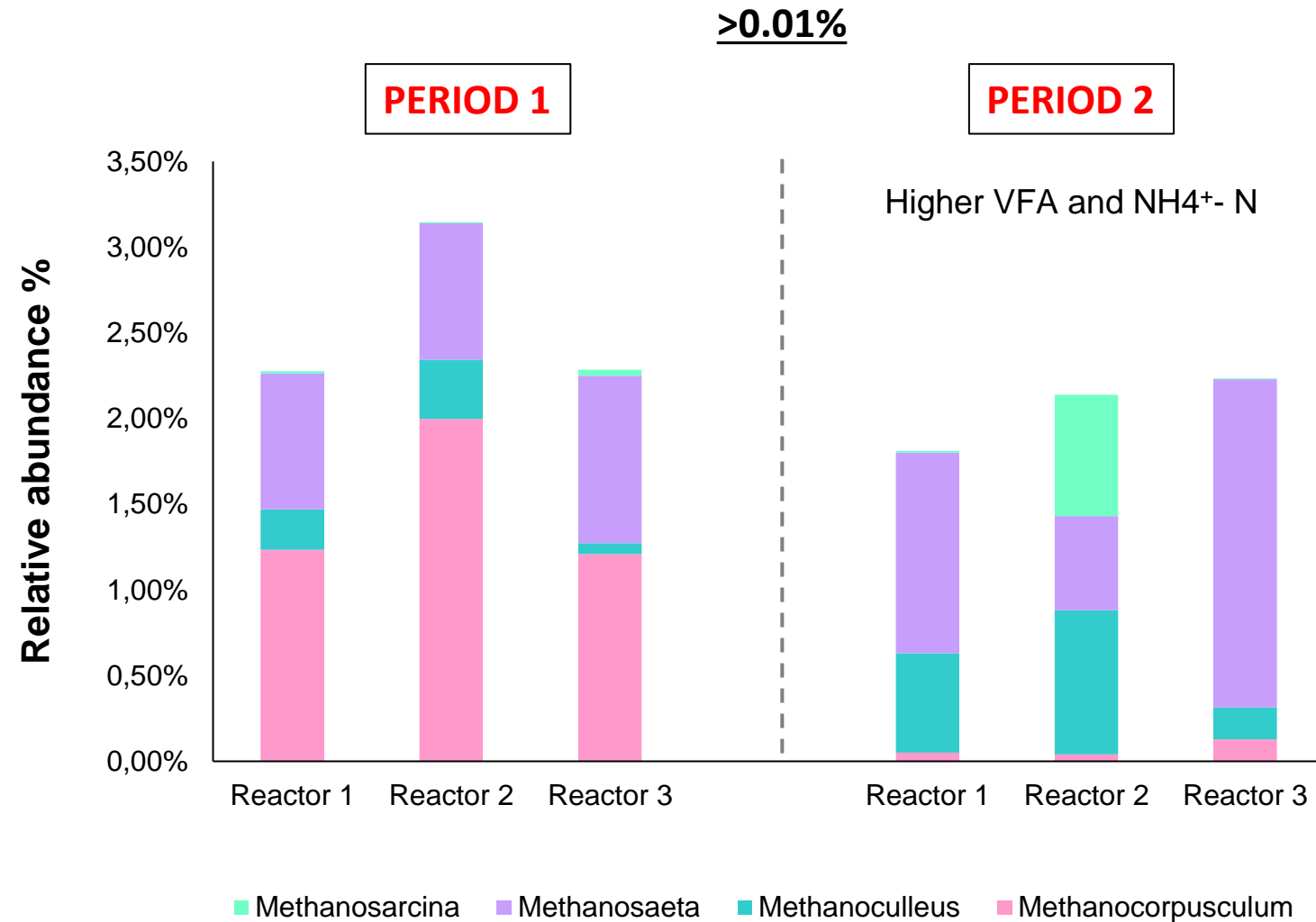
Bacteroidota:

- ❖ *DMER64* sp.: anaerobic mesophilic acetogens usually present in various of digester systems and reported to involve in interspecies hydrogen transfer (IHT) as main H₂ carriers.



Methanogenic composition changes

- ❖ ***Methanocorpusculum*** sp.: hydrogenotrophic methanogens that can be negatively affected by acetate, propionate and total ammonium nitrogen (TAN).
- ❖ ***Methanoculleus*** sp.: Hydrogenotrophic methanogens with positive correlation to TAN.
- ❖ ***Methanosarcina*** sp.: metabolically versatile microorganisms and vulnerable to process inhibitors such as free ammonia and VFAs.
- ❖ ***Methanosaeta*** sp: core genus involved in aceticlastic pathway.



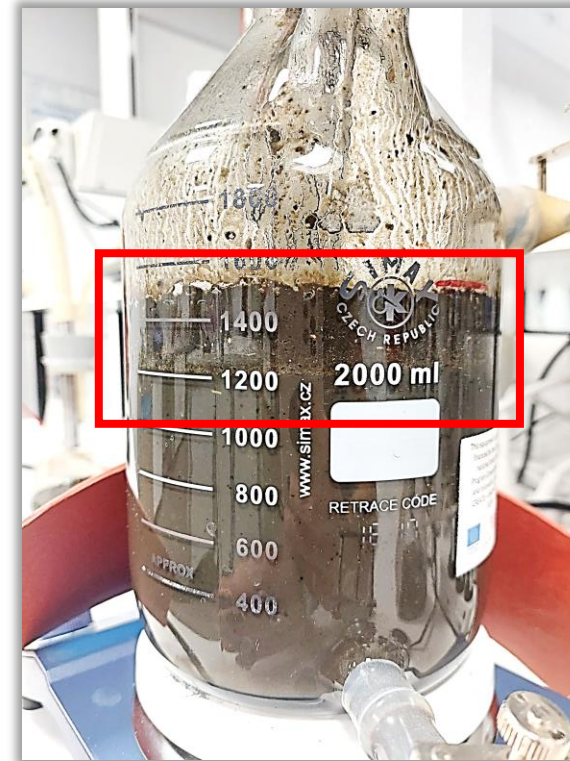
PERIOD 2

1. Foam sampling from **each** effluent bottle
2. DNA extraction & 16S rRNA amplicon sequencing

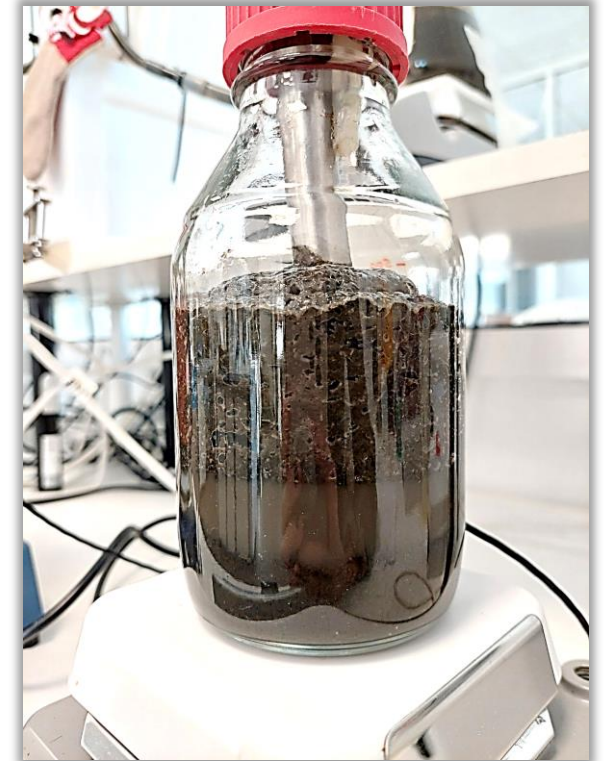
> 0.5% abundance in all the samples

Corynebacteriales order:

- ❖ *Corynebacterium* sp.
- ❖ *Mycobacterium* sp.
- ❖ *Gordonia* sp.



Reactor



Effluent bottle

- ✓ The reactor fed with sludge pretreated at **45° C for 48h and then at 55°C for additional 48h** led to the **highest performance** in terms of methane yield, during all the experimental periods examined.
- ✓ A further increase in organic loading rate up to $2.5 \text{ gVS L}_{\text{Reactor}}^{-1}\text{d}^{-1}$ **did not** lead to higher efficiencies in terms of methane yield.
- ✓ Sludge **thickening altered** the bacterial and archaeal communities of the reactors during the second experimental period, as a response to the incremental change in TS content and unfavorable environment (i.e. VFA stress).
- ✓ **Foam forming filamentous bacteria** were detected in all the samples obtained from effluent bottles during period 2 and OLR equal to $2 \text{ gVS L}_{\text{Reactor}}^{-1}\text{d}^{-1}$.



- ✓ Total volume approx. 800 L
- ✓ Working volume approx. 600 L
- ✓ Mesophilic conditions
- ✓ Two-stage thermal pre-treatment

Acknowledgement



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Thank you for your attention!



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