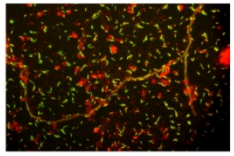
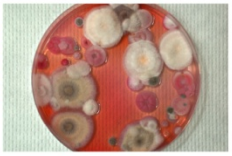
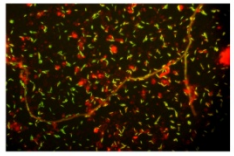
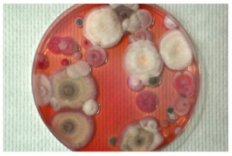
University of Innsbruck - Faculty of Biology
Institute of Microbiology

Prerequisites for reliable VFA quantification from anaerobic digestion systems

Wagner A.O., Markt R., Puempel T., Illmer P., Insam H., Ebner, C.

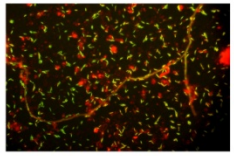
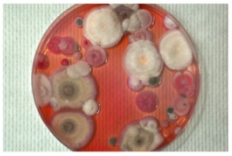
3rd International Conference on Monitoring & Process Control of Anaerobic Digestion Plants (CMP)
Leipzig, March 2017

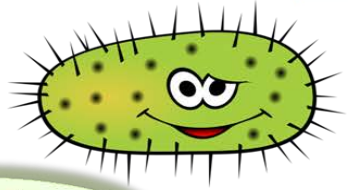
Introduction

Why Anaerobic digestion:

- Locally available resources (**regional**)
- Energy generation from bio-“wastes” (**closed cycles**)
- Digestion of non-fossile resources (**renewable**)
- Energy can be conserved in a chemical form:
  - Storage
  - Availability
  - Mobility

Anaerobic digestion – a complex process



COMPLEX ORGANIC POLYMERS
Carbohydrates Lipids Proteins

1. Hydrolysis — *Hydrolytic bacteria*

SOLUBLE ORGANIC MOLECULES
Sugars amino acids fatty acids

2. Acidogenesis — *Acidogenic and fermentative bacteria*

VOLATILE FATTY ACIDS AND ALCOHOLS

3. Acetogenesis — *Syntrophic acetogens*

Homoacetogenesis

ACETATE ⟷ **$H_2 + CO_2$**

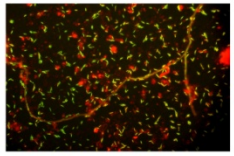
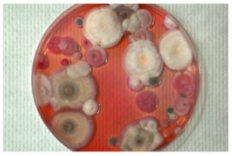
Syntrophic acetate oxidation

4. Methanogenesis — *Hydrogenotrophic methanogens*

Acetoclastic methanogens

BIOGAS
CH_4, CO_2, trace gases

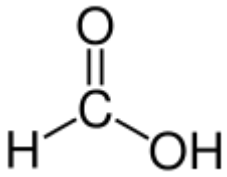
Ruetz, 2016

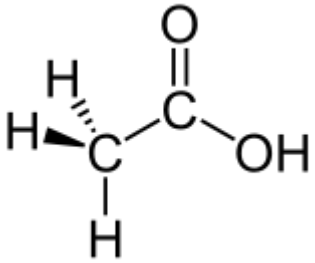
Volatile fatty acids

VFA – volatile fatty acids:

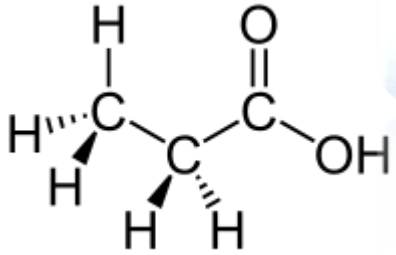
- six or fewer C-atoms: $C_1 – C_6$
- distillable under atmospheric pressure



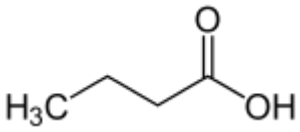
Formic acid – C_1



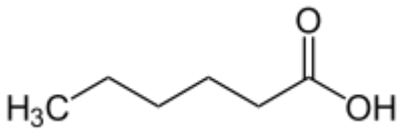
Acetic acid - C_2



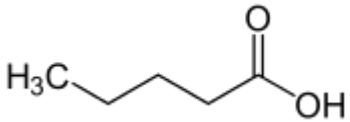
Propionic acid – C_3



Butyric acid – C_4

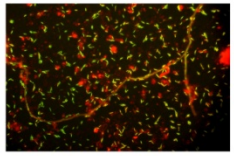
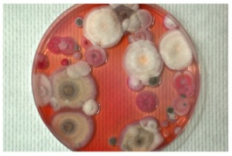


Capronic acid – C_6



Valeric acid – C_5

According to APHO, 1992

Recovery/Quantification of VFA

Separation of solid and liquid phase:

- Filtration: gravity, vacuum
- Centrifugation
- Dialysis

Sample characteristics!!!

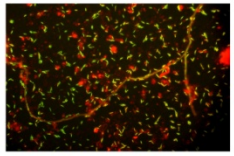
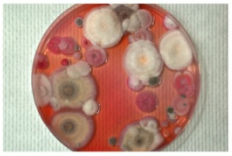
Extraction:

- using an organic solvent
- using an acid: eg. formic acid (10 – 30%)*

Qualification and Quantification:

- Titration
- TLC (DC), IC
- Electrophoresis
- GC
- HPLC

*patented??

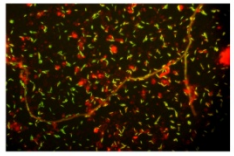
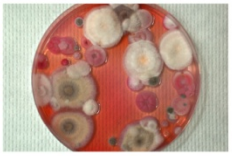



Aims and setup

Evaluation[1] of:

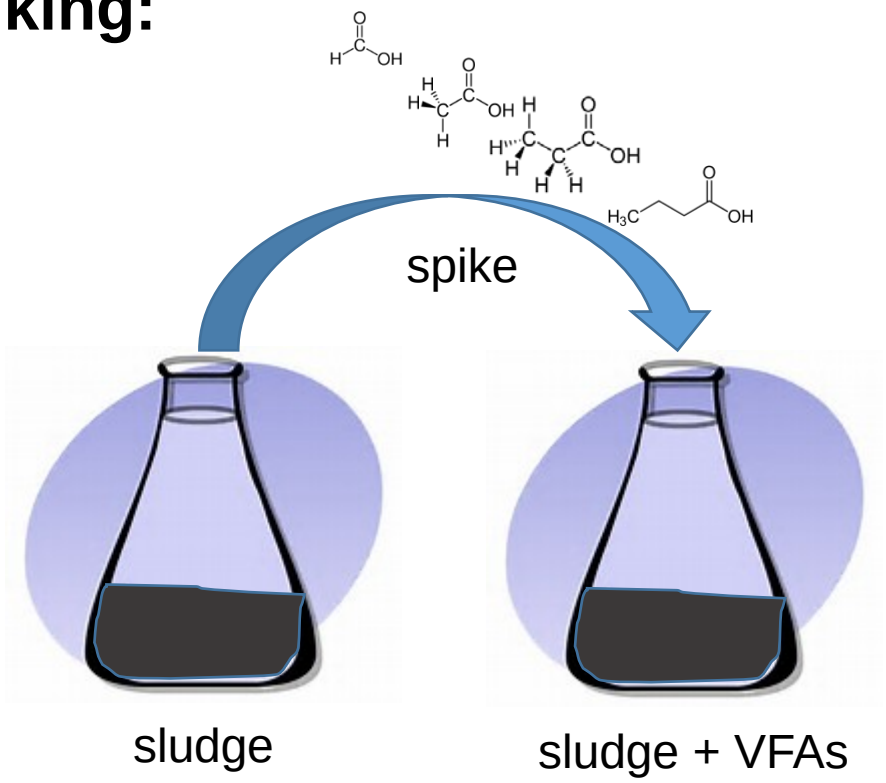
1. separation techniques on VFA recovery
2. decomposition of VFA under original reactor temperature conditions and at +4 °C
3. preservation and precipitation/coagulation agents on VFA recovery
4. sample storage at +4 and -20 °C

[1]: published in: Engineering in Life Science 2017, 17, 132-139. DOI: 10.1002/elsc.201600095

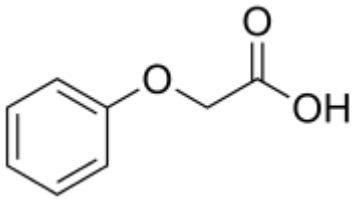



Material and Methods

Spiking:

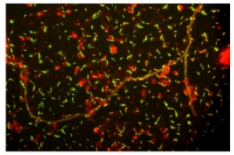
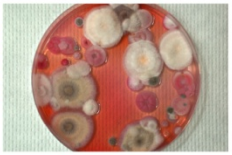


Addition of **Phenoxy acetic acid** as inert tracer



1. Separation techniques
2. Sampling (4 vs 38 °C)
3. Preservation
4. Storage

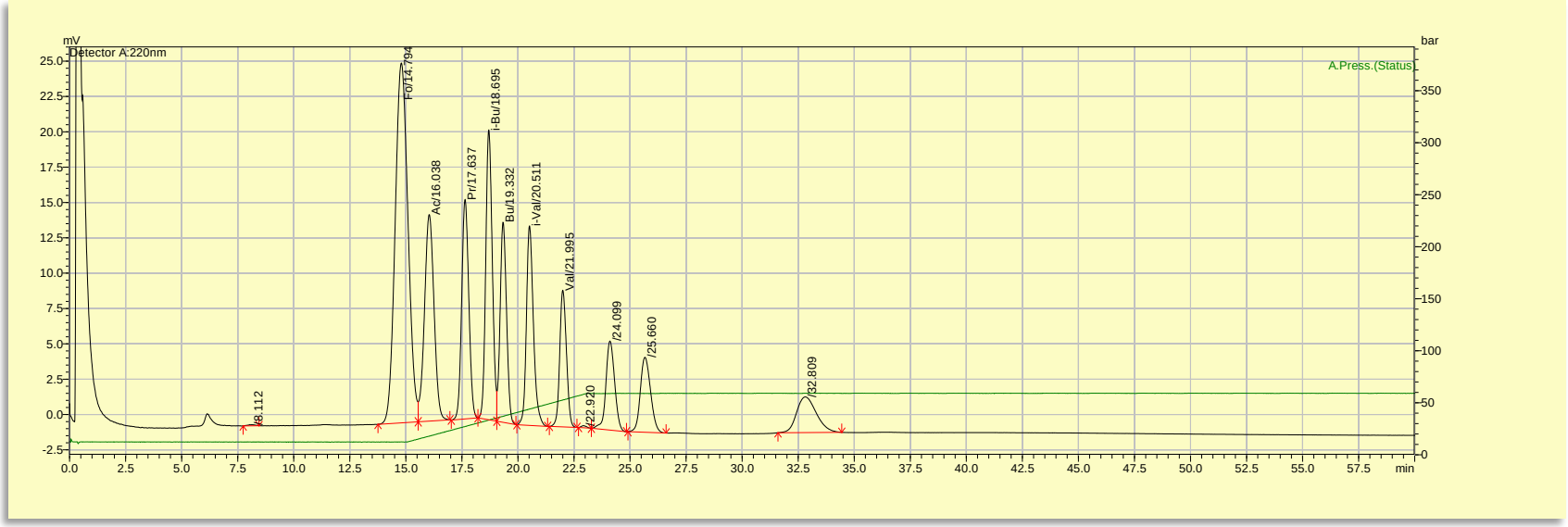
Sludge: from full-scale WWTP Zirl, Austria (mesophilic)

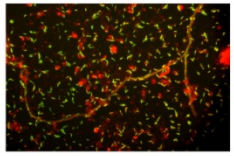
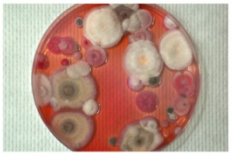



Material and Methods

Detection :

- HPLC: Phenomenex Fast Fruit Column
- Detection: UV@220 nm
- Using an external standard (Sigma, Germany):
  C_1 – C_7; 5 mM each

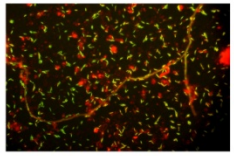
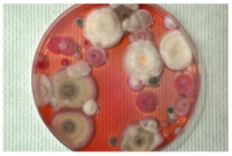


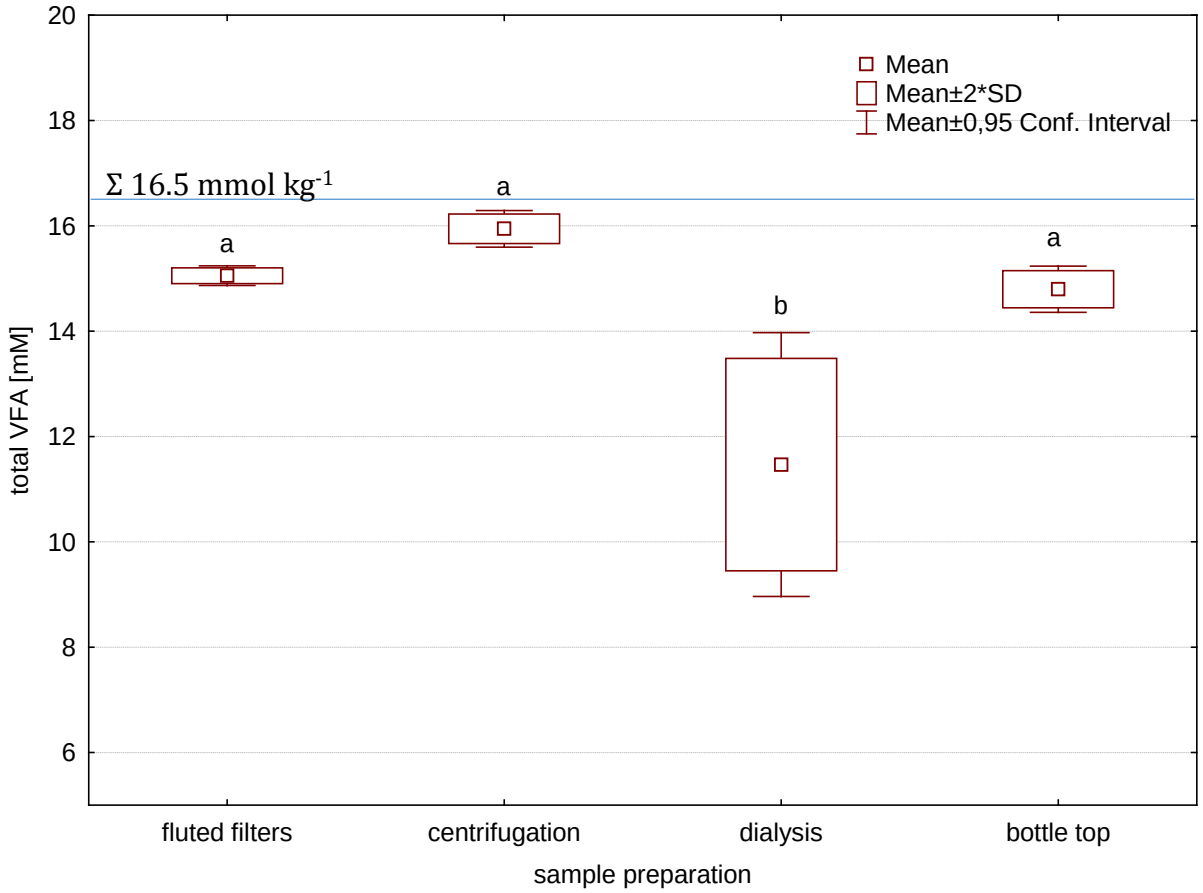
Material and Methods

1. Separation:

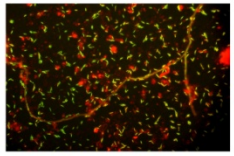
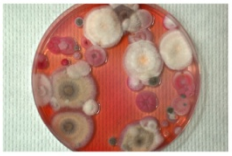
- Gravity filtration (MN 615)
- Centrifugation: 15 min@15 000 x g
- Dialysis tube: Visking #44114 - 24h on ice
- Vacuum filtration: Rapid-Flow (Thermo)

Results – 1. Extraction/Separation



- Total spike: 16.5 mmol kg^{-1}
- Maximum recovery with centrifugation yielding 94.9% of theoretically added sum
- Filtration methods work surprisingly good
- High losses by application of dialysis

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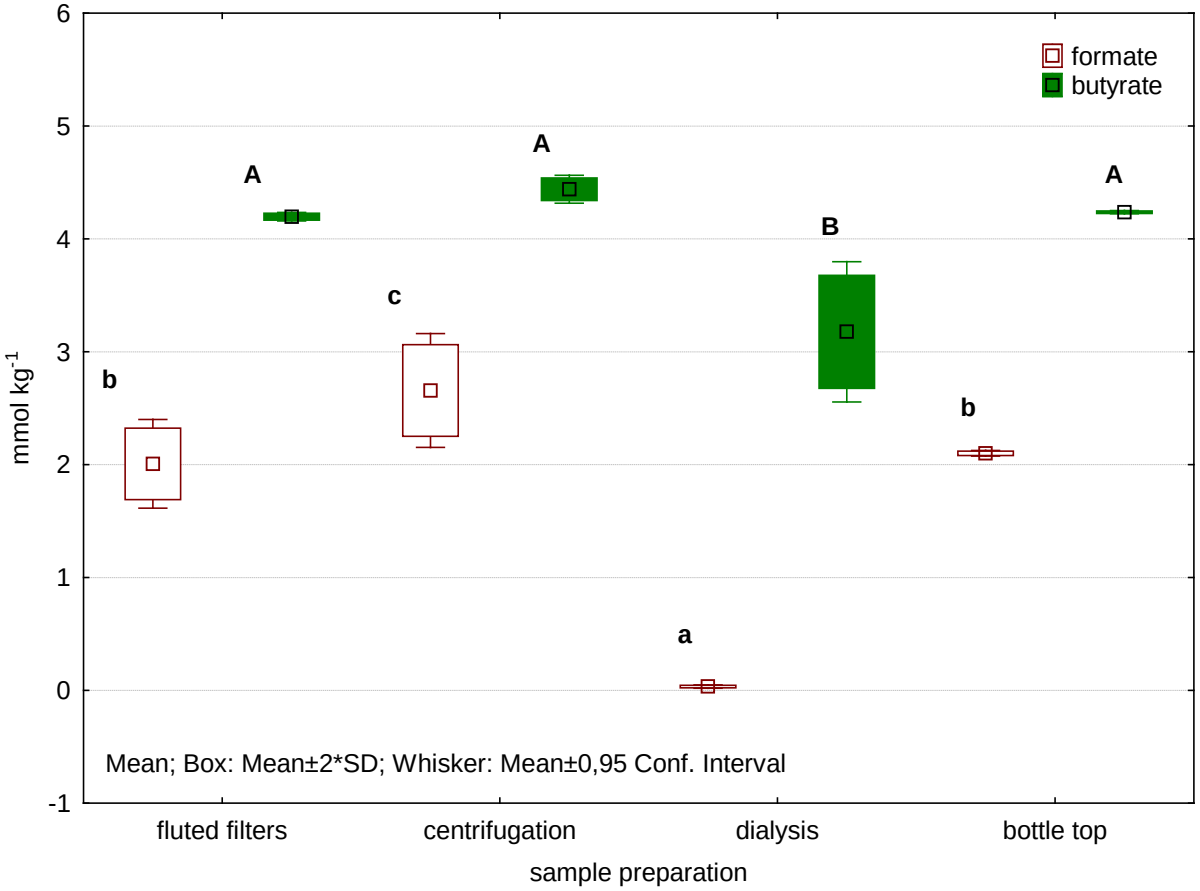
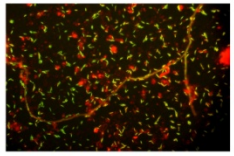
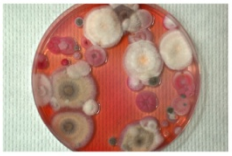


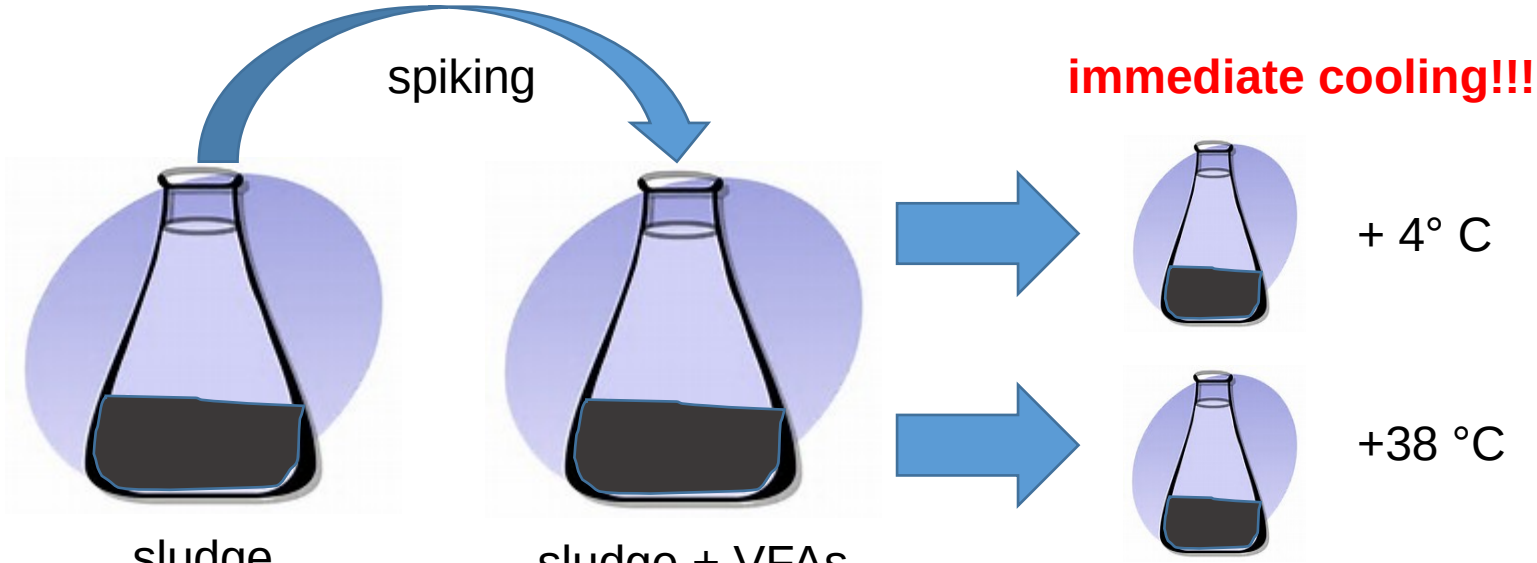
- Dialysis showed a poor recovery of butyrate and failed to recover formate
- Nondiffusible volume fraction of larger solids particles and/or nondiffusible microbial biomass

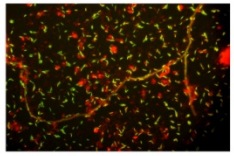
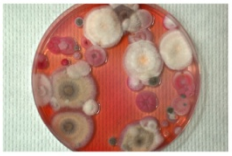



Material and Methods

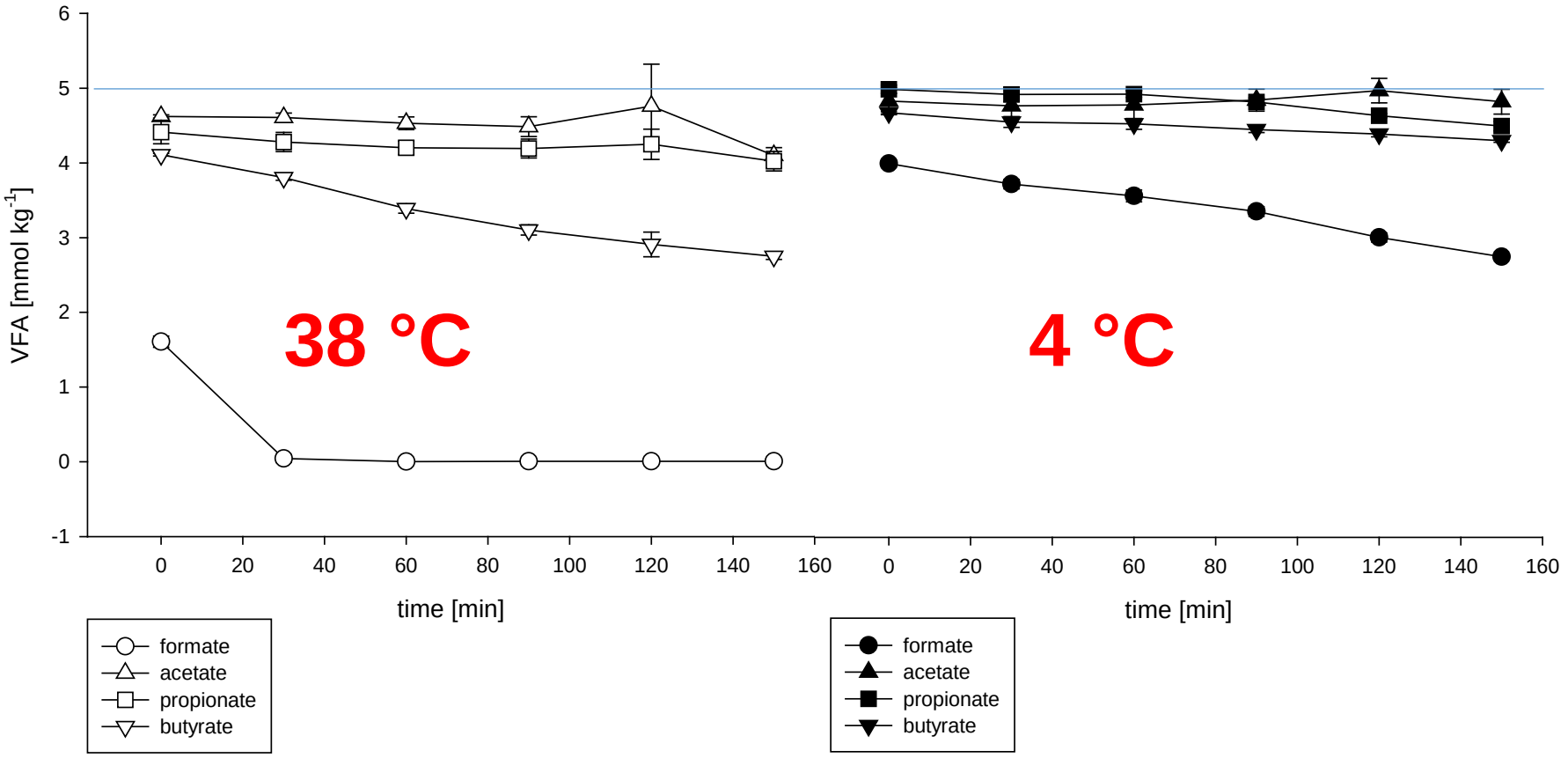
2. Decomposition:

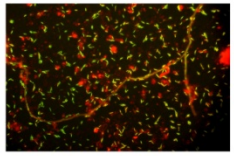
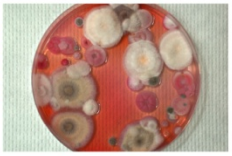
- Spiking of sludge with 5.0 mmol kg^{-1} Fo – Bu
- Sampling in 30 min intervals for 2.5 hours
- Incubation of sludge at 4 °C and 38 °C, respectively



Results – 2. Decomposition



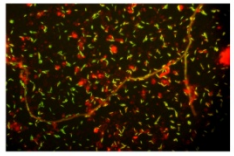
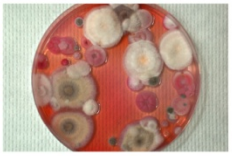



Results – 2. Decomposition

Recovery [%] from initial concentrations of spiked VFA and loss of VFA h^{-1} at 4 °C and 38 °C, respectively.

	38 °C				4 °C			
Min	**Formate**	**Acetate**	**Propionate**	**Butyrate**	**Formate**	**Acetate**	**Propionate**	**Butyrate**
30	2.7	97.0	99.7	92.5	94.5	98.9	98.9	97.8
60	0.2	95.2	98.0	82.5	91.3	99.1	98.9	97.4
150	0.4	91.2	88.7	66.9	75.1	99.9	91.8	93.4
Loss [mmol kg^{-1} h^{-1}]*	**	0.172	0.118	0.626	0.372		0.114	0.123

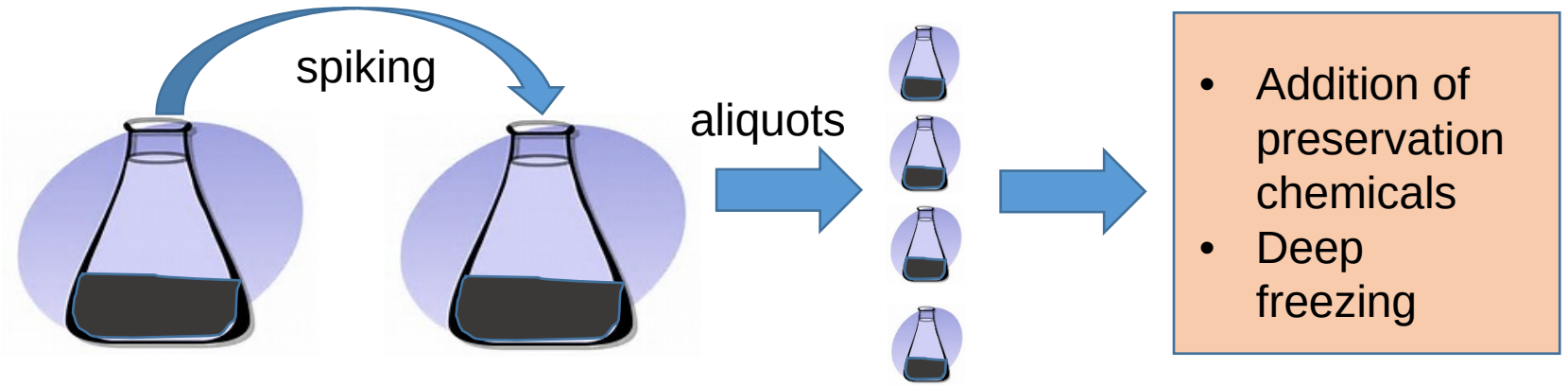
*: for linear regression with $R^2 > 0.5$.
**: completely lost

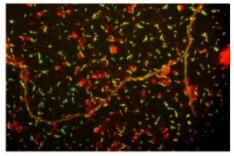
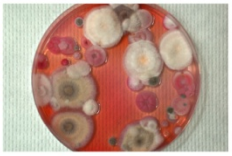



Material and Methods

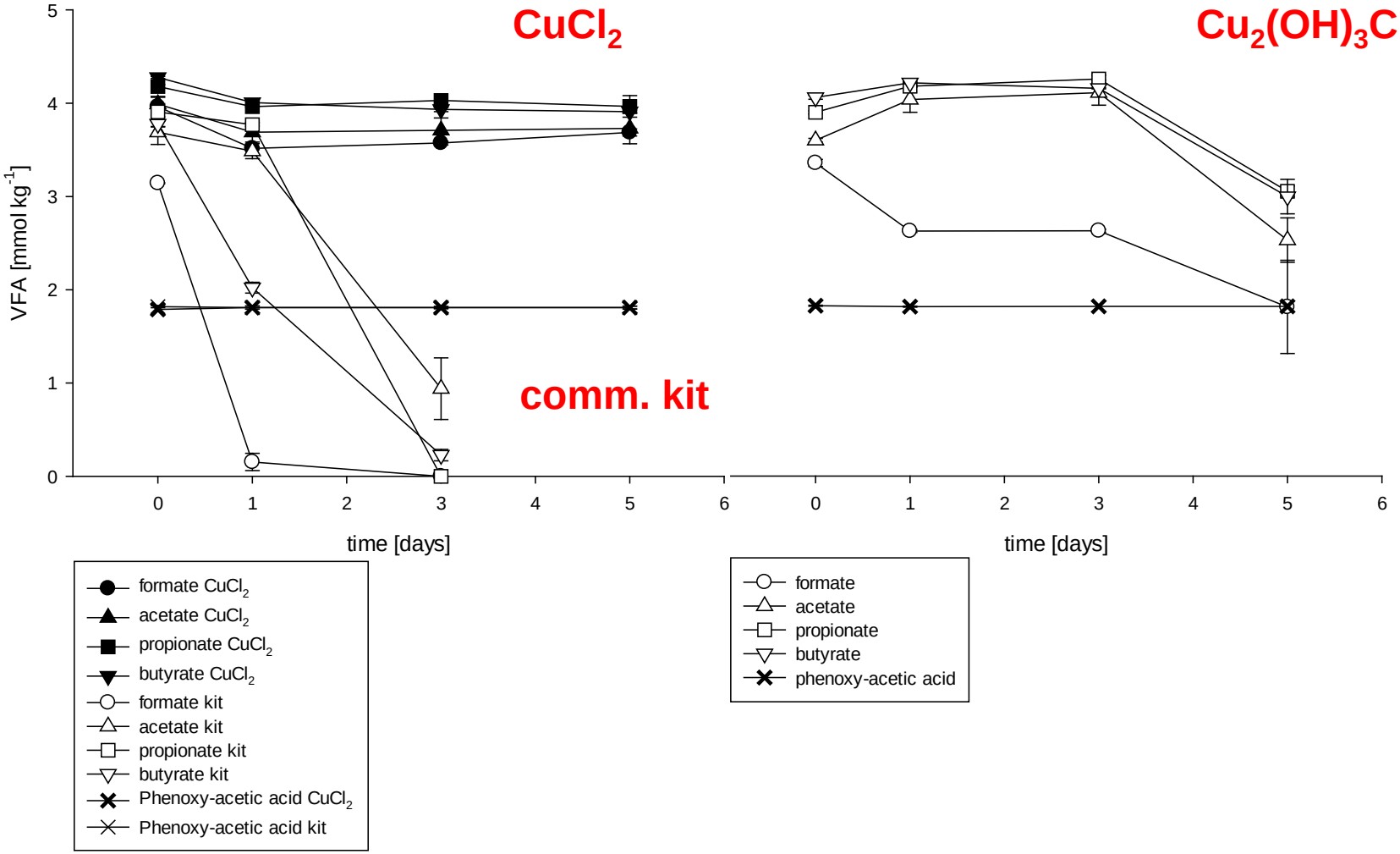
3. Preservation:

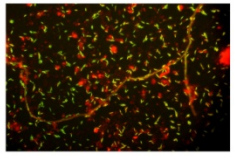
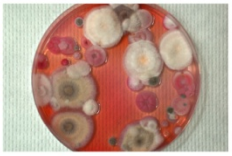
- Deep freezing (-20 °C)
  - Aliquoted 1 g samples
- Chemical:
  - $ZnCl_2$ [7.5 mM]
  - Cu: $CuCl_2$-$3Cu(OH)_2$ [50 mM], $CuSO_4$ [1 mM], $CuCl_2$ [50 mM], commercial kit [??]



Results – 3. Preservation



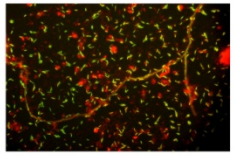
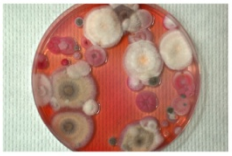
Results – 3. Preservation

Recovery [%] of spiked VFAs.

day	$Cu_2(OH)_3Cl$	Commercial kit	$CuCl_2$	-20° C
1	101	60.8	95.1	nd
3	102	7.51	95.5	nd
5	69.7	nd	95.8	92.7

- $CuCl_2$: >95% recovery after 5 days, but loss of approx. 5% within 24 h (lag phase Cu toxicity?)
- Deep freezing: >92% recovery for all spiked VFA, approx. 98% recovery for Ac, Pr, Bu

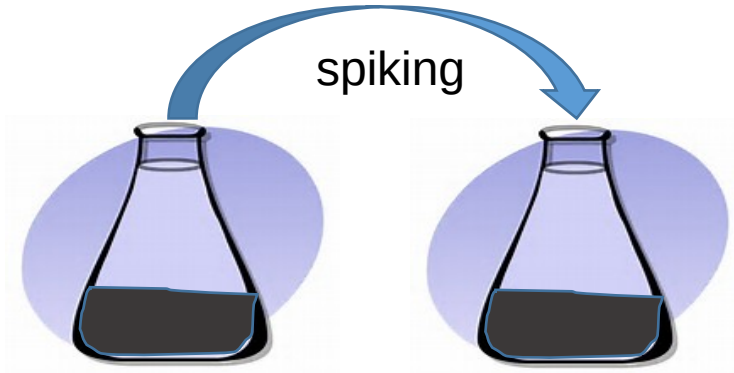
- $ZnCl_2$ and $CuSO_4$ inappropriate in the applied concentrations (reducing the extractable VFA concentration at t=0)
- Commercial kit failed to preserve the sample
- $Cu_2(OH)_3Cl$: onset of microbial acitivity after 3 days

Material and Methods

4. Sample storage:

- Spiking of sludge: cooling (4° C)
- Extraction by centrifugation 15 min@15 000 x g
- Filtration (0.2 µm RC) into HPLC glass vials
- Storage of extracted samples at +4 °C and -20 °C



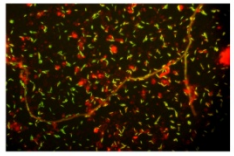
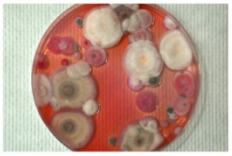
15 min @ 15 000 g

Filtered (0.2 µm RC)

Storage at

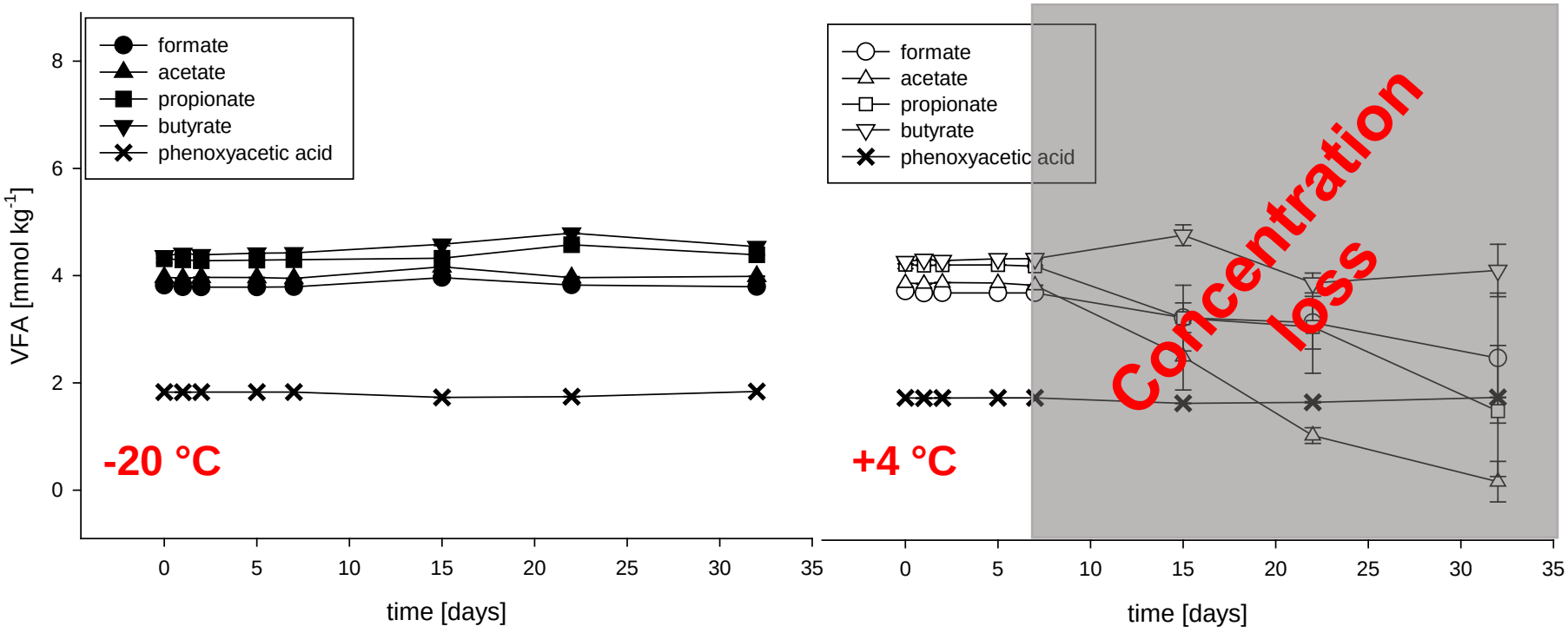
+ 4 °C

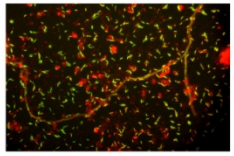
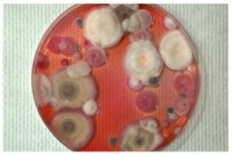
-20 °C

Results – 4. Storage

Storage of extracted, filtered samples at -20 °C and +4 °C



Conclusions and future prospects

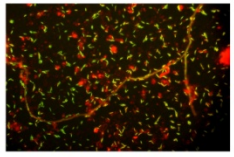
Conclusions:

1. "*The simpler the better*" **centrifugation for "extraction"**
2. "*The faster the better*" **immediate cooling after sampling**
3. "*Chemistry can help*" **$CuCl_2$ or deep freezing at -20 °C**
4. "*The cooler the better*" **storage of extr. samples at -20 °C**

Current projects and further prospects:

- Biological pre-treatment strategies
- Inhibition of AD processes

FWF Der Wissenschaftsfonds.

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… many thanks to …

tirol

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alp·S

… and the team of Ecophysiology WG