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RECYCLES WORKSHOP

Metagenomics and
metabarcoding approaches to
describe ecological systems
and infer their development

5th, 6th & 7th of July 2022

**Microbial diversity monitoring in an
anaerobic digester treating tannery fleshing**

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GA: 872053 — H2020 - MSCA - RISE-2019



European
Commission



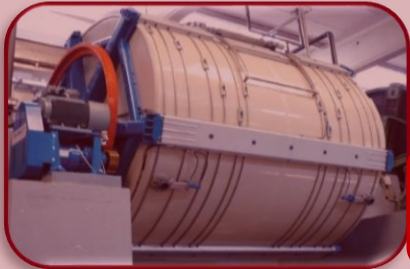
1. Context: Tannery Industry



Leather tanning process



Raw Hides



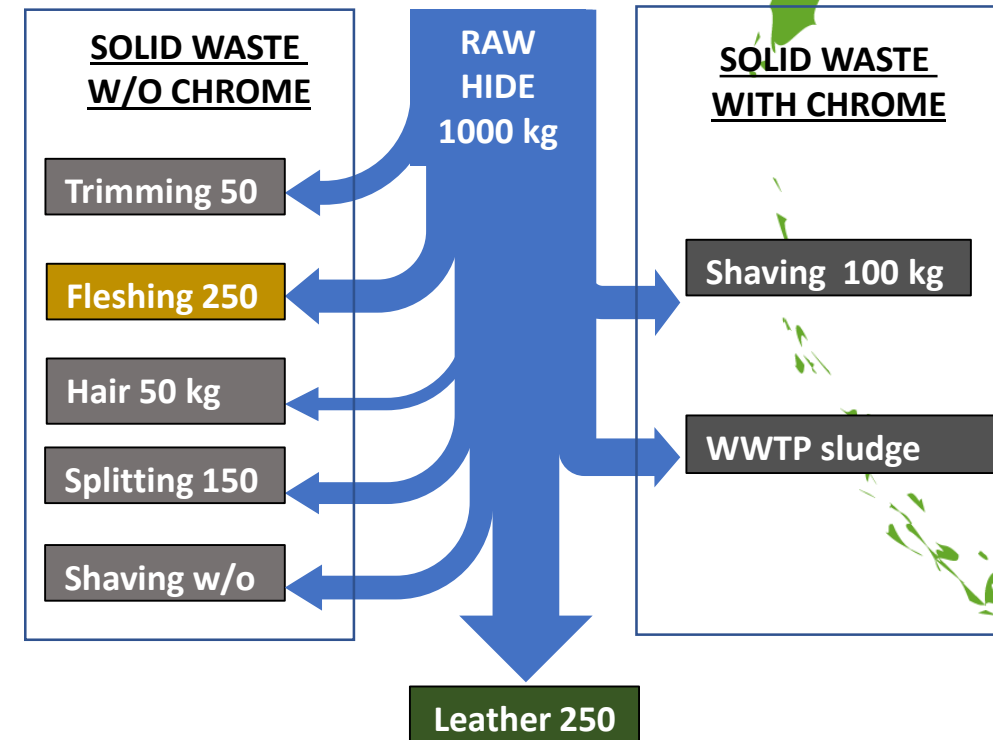
Tannery industry



Finished product

Tanning process transform putrescible raw hides in leather through chemical and mechanical processes

ONLY 25% of the weight of the raw hide is transformed in leather.



2. Fleshing characteristics



SOURCE: chrome-free solid residues which are mechanically removed from the hides after unhairing treatment

ASPECT: greasy tissue , bad smelling, not homogeneous, slimy

CLASSIFICATION: ABP Cat.3 (Animal By-Product)

Wetlimed fleshing characteristics

Water	%	67-72%
Dry matter (TS)	%	28-33%
Ashes	%	8,5-11,5%
Organic matter	%	18,5-21%
VS/TS	%	60-65%
pH	-	11,5 - 13
COD	g/kg	300-320
COD	kg/kgVS	1,5
C/N ratio		3-5
S	g/kgDM	2-4



Fibres	%	0,0%
Fats	%	6,0-6,5%
Proteins	%	11,5-12,5%
Carbohydrates	%	1-2%

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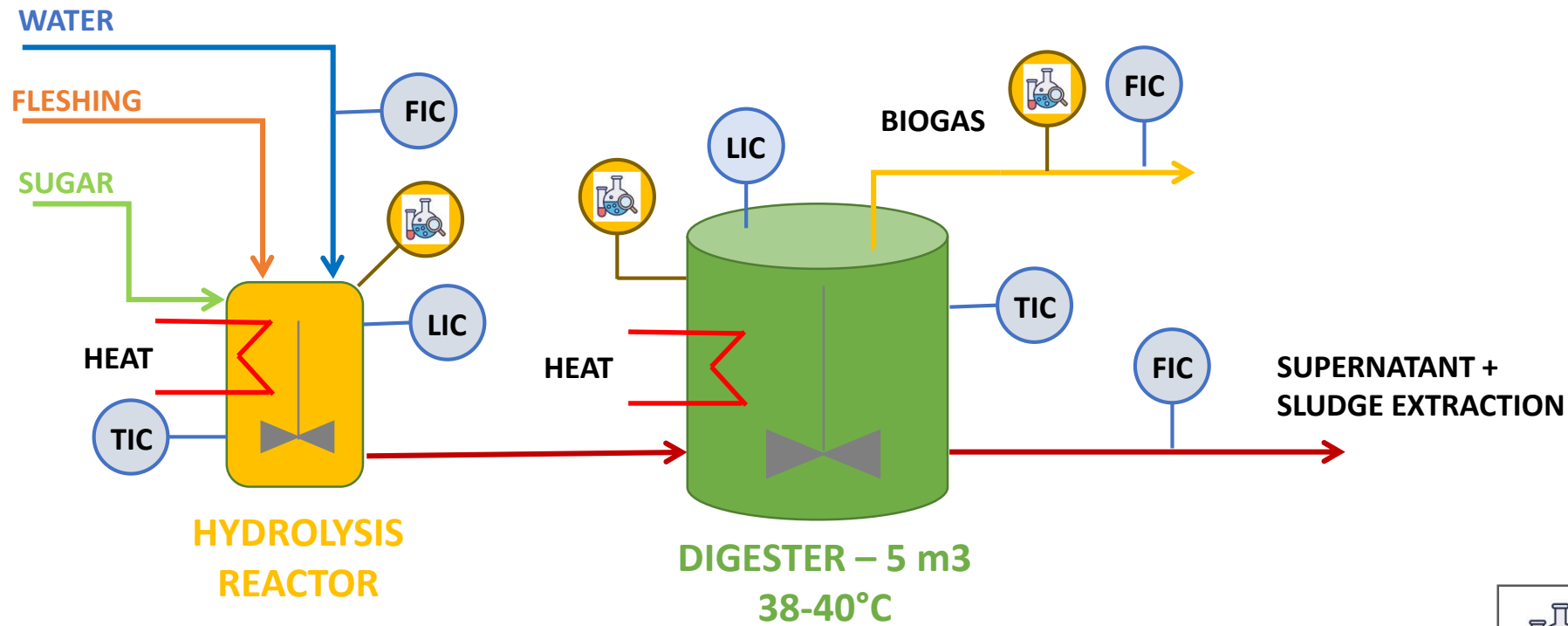
High fats concentration, so high BMP



INHIBITION
CO-INHIBITION

Low C/N ratio
High Organic Nitrogen (TKN)
High salinity
High Sulphide concentration
LCFA (from lipid hydrolysis)

3. Pilot plant configuration



	Chemical Analysis point
TIC	Temperature transmitter
LIC:	Level transmitter
FIC	Flow transmitter

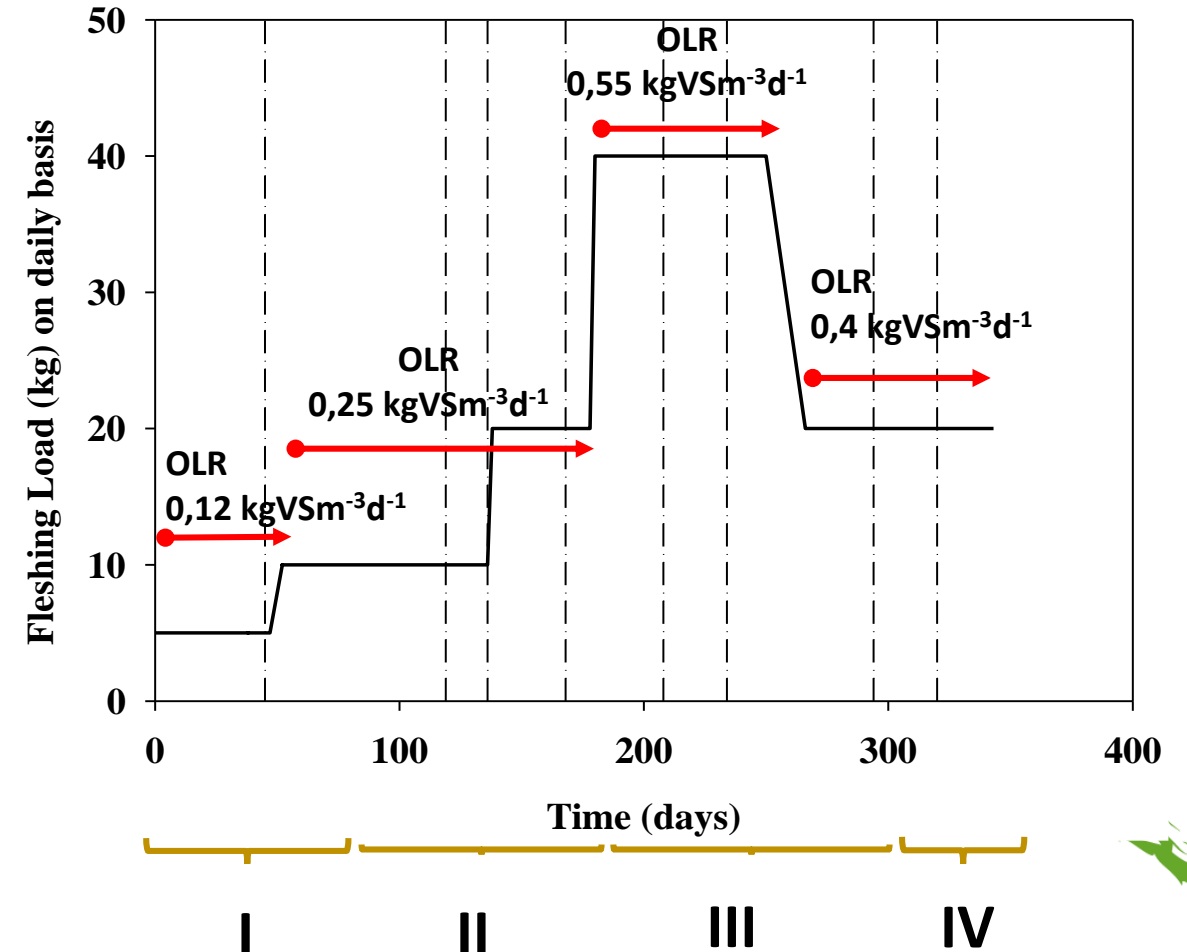
4. Pilot plant configuration



Type	CSTR (Single stage AD)
Temperature	38-42 °C (Mesophilic)
Useful volume	5 m3
Mixing	By vertical mixer (1,5 kW VFD)
Biomass concentration	Approx 5-7 g/l as VS
HRT	Range 50-100 d

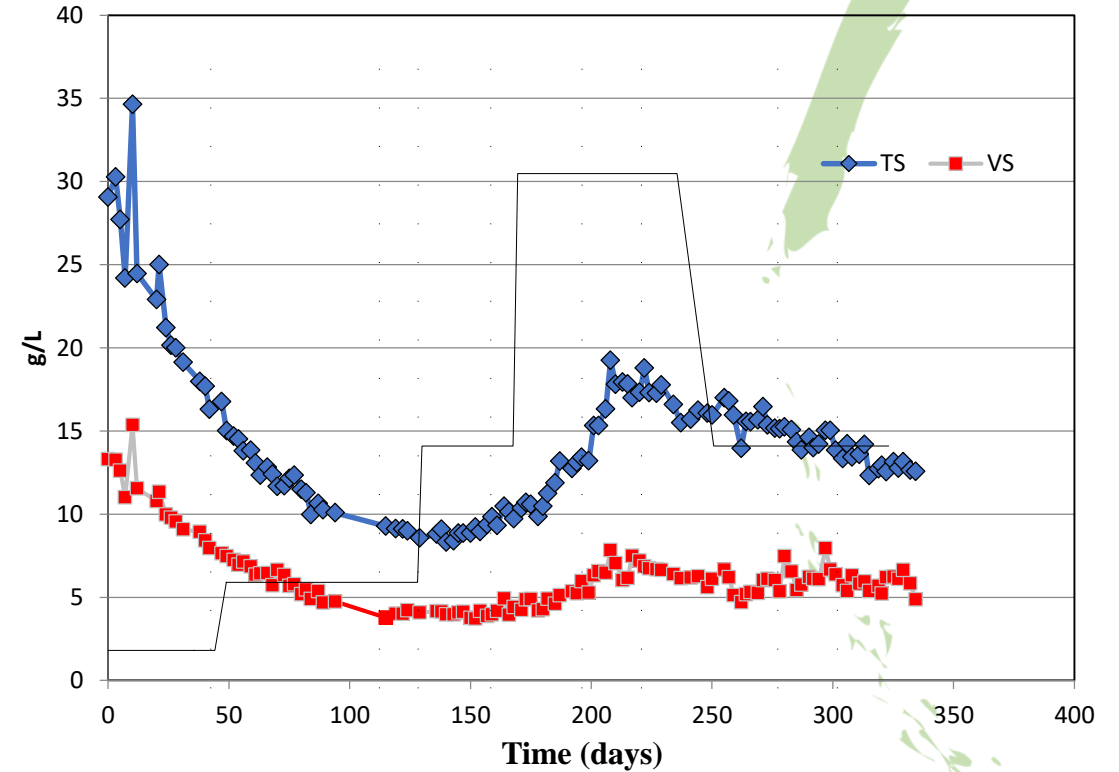
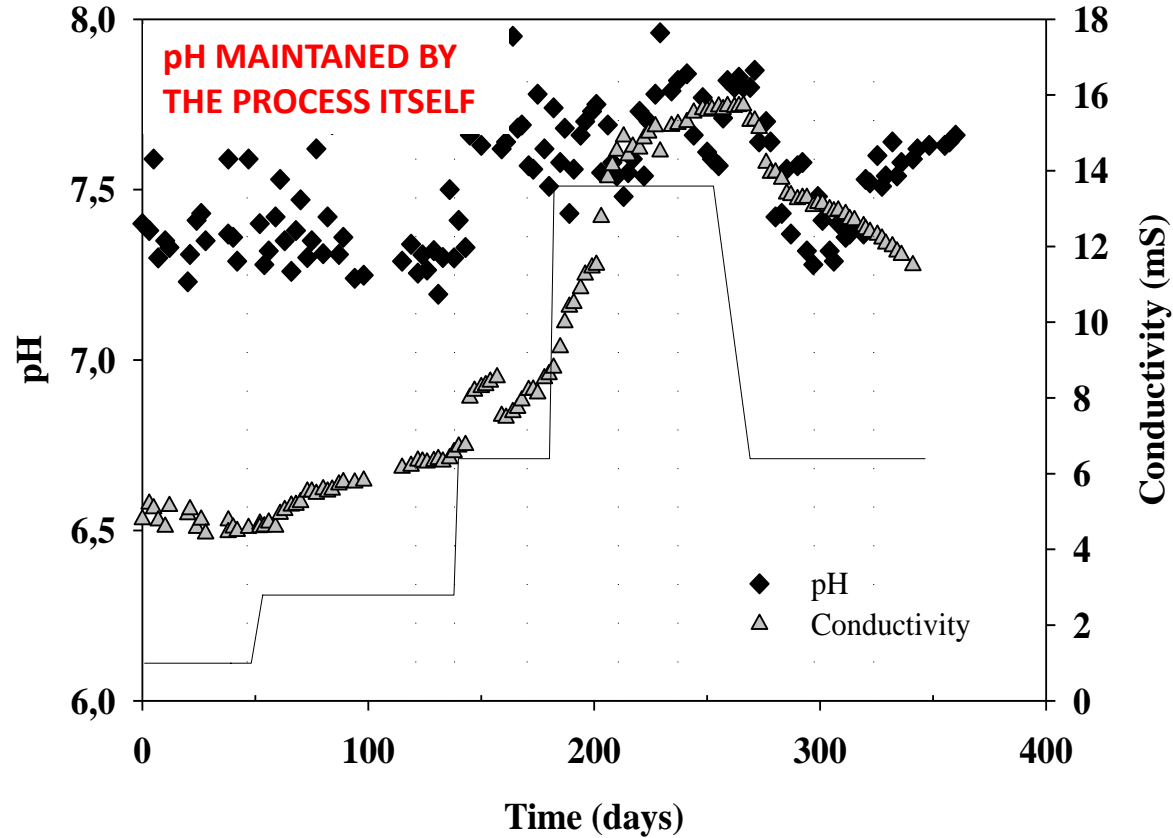
WP 5. – 20/012021

DIGESTER FEEDING TREND (semi-continuous mode)



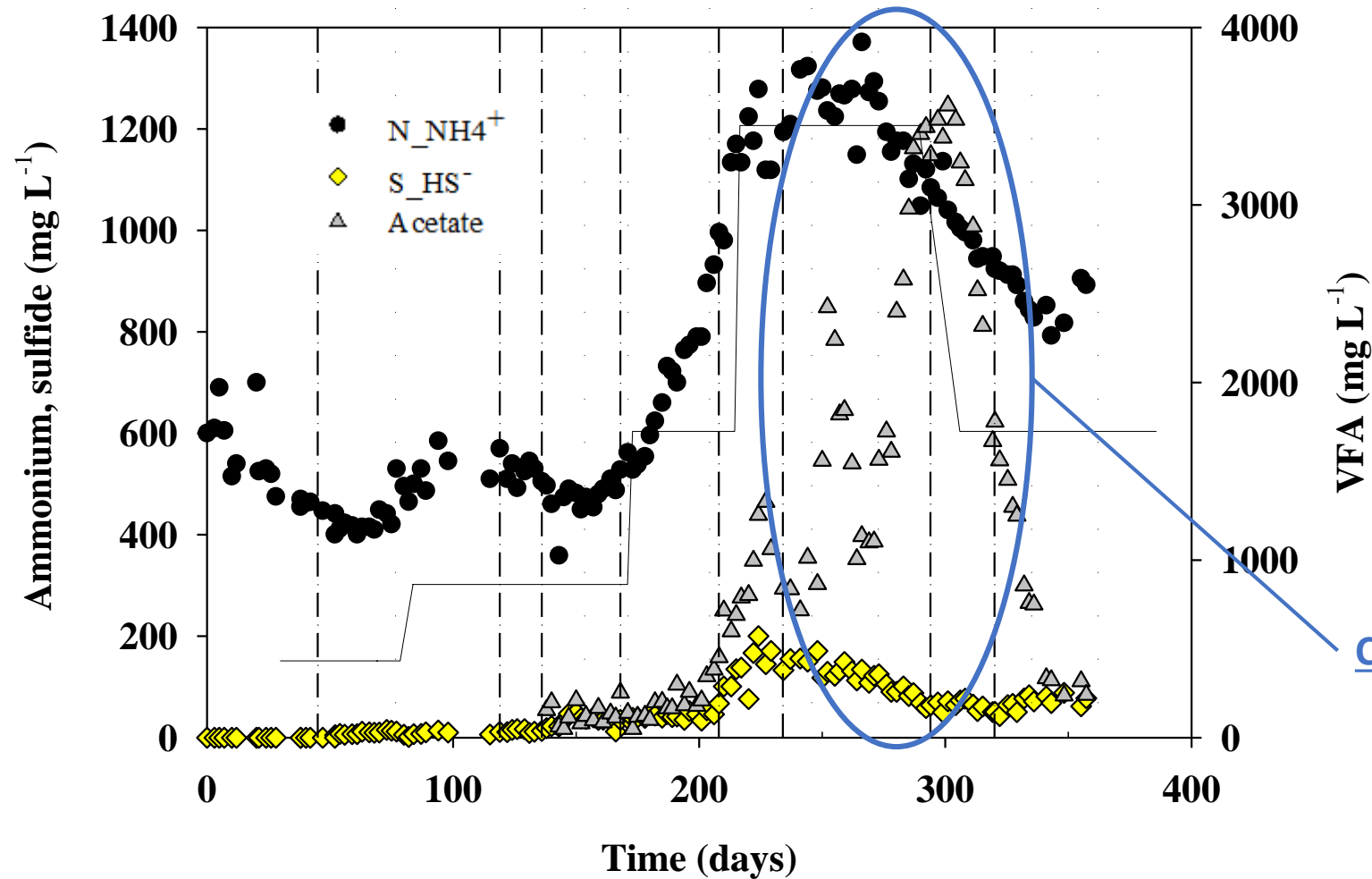
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4. Physical-chemical data

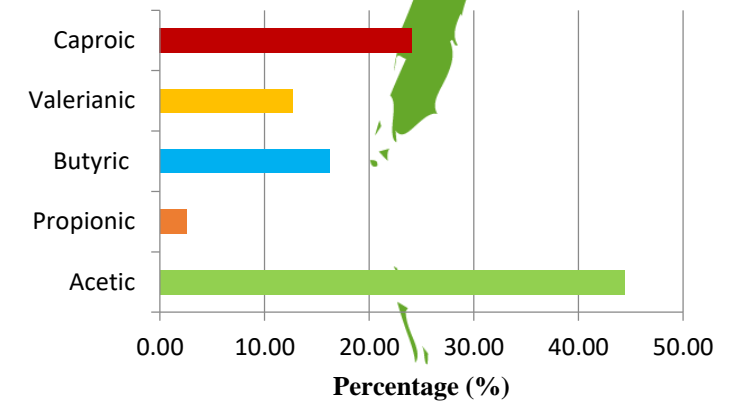


VS/TS : 0.35 – 0.48

4. Physical-chemical data

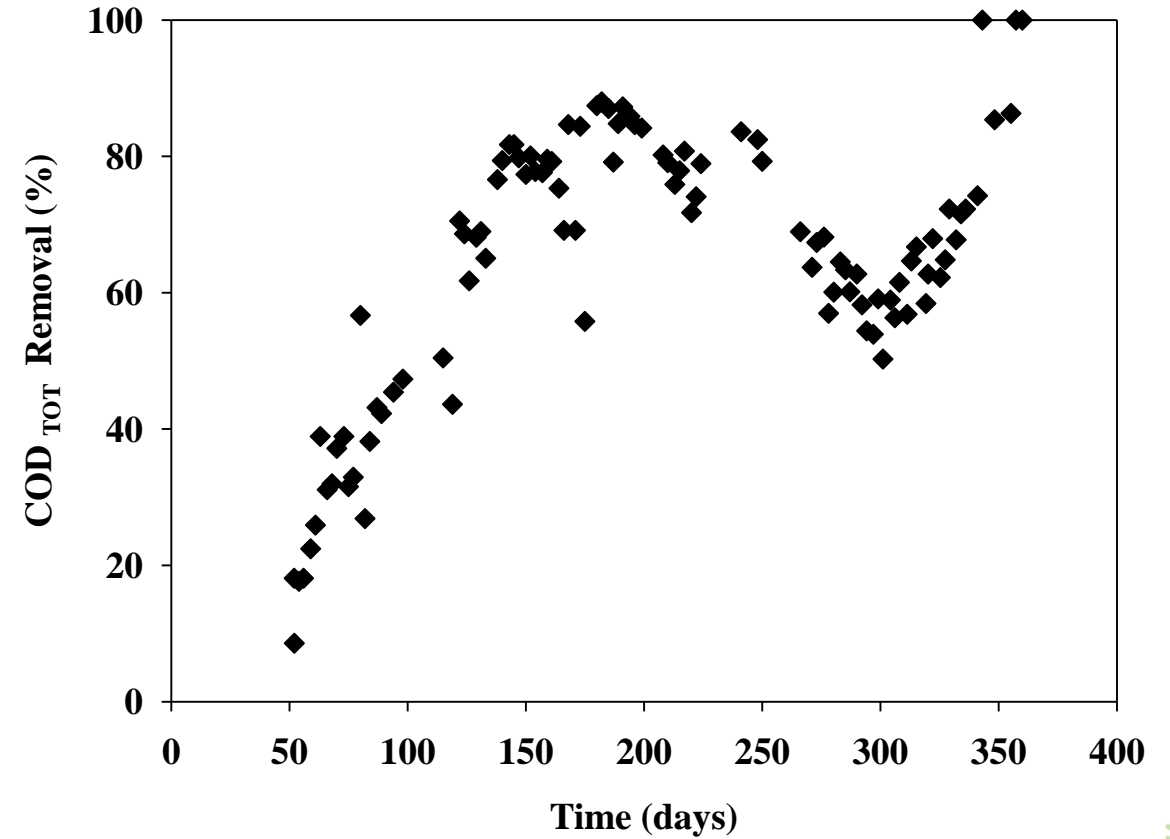
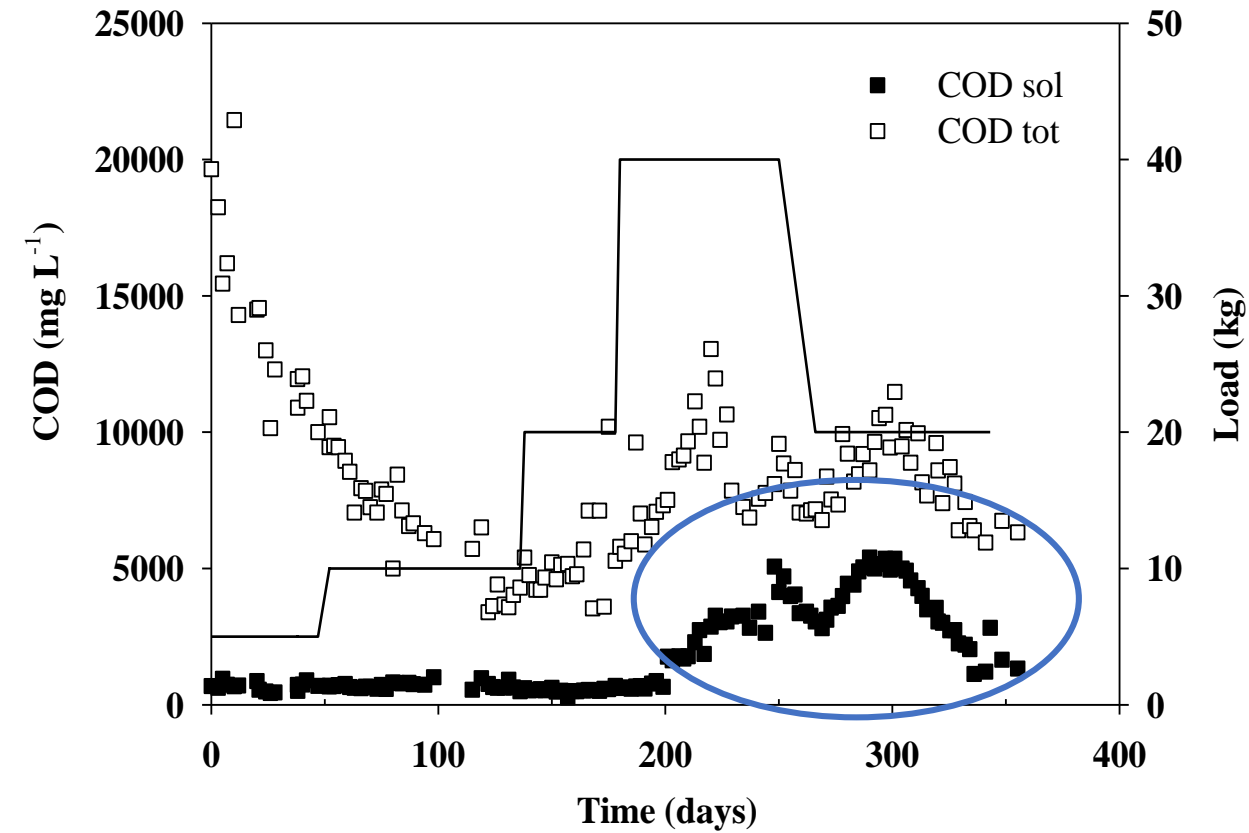


VFA IN DIGESTATE

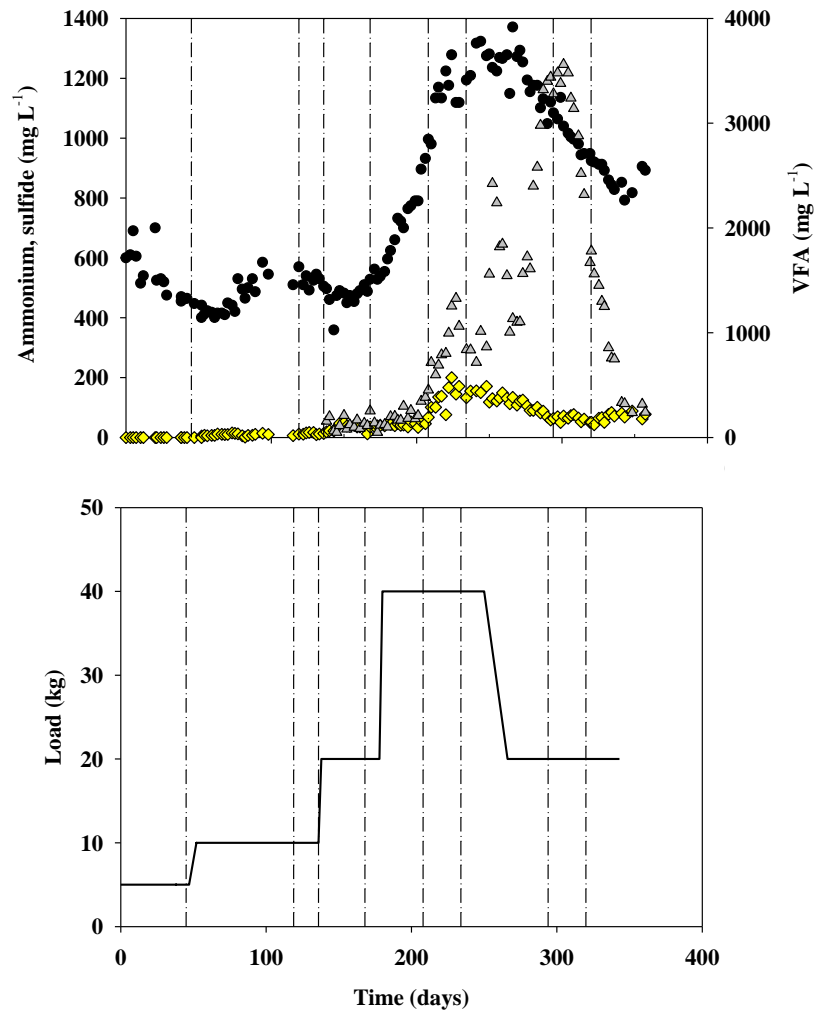


CO-INHIBITION: NH_4^+ / HS^- / HIGH SALINITY

4. Physical-chemical data



5. Monitoring of the microbial community



AIM

Monitor microbial community evolution at different operational conditions

MAT&MET:

Samples collection over 1 year (every 30-60 days)

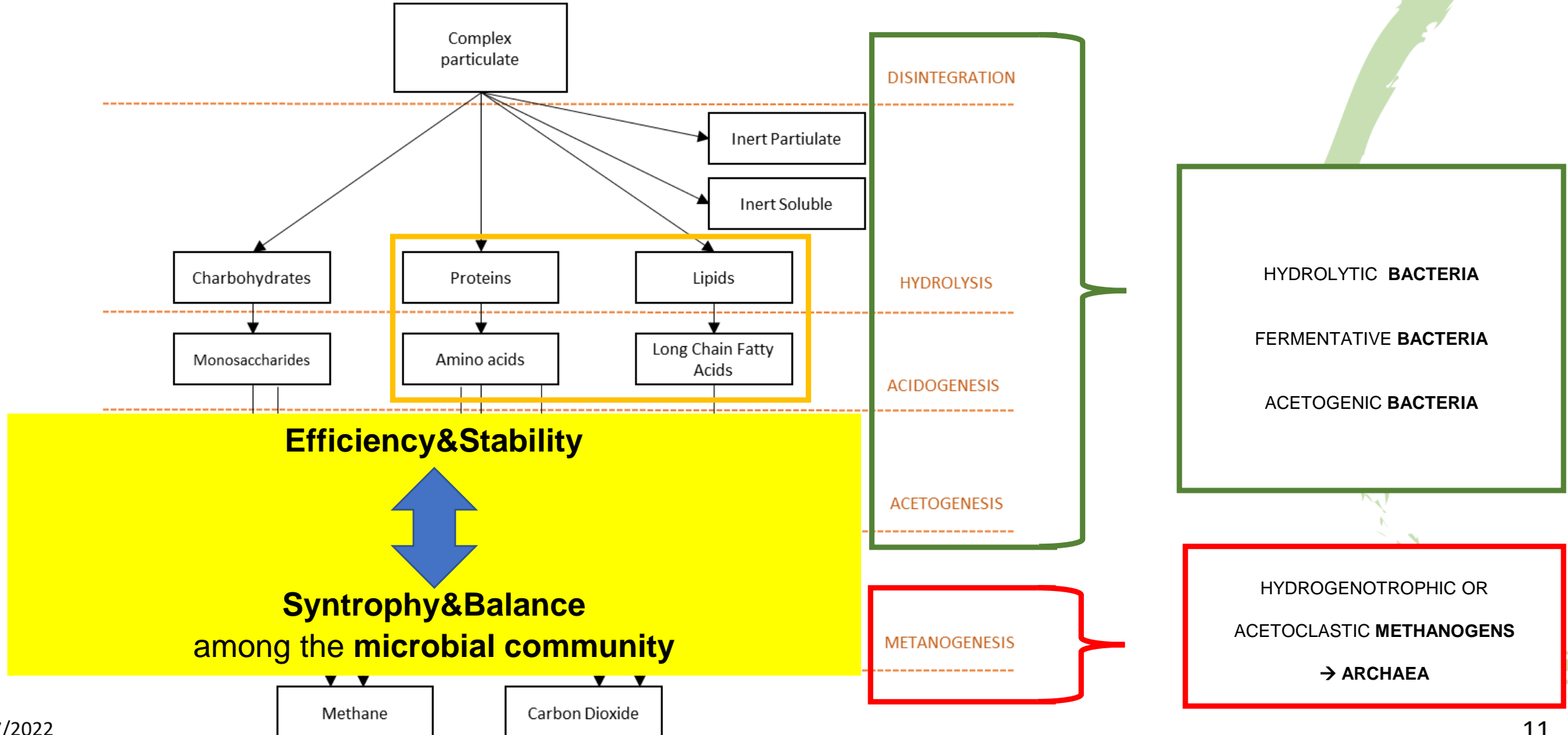
DNA extraction (on fresh sludge samples) and PCR by UNIFI Biology

Department, Prof. M. Ramazzotti

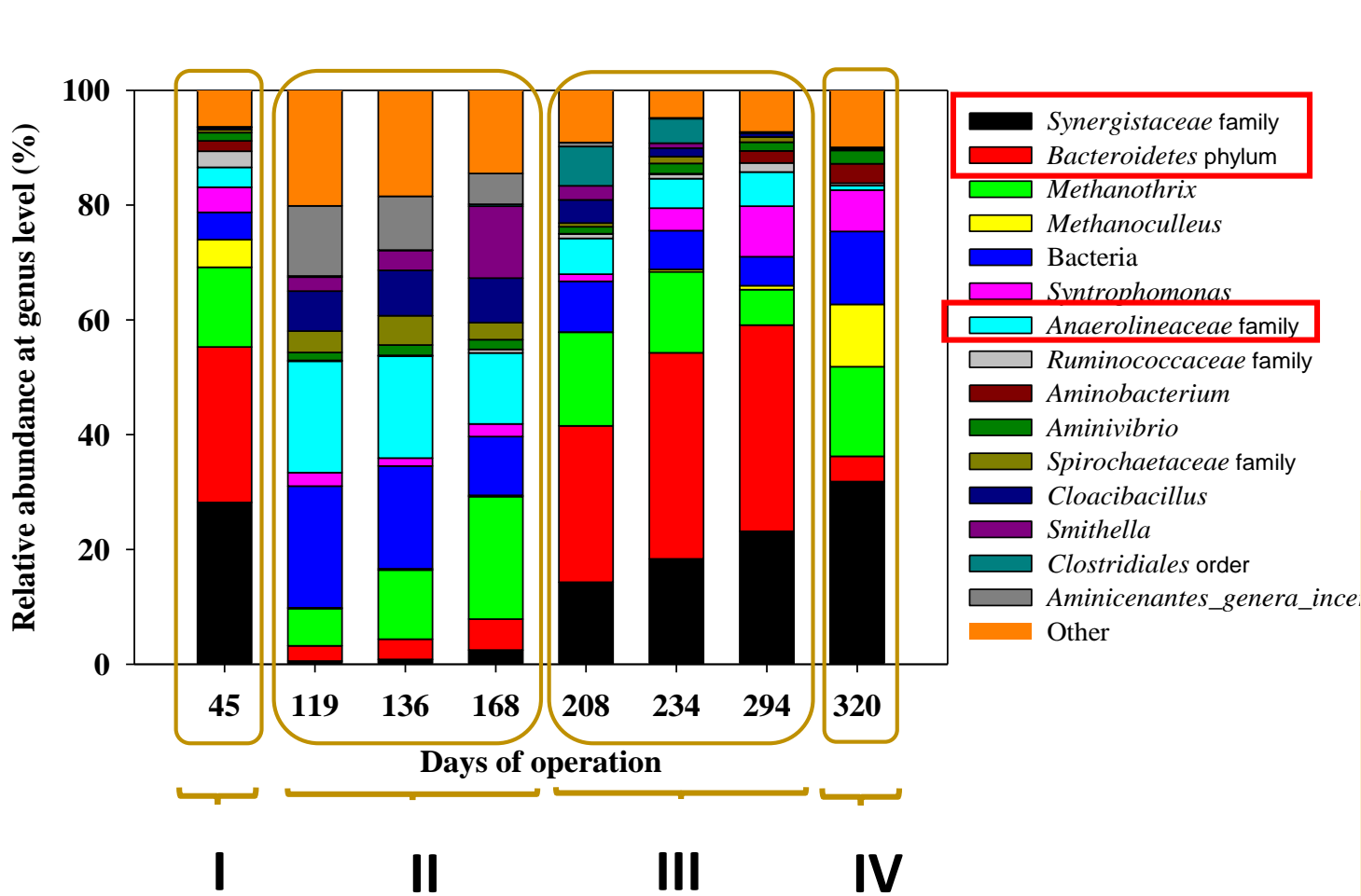
Amplicon Sequencing, External lab

Data elaboration by previous secondee Dr Eva Fernandez from UAB to Italprogetti

5.1 Anaerobic digestion reactions



5.2 Evolution of the microbial populations

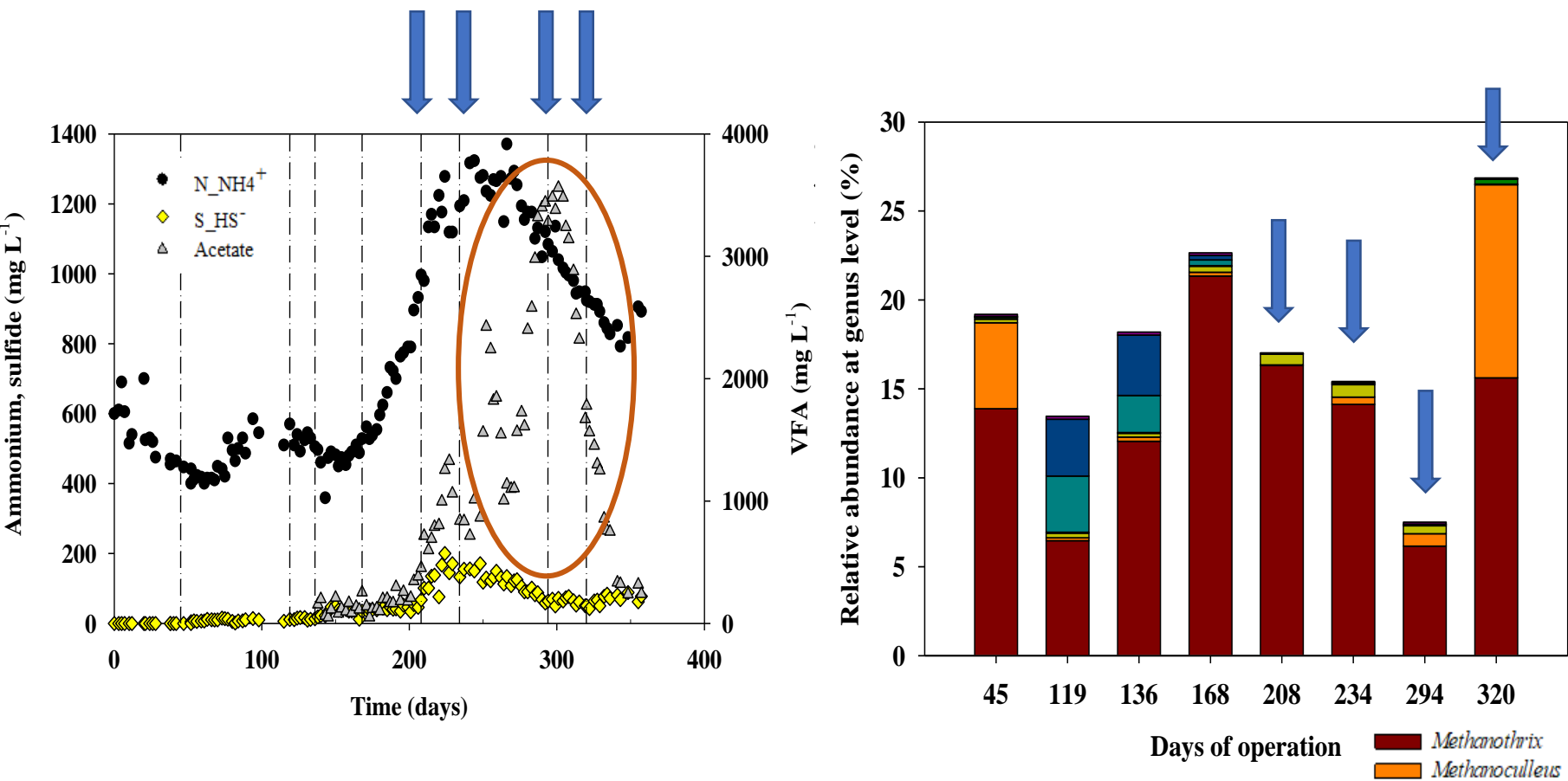


Most remarkable changes in the bacterial community				
	I	II	III	IV
Synergistaceae fam. (Synergistetes phylum)	28	1	19	32
Bacteroidetes phylum	27	4	33	4
Anaerolineaceae fam. (Chloroflexy phylum)	5	17	6	1

Bacteroidetes and Chloroflexy
→ hydrolytic fermentative bacteria encountered at different operational conditions/substrates

Synergistetes → AD of lipid-rich substrates
LCFA β-oxidizing acetogenic bacteria and methanogenic archaea syntrophy

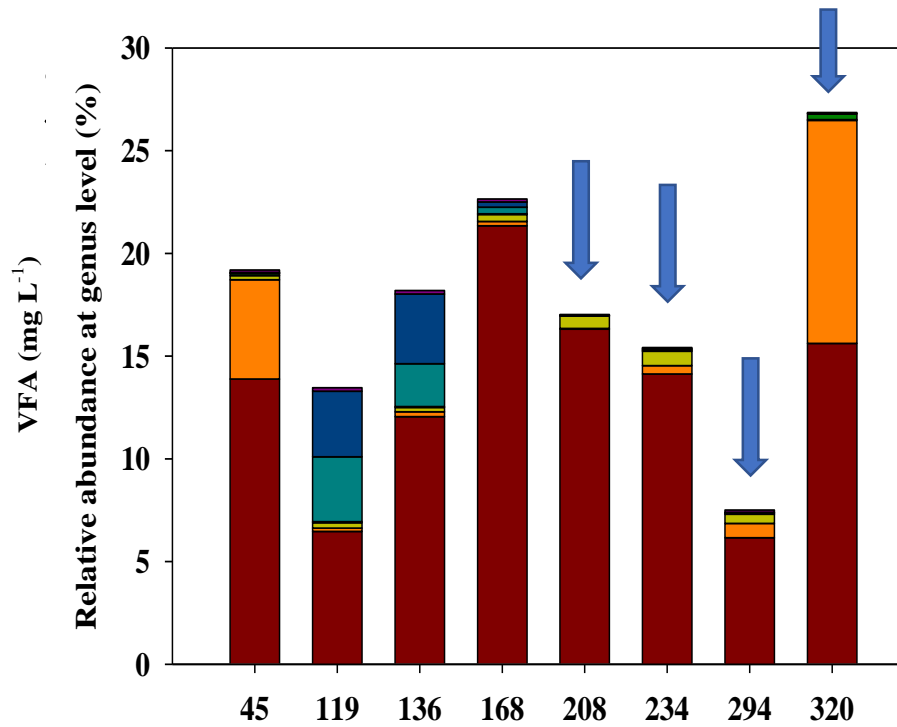
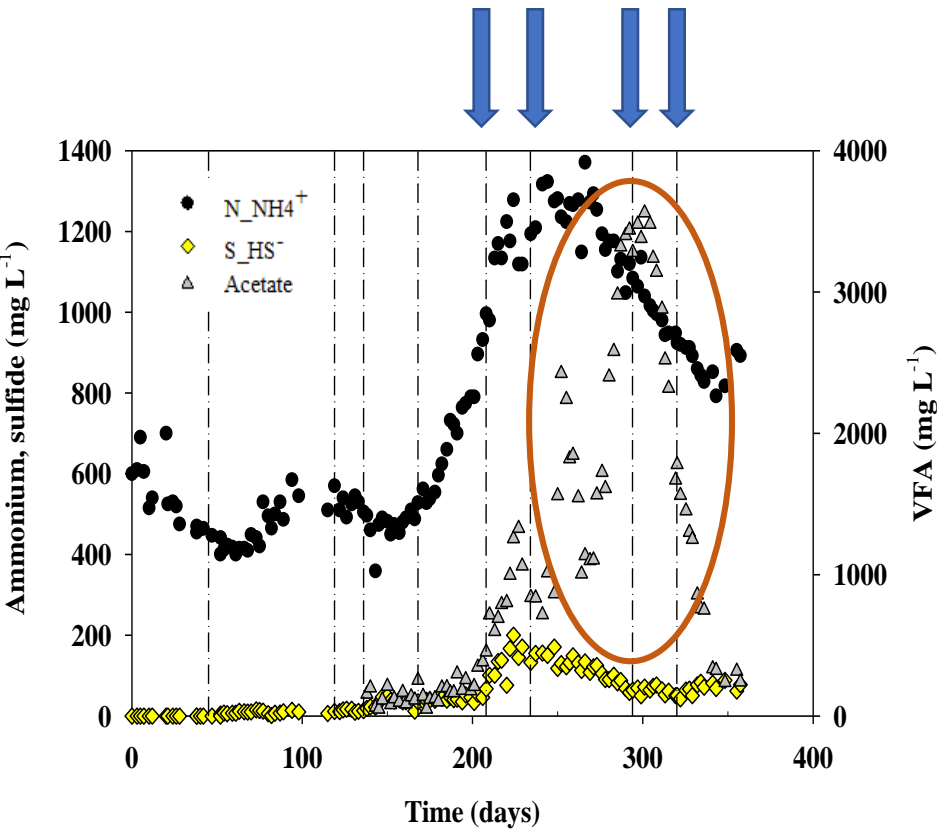
5.2 Evolution of the microbial populations - Archaea



Day of operation	Total % of Archaea
45	19.2
119	13.5
136	18.2
168	22.6
208	17.0
234	15.4
294	7.5
320	26.9

Environmental pressure on Methanogens
→ generally considered **the most sensitive** microbial group to ammonia inhibition.

5.2 Evolution of the microbial populations - Archaea



Methanothrix

(Methanosarcinales order)

Aceticlastic methanogens → more susceptible to ammonia inhibition

- Tolerant to moderate-high salinity

Methanocullelus

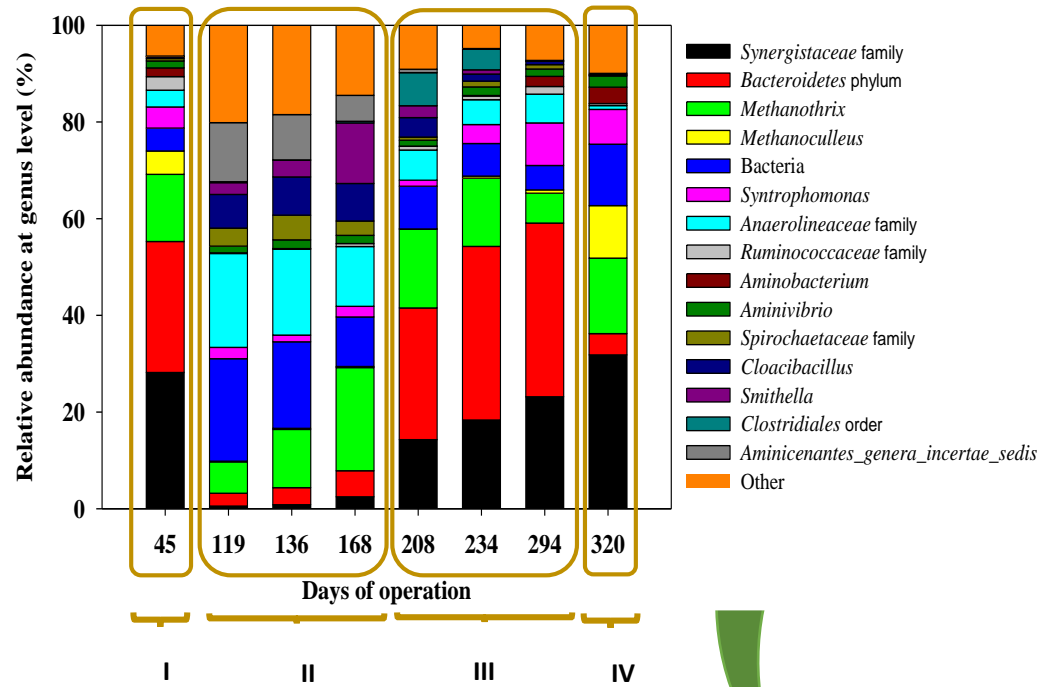
(Methanomicrobiales order)

- **Hydrogenotrophic methanogens**
→ **dominant at high ammonia levels**
- Reported as coupled with syntrophic acetate oxidation bacteria (SAOB)

Ammonia exposure is reported to promote a shift of methane production pathway towards **syntrophic acetate oxidation** and **hydrogenotrophic methanogenesis, SAO-HM**

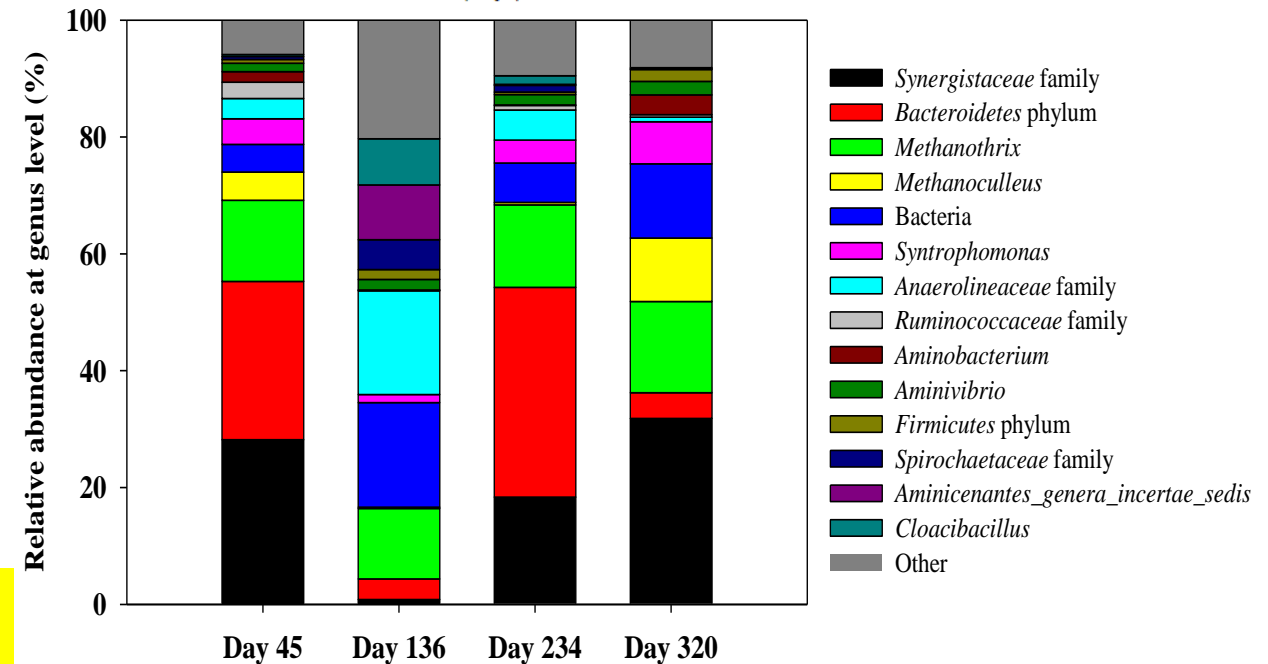
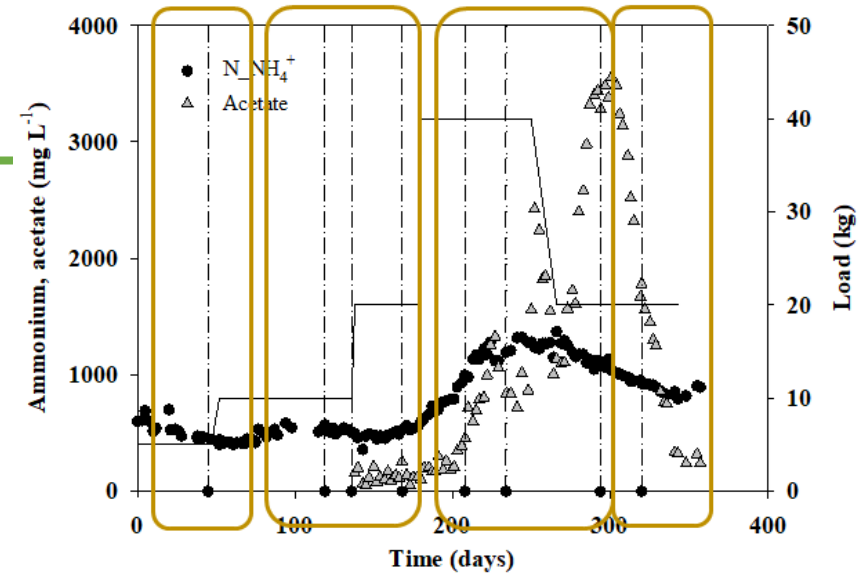
Other Archaea

6. Sampling frequency



Similar results in samples collected monthly within the same operational phase

Reduce sampling frequency?



7. Conclusions and critical aspects



- Digester start-up with fleshing as sole substrate showed promising results on methane production, but warned on overloading conditions and inhibition drawbacks
- The observed evolution of the microbial community is in line with the operational conditions in the digester. Pressure on archaeal community was evident during stress conditions, leading to a shift towards ammonia-tolerant genus



Results suggest that sampling intervals of 2-3 months could have been sufficient to collect sludge sample representative of each operational phase

→ **Proper sampling frequency? What about replicates?**

Results were available more than one year after the beginning of the digester operation, when Ammonia and VFA accumulation issues were already solved.

→ **Nice to monitor microbial evolution, but what's the point for technicians?**



Thank you!

REGIONE
TOSCANA



ITALPROGETTI

Progetto Biorec

Processi Biologici innovativi per il Recupero di Energia
dai rifiuti dell'industria Conciaria. Regione Toscana FSC
2021



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	I	II			III			IV
Genus	Day 45	Day 119	Day 136	Day 168	Day 208	Day 234	Day 294	Day 320
Synergistaceae family	28,19	0,56	0,83	2,48	14,30	18,36	23,15	31,81
Bacteroidetes phylum	27,10	2,66	3,53	5,39	27,24	35,90	35,95	4,40
Methanothrix	13,88	6,47	12,05	21,34	16,31	14,13	6,16	15,62
Methanoculleus	4,83	0,15	0,24	0,22	0,03	0,41	0,70	10,85
Bacteria	4,73	21,20	17,88	10,25	8,82	6,75	5,04	12,71
Syntrophomonas	4,38	2,33	1,39	2,18	1,25	3,94	8,82	7,21
Anaerolineaceae family	3,46	19,43	17,76	12,37	6,22	5,12	5,96	0,78
Ruminococcaceae family	2,80	0,13	0,12	0,64	0,81	0,81	1,59	0,42
Aminobacterium	1,80	0,01	0,01	0,00	0,03	0,08	2,08	3,39
Aminivibrio	1,45	1,37	1,82	1,68	1,21	1,78	1,54	2,30
Spirochaetaceae family	0,51	3,74	5,11	2,95	0,64	1,18	0,90	0,16
Cloacibacillus	0,35	6,96	7,92	7,78	4,03	1,47	0,69	0,16
Smithella	0,08	2,43	3,45	12,53	2,47	0,88	0,06	0,00
Clostridiales order	0,07	0,21	0,06	0,32	6,83	4,25	0,11	0,23
Aminicenantes_genera_incertae_sedis	0,01	12,23	9,37	5,37	0,71	0,15	0,01	0,00
Other	6,36	20,13	18,48	14,49	9,09	4,82	7,26	9,94

Genus	I	II			III			IV
		Day 119	Day 136	Day 168	Day 208	Day 231	Day 252	Day 270
Synergistaceae family		0,56	↓ 1,83	2,48	14,30	↑ 15,05	23,15	31,41
Bacteroidetes phylum		2,66	↓ 2,23	5,39	27,24	↑ 29,00	35,95	47,00
<i>Methanothrix</i>		6,47	12,05	21,34	16,31	14,13	6,16	15,62
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