

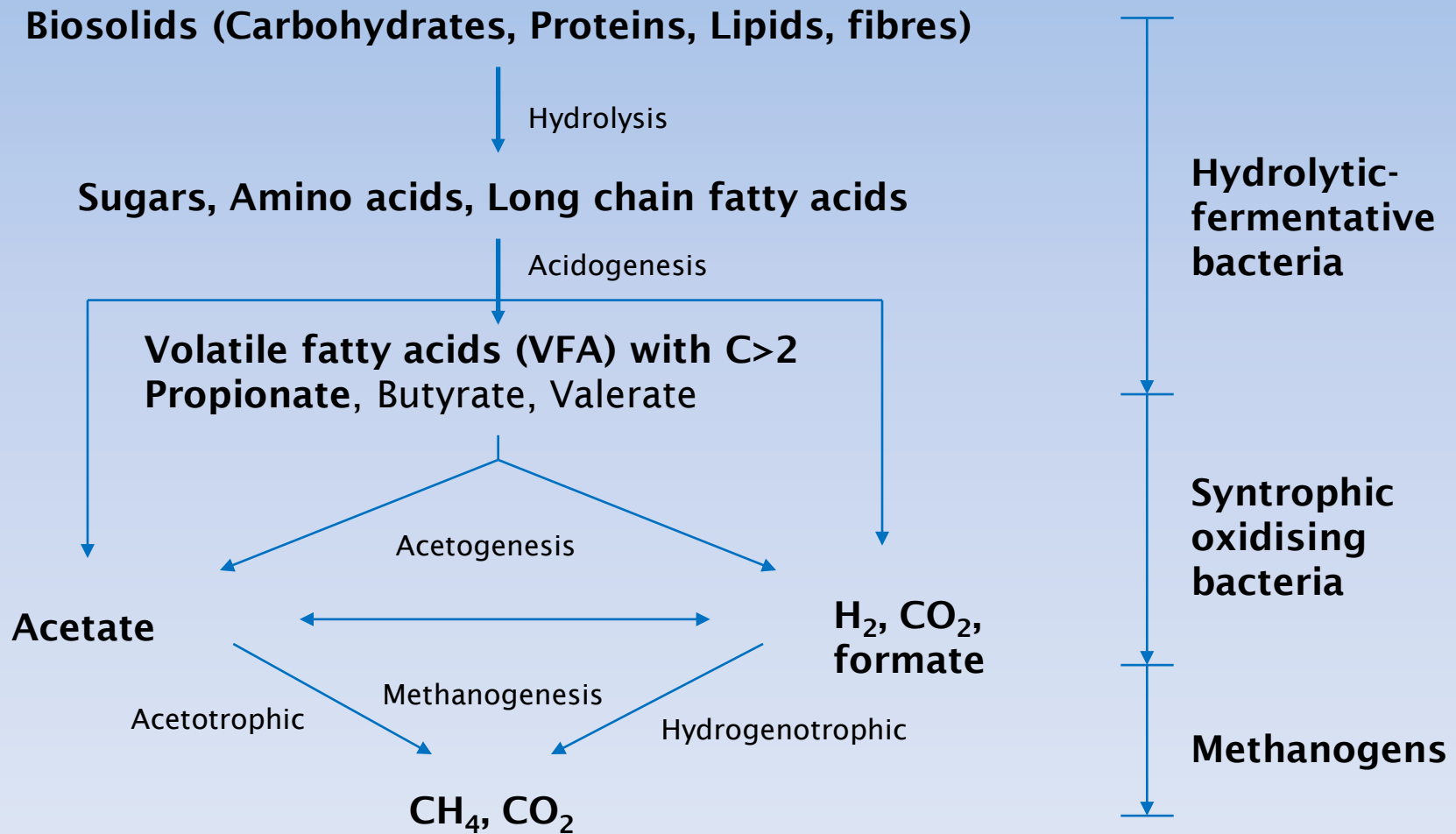
Anaerobic digestion of food waste

Supporting Research

Dr Yue Zhang

Anaerobic conversion of biomass to CH₄

Strict syntrophic manner



Anaerobic digestion operation

Input

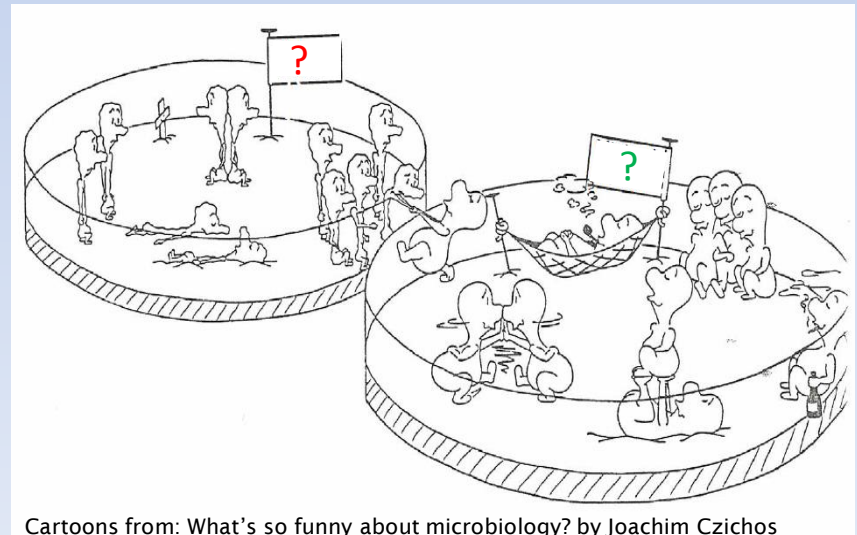
- Calorific value
- Biochemical composition
- Elemental composition
- Chemical oxygen demand
- Biodegradable carbon : TKN
- Macro-nutrients
- Essential trace elements
- Potentially toxic elements
- etc

AD process

- *Temperature*
- *Hydraulic retention time*
- *Solid retention time*
- *Flow mode*
- *Feeding regime*
- *Low solids, high solids*
- *One stage, two stage*
- *etc*

Output

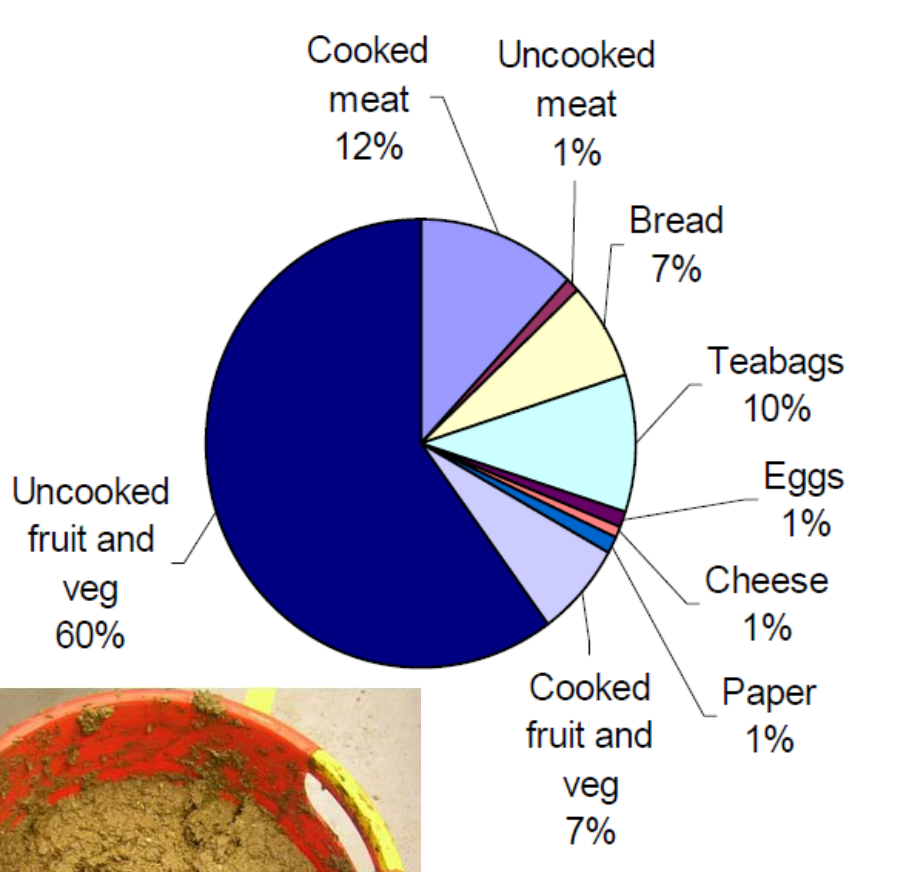
- Optimised Biogas production
- Stabilised and useful digestate



Why separated domestic food waste?

- Food waste between 20~30% of kerbside collected material
- Removes wet and putrescible waste and makes recycling of dry wastes easier
- Reduces vermin and smell problems
- Biofertiliser is free of contaminants and can be applied to agricultural land
- Keeps the AD process simple

Collected food waste



Laboratory digesters for food waste trials

CSTR-type digesters:

2-litre

5-litre

40-litre

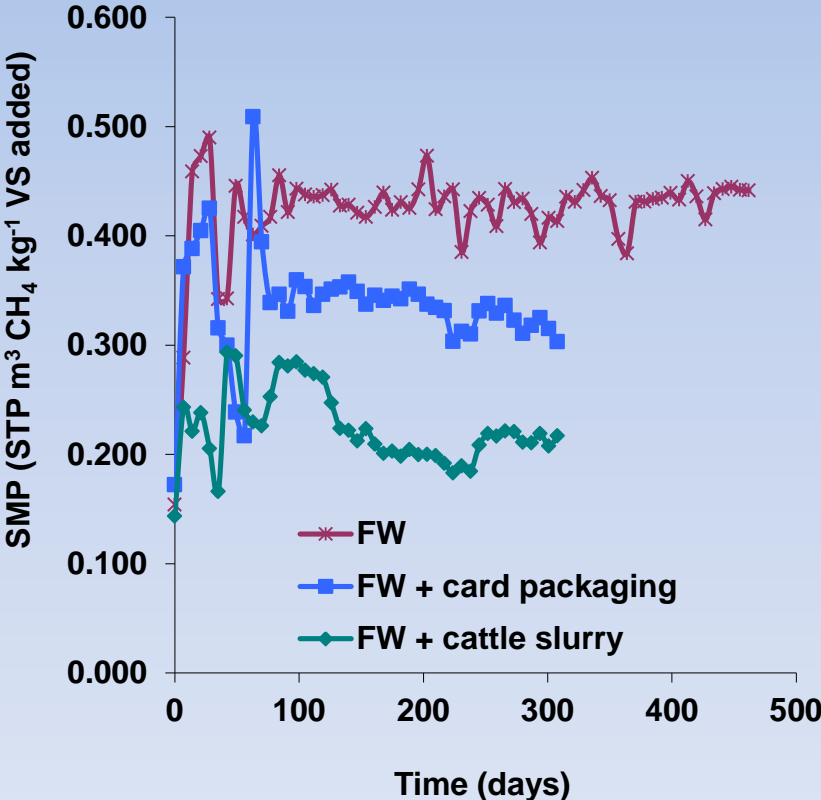
100-litre



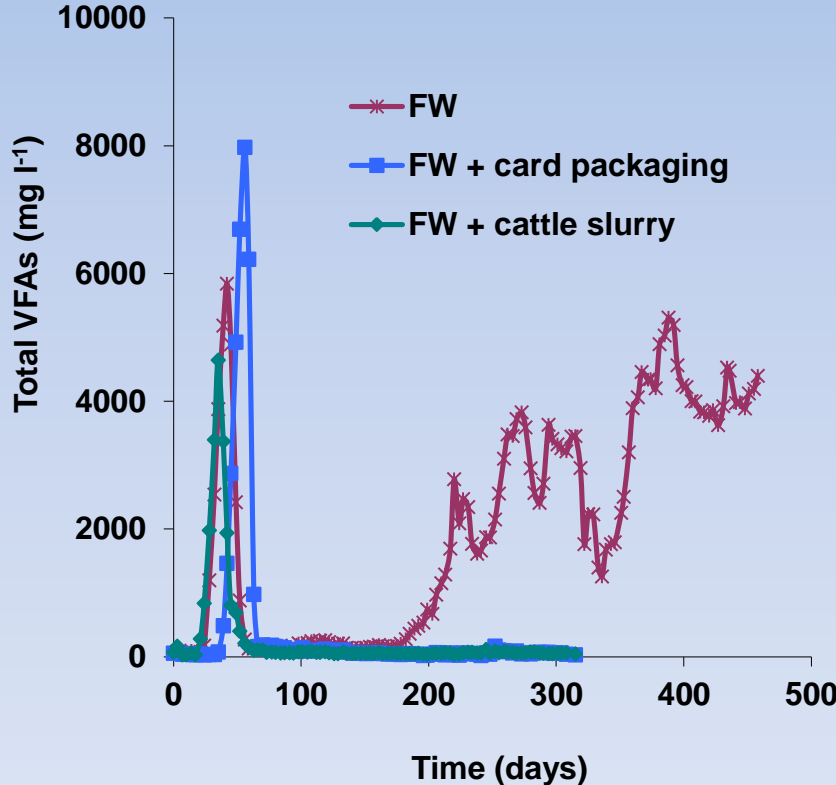
Mesophilic food waste digestion performance

Inoculum: sewage sludge digestate; **Temperature:** 36 ± 1 ° C; **Organic loading rate:** 2 kg VS m⁻³ d⁻¹

Specific Methane Production



Volatile Fatty Acids



Long chain fatty acids (LCFA) accumulation

X-ray diffraction analysis



Instability issue of food waste digestion

- **Negative response**

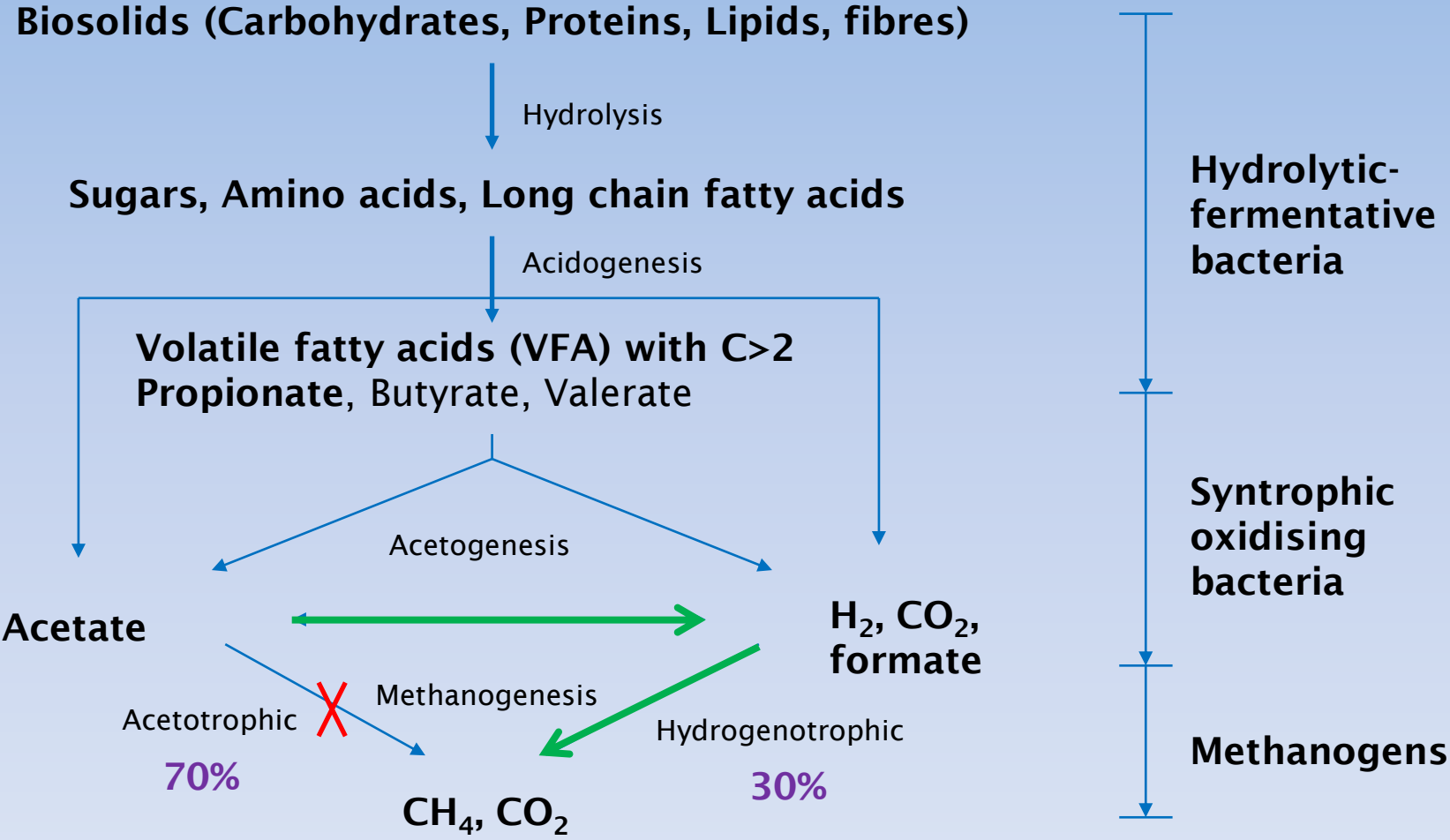
 - accumulation of long chain and volatile fatty acids

- **Loading limit**

 - less than 2 kg VS m⁻³ day⁻¹

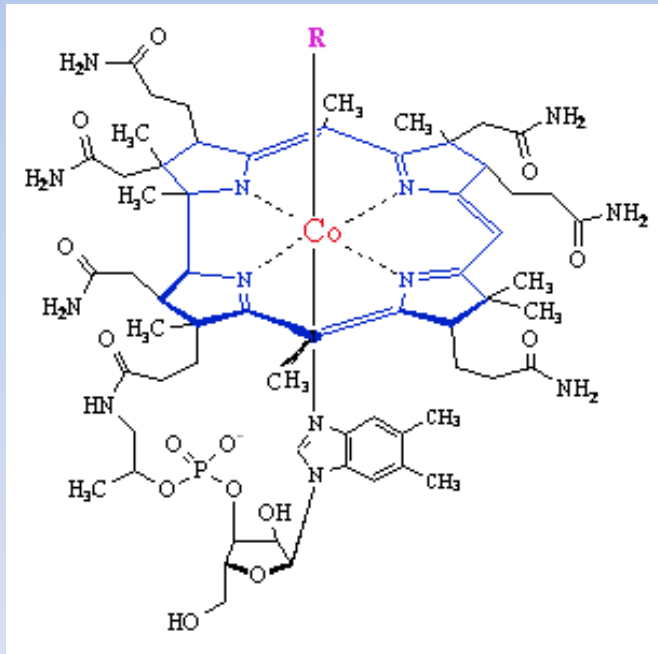
Sub-healthy

Anaerobic conversion of food waste to CH₄

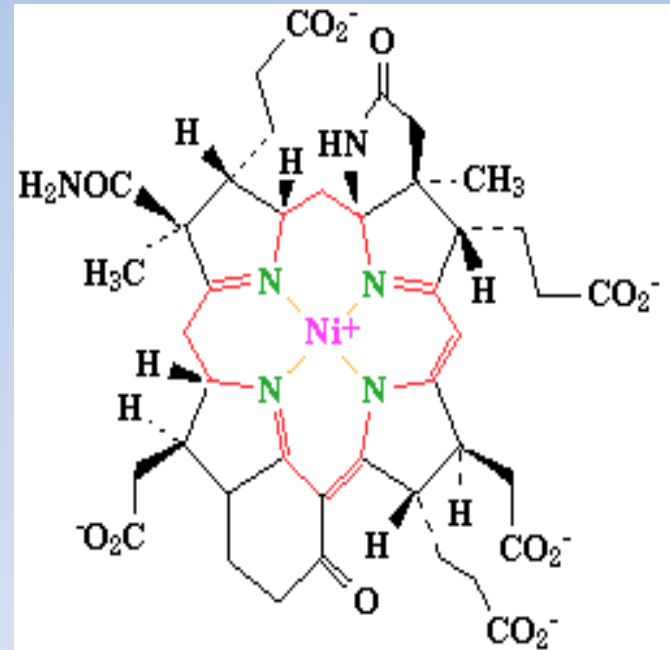


Function of trace elements in AD

- Active centre of enzymes and electron carrier molecules
- Trace elements: Co, Ni, Fe, Se, Mo, W, Zn, Cu, Mn, Al, B



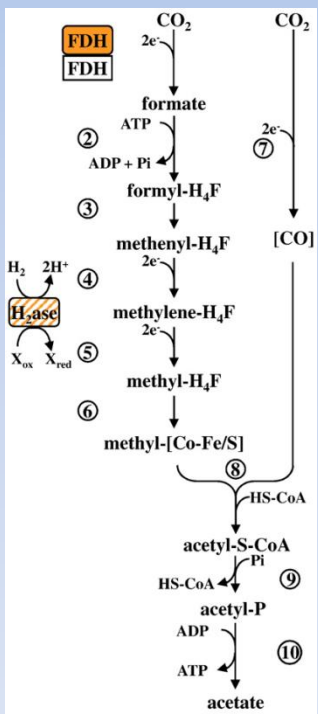
Corrinoid



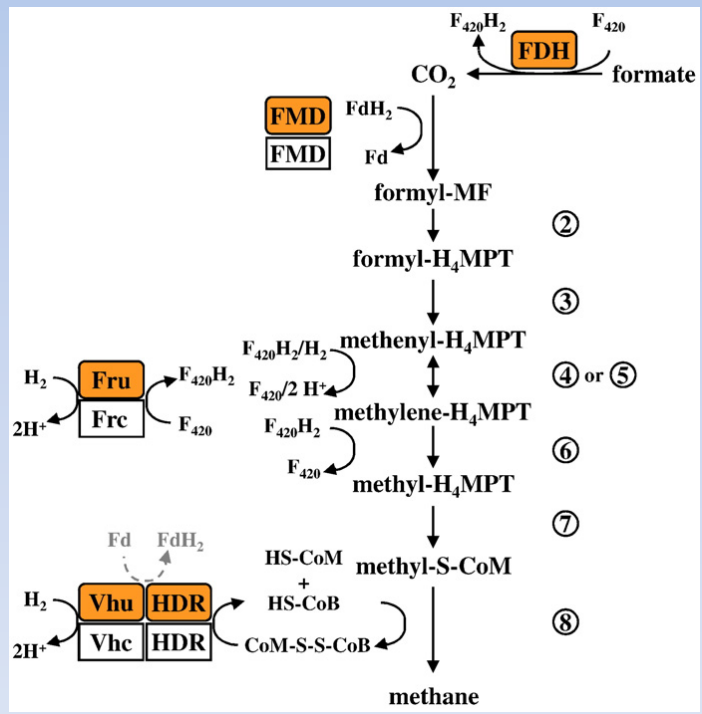
Cofactor F430

Function of trace elements in food waste AD

- Active centre of enzymes and electron carrier molecules
- Ammonia toxicity: 4000~7000 mg N l⁻¹
- Trace elements deficiency: Co, Ni, Fe, Se, Mo, W, Zn, Cu, Mn, Al, B



The reductive Wood-Ljungdahl pathway of acetogenesis



The path of CO2 reduction to methane in *Methanococcus* species

Reference: Stock T. and Rother M. (2009) Selenoproteins in Archaea and Gram-positive bacteria. *Biochimica et Biophysica Acta* 1790, 1520-1532.

Note: selenoproteins involved are round-boxed in orange, and the potential involvement of a Sec-containing hydrogenase is indicated by the dashed round box.

Stable performance of food waste digestion

- Trace element supplementation research

Aim - Optimising trace element supplementation strategy

- Distinguish essential trace elements for stable food waste digestion
- Identify optimal trace element supplementation strength

Research approaches

- Batch flask trials for screening purpose
- Semi-continuous digester operation to monitor the long-term effect

Key monitoring parameters

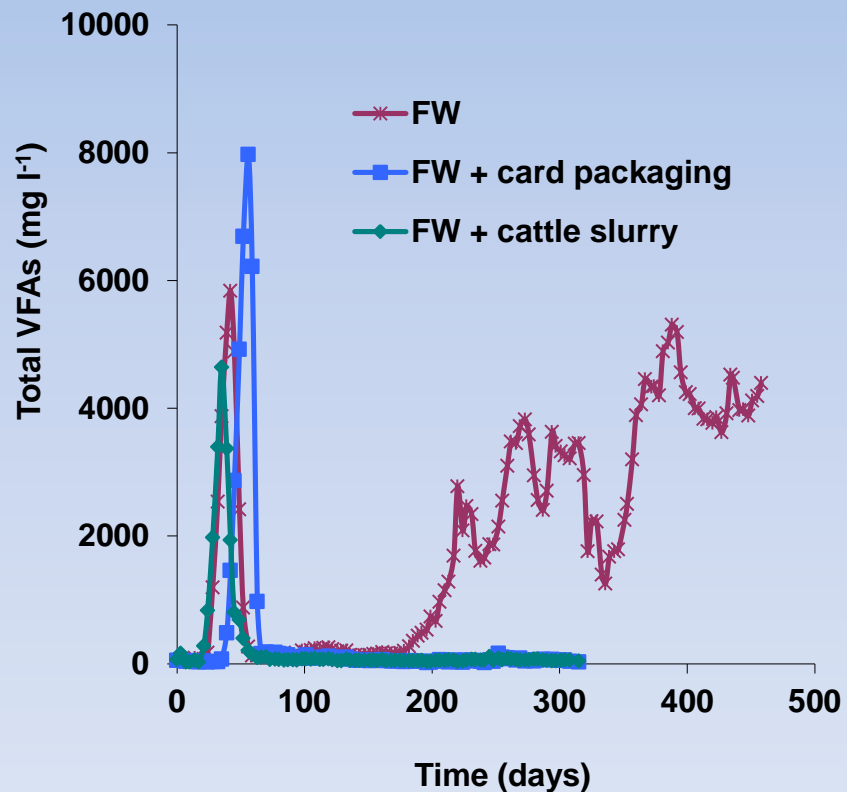
- Trace elements (TE) concentrations
- Volatile fatty acids (VFA) concentrations

Batch screening tests

Food waste digestate used



Volatile Fatty Acids



Food waste digestate parameters

| | |
|---|------|
| pH | 8.0 |
| Total ammonium nitrogen (mg-N l ⁻¹) | 4700 |
| Total volatile fatty acid (mg l ⁻¹) | 4400 |
| Acetic acid (mg l ⁻¹) | 4100 |
| Propionic acid (mg l ⁻¹) | 100 |

| Substrate pike | Concentration (mg l ⁻¹) |
|-------------------|-------------------------------------|
| Sodium acetate | 4500 as acetic acid |
| Sodium propionate | 8000 as propionic acid |
| Glucose | 4000 |
| Starch | 4000 |

Fractional factorial design

| Run | Pattern | Co | Ni | Mo | Se | Fe | W | Zn | Cu | Mn | Al | B |
|-----|-------------|----|----|----|----|----|---|----|----|----|----|---|
| 1 | ----- | - | - | - | - | - | - | - | - | - | - | - |
| 2 | ---+++----- | - | - | - | Se | Fe | W | - | - | - | - | - |
| 3 | --++++----- | - | - | Mo | - | Fe | W | - | - | - | - | - |
| 4 | ---++----- | - | - | Mo | Se | - | - | - | - | - | - | - |
| 5 | +---+----- | - | Ni | - | - | Fe | - | - | - | - | - | - |
| 6 | +++++----- | - | Ni | - | Se | - | W | - | - | - | - | - |
| 7 | ---++----- | - | Ni | Mo | - | - | W | - | - | - | - | - |
| 8 | +++++----- | - | Ni | Mo | Se | Fe | - | - | - | - | - | - |
| 9 | +---+----- | Co | - | - | - | - | W | - | - | - | - | - |
| 10 | +++++----- | Co | - | - | Se | Fe | - | - | - | - | - | - |
| 11 | +++++----- | Co | - | Mo | - | Fe | - | - | - | - | - | - |
| 12 | +++++----- | Co | - | Mo | Se | - | W | - | - | - | - | - |
| 13 | +++++----- | Co | Ni | - | - | Fe | W | - | - | - | - | - |
| 14 | +++++----- | Co | Ni | - | Se | - | - | - | - | - | - | - |
| 15 | +++++----- | Co | Ni | Mo | - | - | - | - | - | - | - | - |
| 16 | +++++----- | Co | Ni | Mo | Se | Fe | W | - | - | - | - | - |
| 17 | +++++----- | Co | Ni | Mo | Se | Fe | W | Zn | - | - | - | - |
| 18 | +++++----- | Co | Ni | Mo | Se | Fe | W | Zn | Cu | Mn | - | - |
| 19 | +++++----- | Co | Ni | Mo | Se | Fe | W | Zn | Cu | Mn | Al | B |

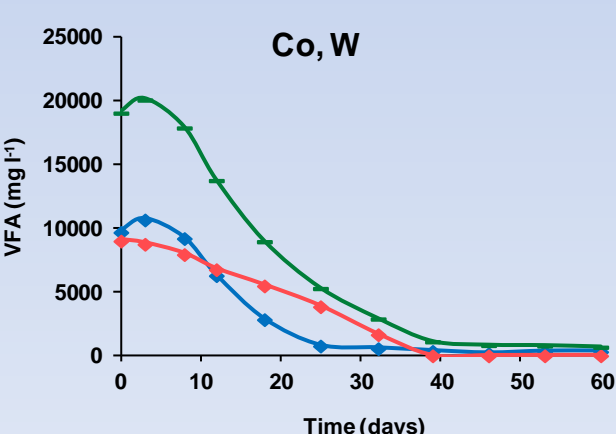
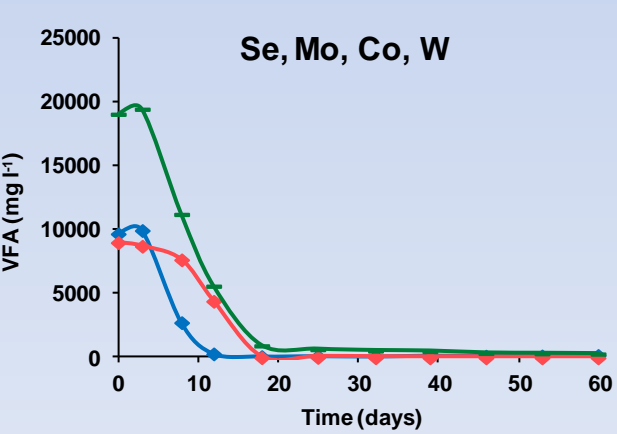
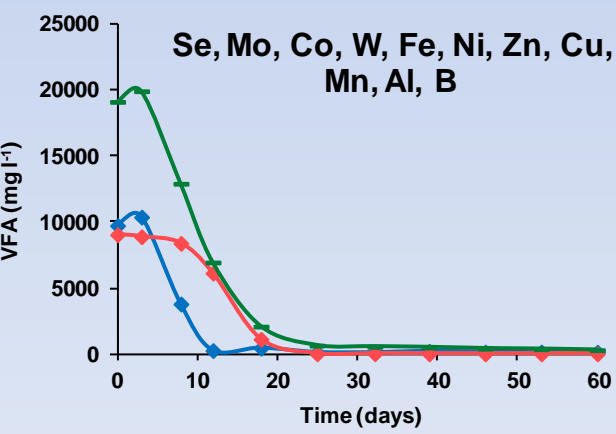
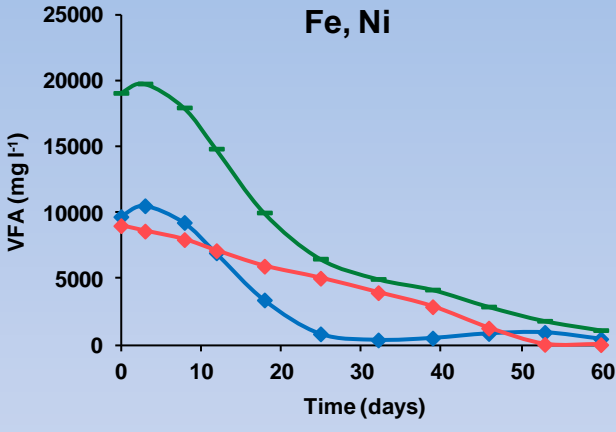
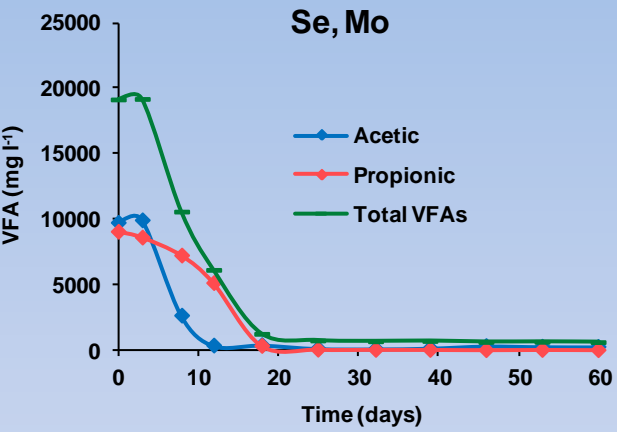
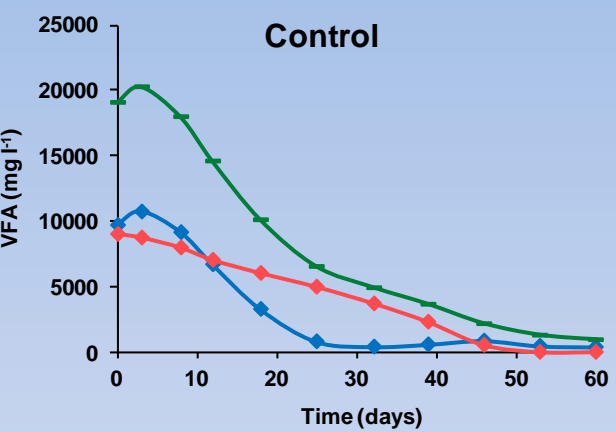
Trace element concentrations spiked

| Essential element | Compound used | Trace element concentration (mg l ⁻¹) | |
|-------------------|--|---|--------------------------------|
| | | Supplemented at the beginning of the tests | Existing in the test digestate |
| Cobalt (Co) | CoCl ₂ ·6H ₂ O | 1.0 | 0.083 |
| Nickel (Ni) | NiCl ₂ ·6H ₂ O | 1.0 | 2.9 |
| Molybdenum (Mo) | (NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O | 0.2 | 0.29 |
| Selenium (Se) | Na ₂ SeO ₃ | 0.2 | 0.050 |
| Tungsten (W) | Na ₂ WO ₄ ·2H ₂ O | 0.2 | <0.035 |
| Iron (Fe) | FeCl ₂ ·4H ₂ O | 5.0 | 173.7 |
| Zinc (Zn) | ZnCl ₂ | 0.2 | 8.11 |
| Copper (Cu) | CuCl ₂ ·2H ₂ O | 0.1 | 5.75 |
| Manganese (Mn) | MnCl ₂ ·4H ₂ O | 1.0 | 18.5 |
| Aluminium (Al) | AlCl ₃ ·6H ₂ O | 0.1 | 63.3 |
| Boron (B) | H ₃ BO ₃ | 0.1 | 2.5 |

Experimental set up



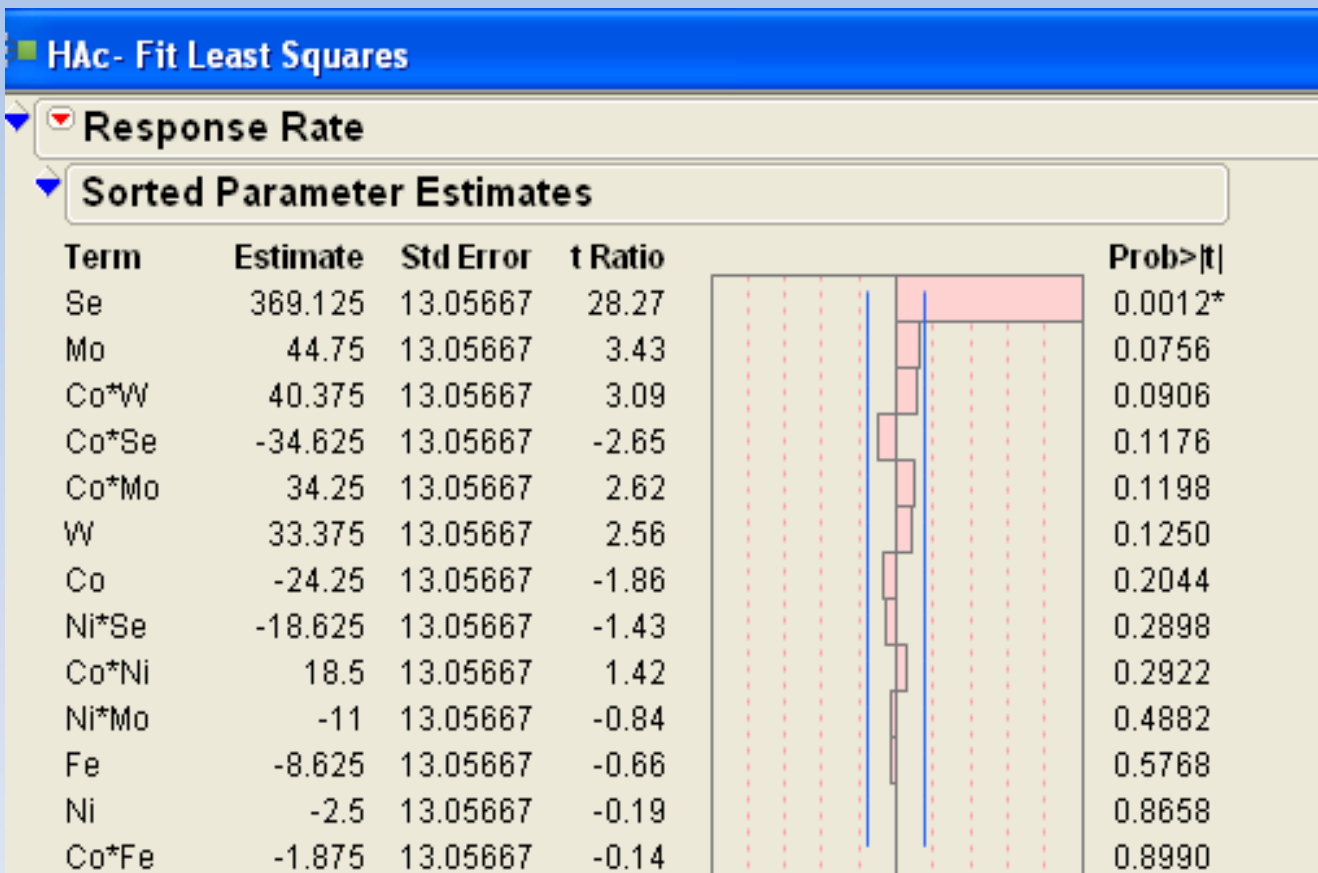
Volatile fatty acids degradation profiles



Statistical analysis

- TE on acetic acid degradation

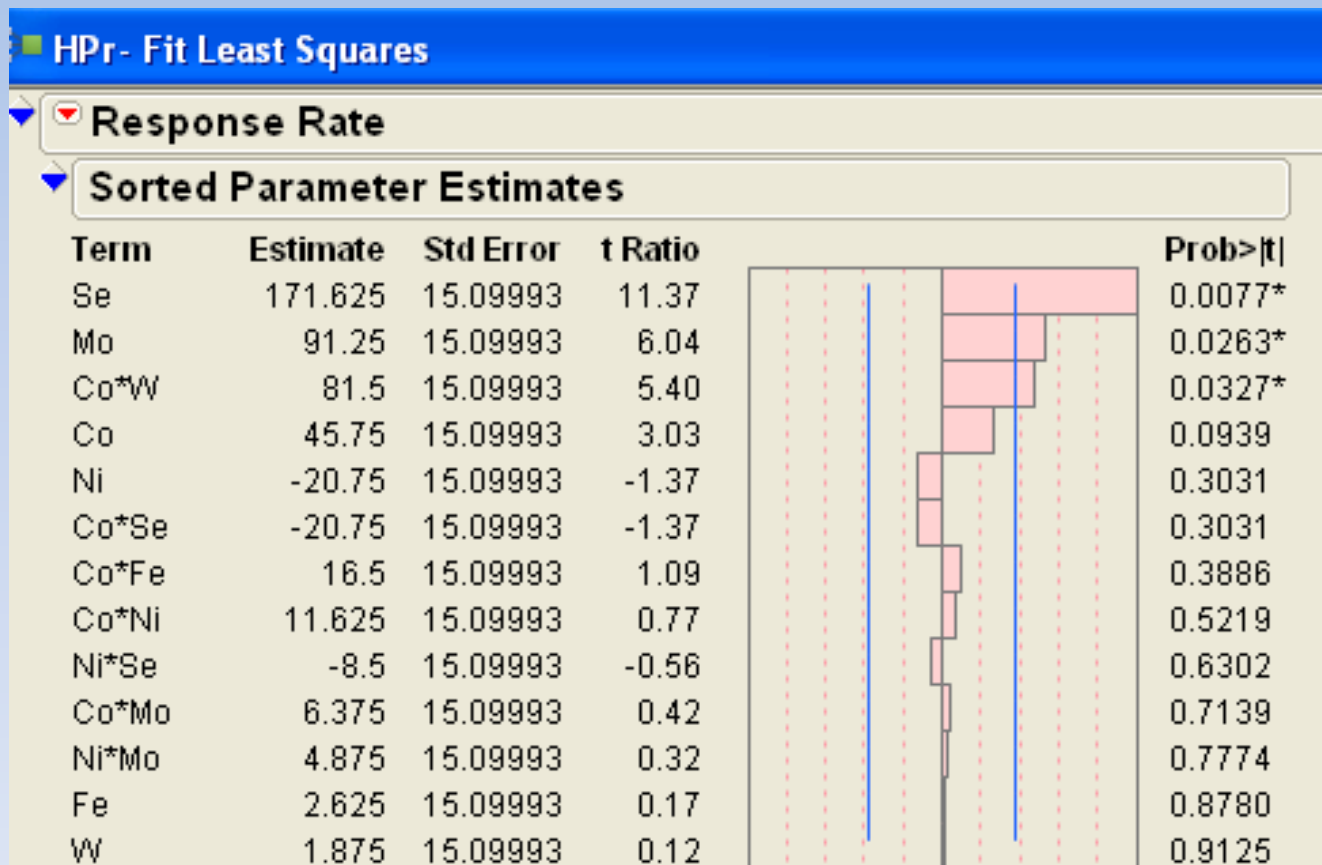
Effect of trace elements on acetic acid degradation



Statistical analysis








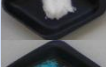



- TE on propionic acid degradation

Effect of trace elements on propionic acid degradation



Batch experiments - result

Essential trace element for acetic and propionic acid degradation in food waste digestate

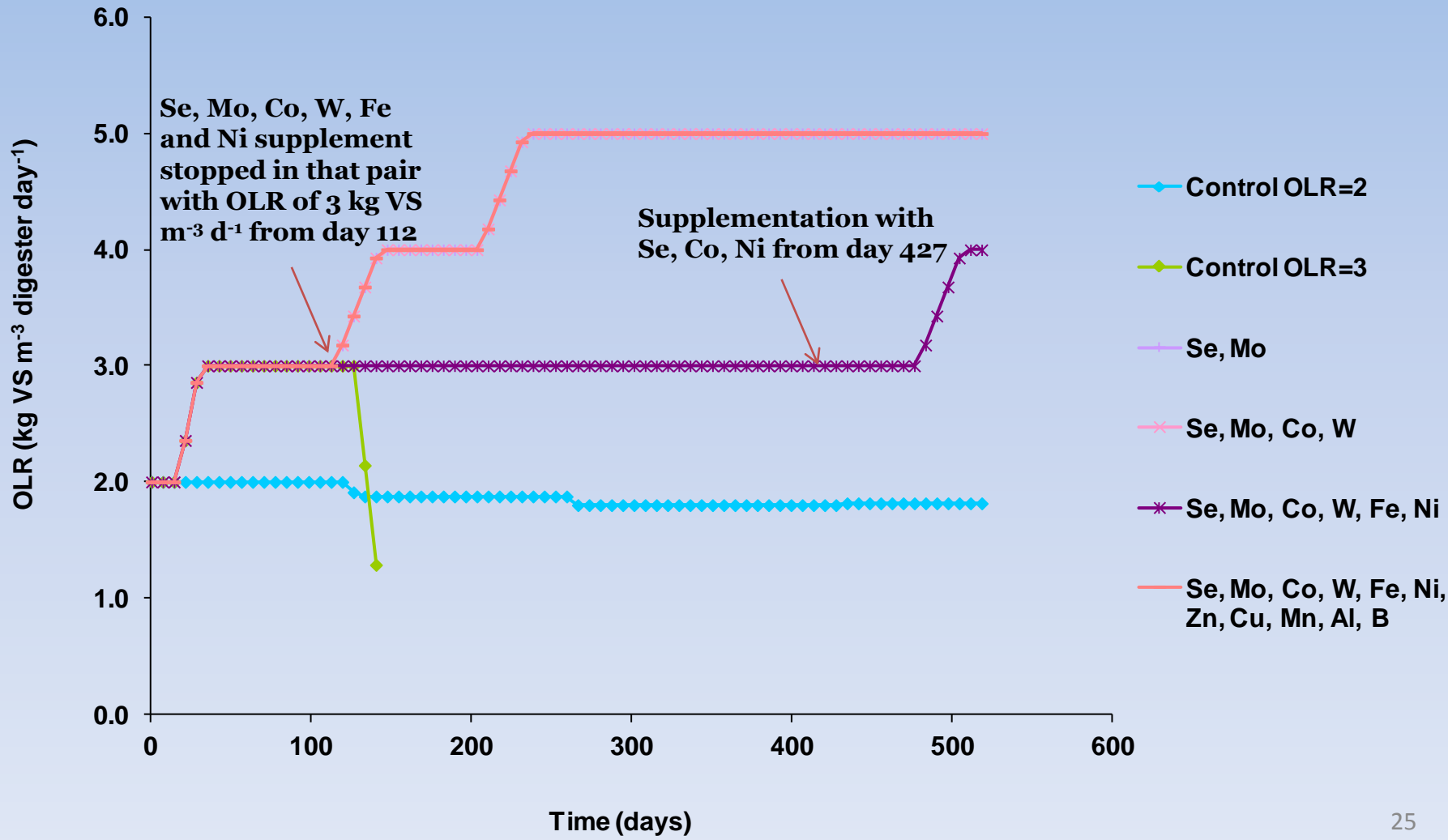
| Tier | Trace element | Compound | Dosing strength (g tonne ⁻¹) |
|-----------------|-----------------|--|--|
| 1 st | Selenium (Se) |  Na_2SeO_3 | 0.2 |
| | Molybdenum (Mo) |  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ | 0.2 |
| 2 nd | Cobalt (Co) |  $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ | 1.0 |
| | Tungsten (W) |  $\text{Na}_2\text{WO}_4\cdot 2\text{H}_2\text{O}$ | 0.2 |
| 3 rd | Iron (Fe) |  $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$ | 5.0 |
| | Nickel (Ni) |  $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ | 1.0 |
| 4 th | Zinc (Zn) |  ZnCl_2 | 0.2 |
| | Copper (Cu) |  $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$ | 0.1 |
| | Manganese (Mn) |  $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ | 1.0 |
| | Aluminium (Al) |  $\text{AlCl}_3\cdot 6\text{H}_2\text{O}$ | 0.1 |
| | Boron (B) |  H_3BO_3 | 0.1 |

Semi-continuous trials

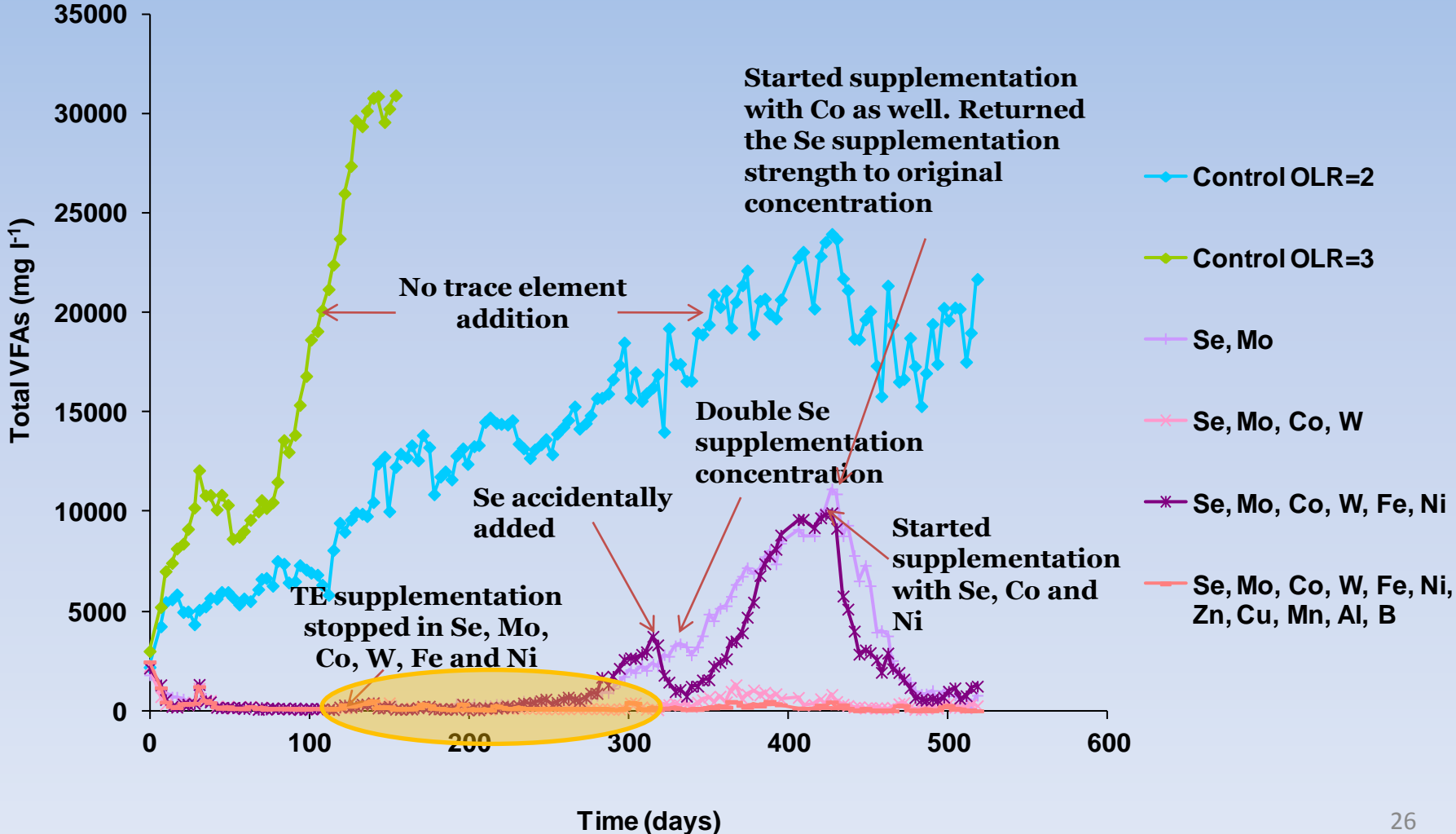
Digesters



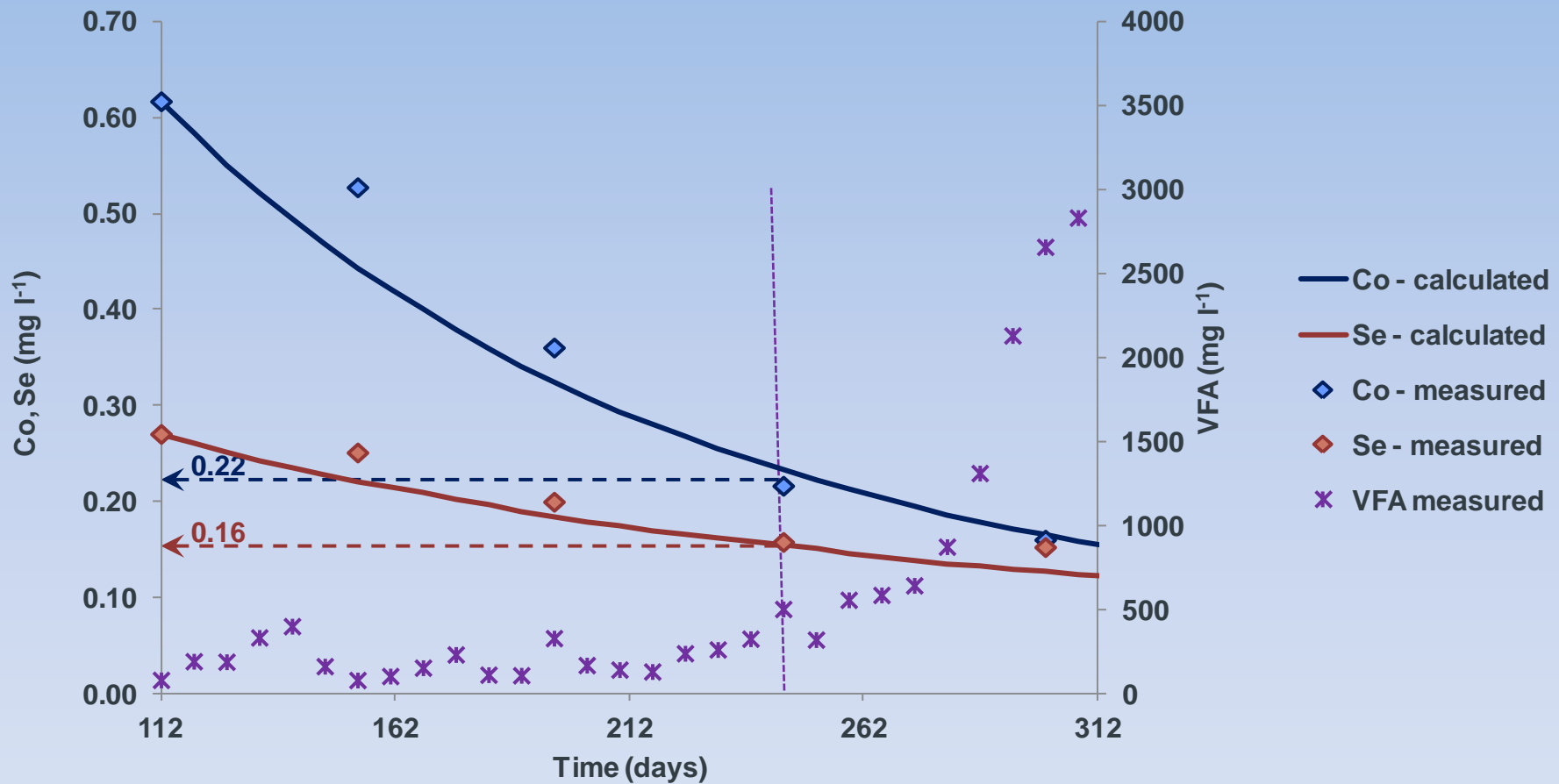
Organic loading rate (OLR)



Volatile fatty acids (VFA) profile



Co and Se dilute-out curves vs VFA profile



Se: $0.16 \text{ mg l}^{-1} = 0.16 \text{ g m}^{-3} = 10^{21} \text{ Se m}^{-3}$

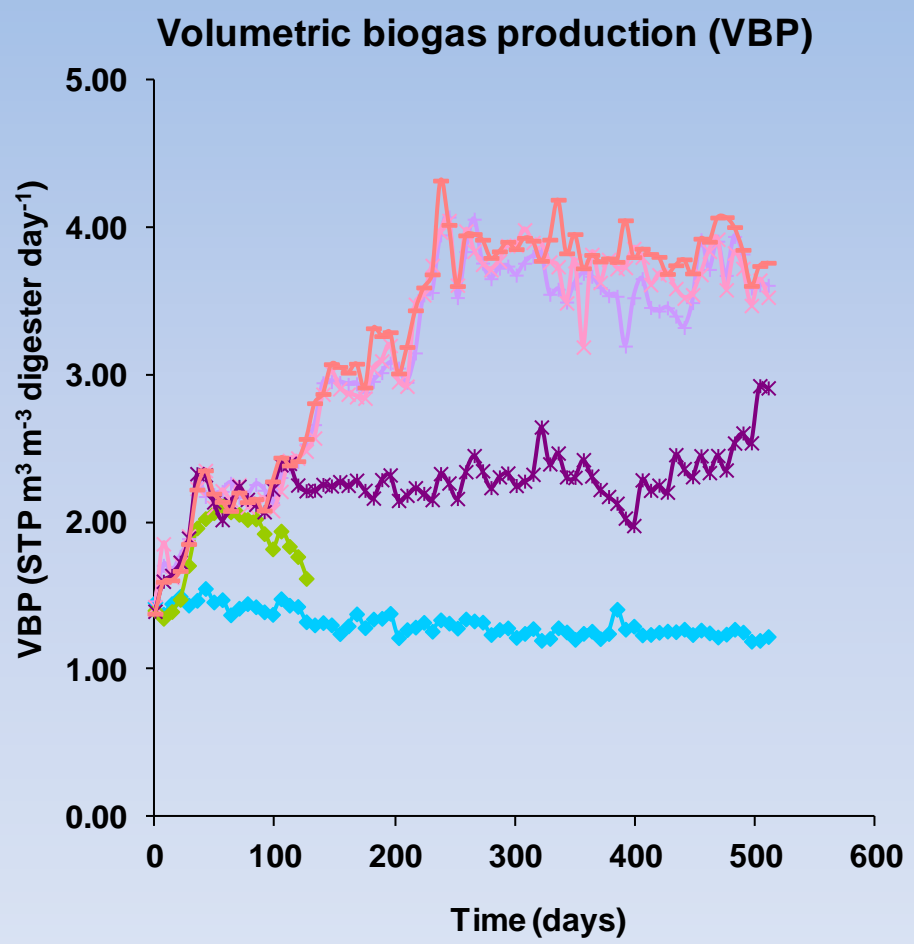
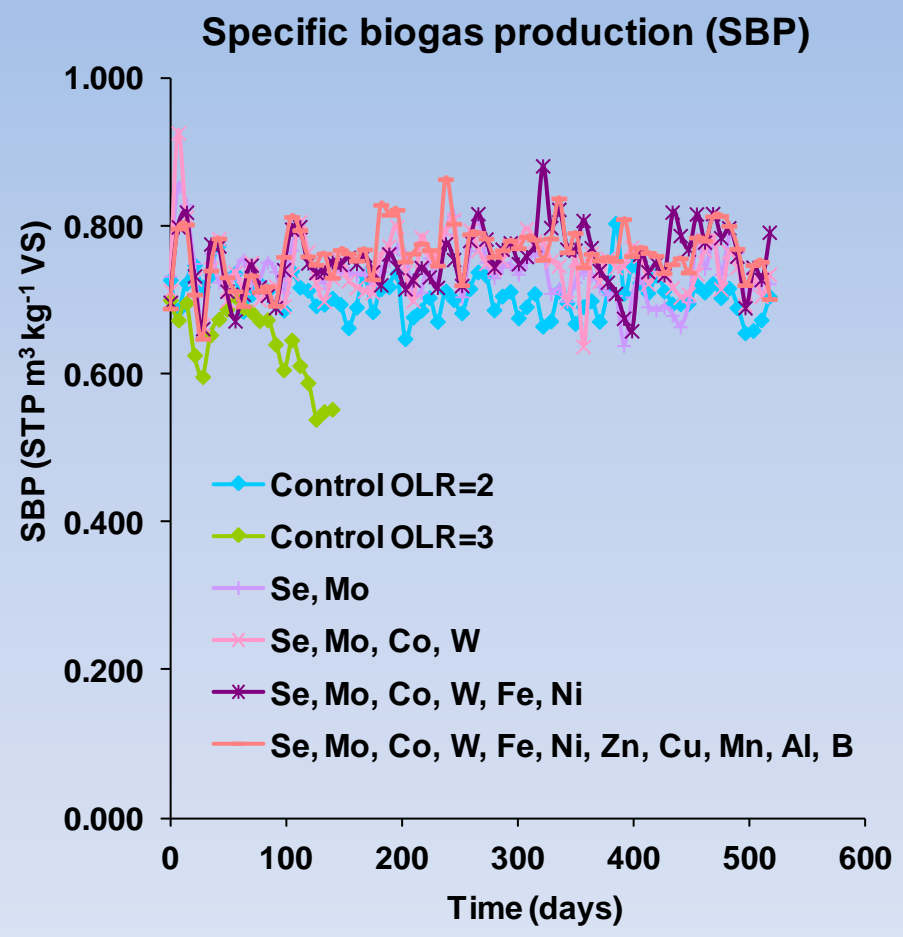
Microorganisms: 10^{16} m^{-3}

TE required vs TE in the UK food waste

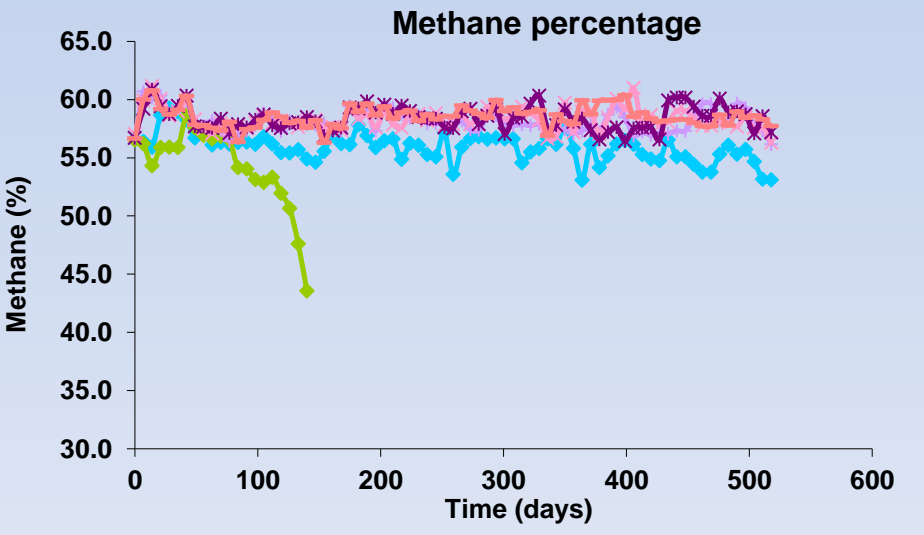
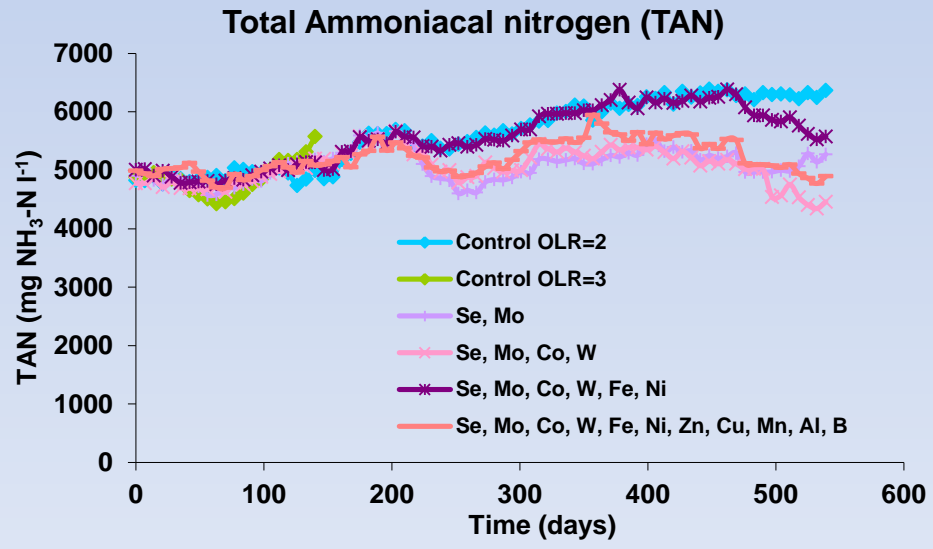
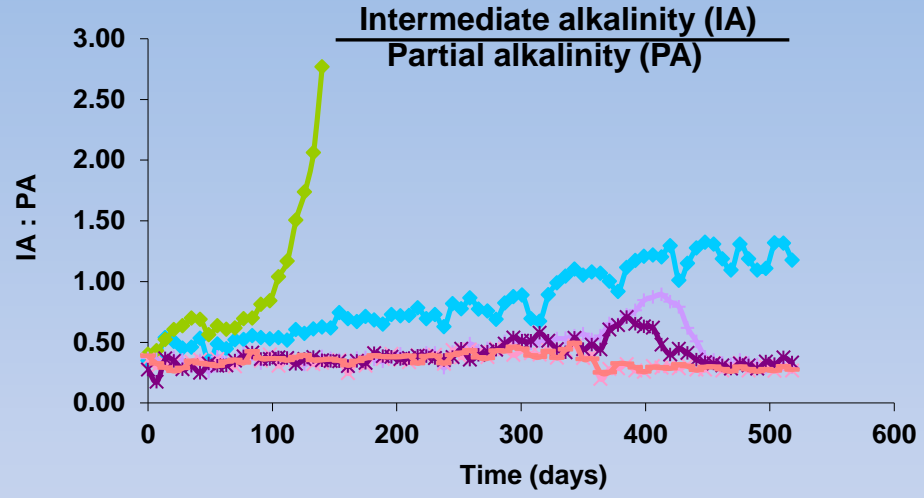
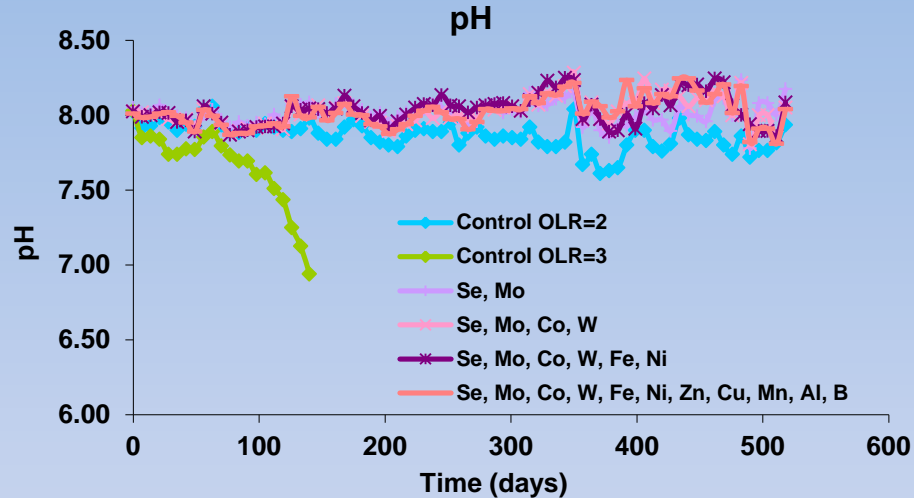
| | Minimum requirement at a moderate loading rate | Hackney, London | Eastleigh, Hampshire | Luton, South Bedfordshire | Ludlow, Shropshire |
|-------------------------------|--|--------------------|----------------------|---------------------------|--------------------|
| Cobalt (Co) | 0.22 | 0.09 ± 0.05 | 0.02 ± 0.01 | 0.02 ± 0.00 | < 0.06 |
| Selenium (Se) | 0.16 | 0.10 ± 0.08 | 0.03 ± 0.00 | 0.28 ± 0.14 | < 0.07 |
| Total Kjeldahl Nitrogen (TKN) | | 8100 | 7500 | 7400 | 8100 |

Unit: mg kg⁻¹ fresh matter

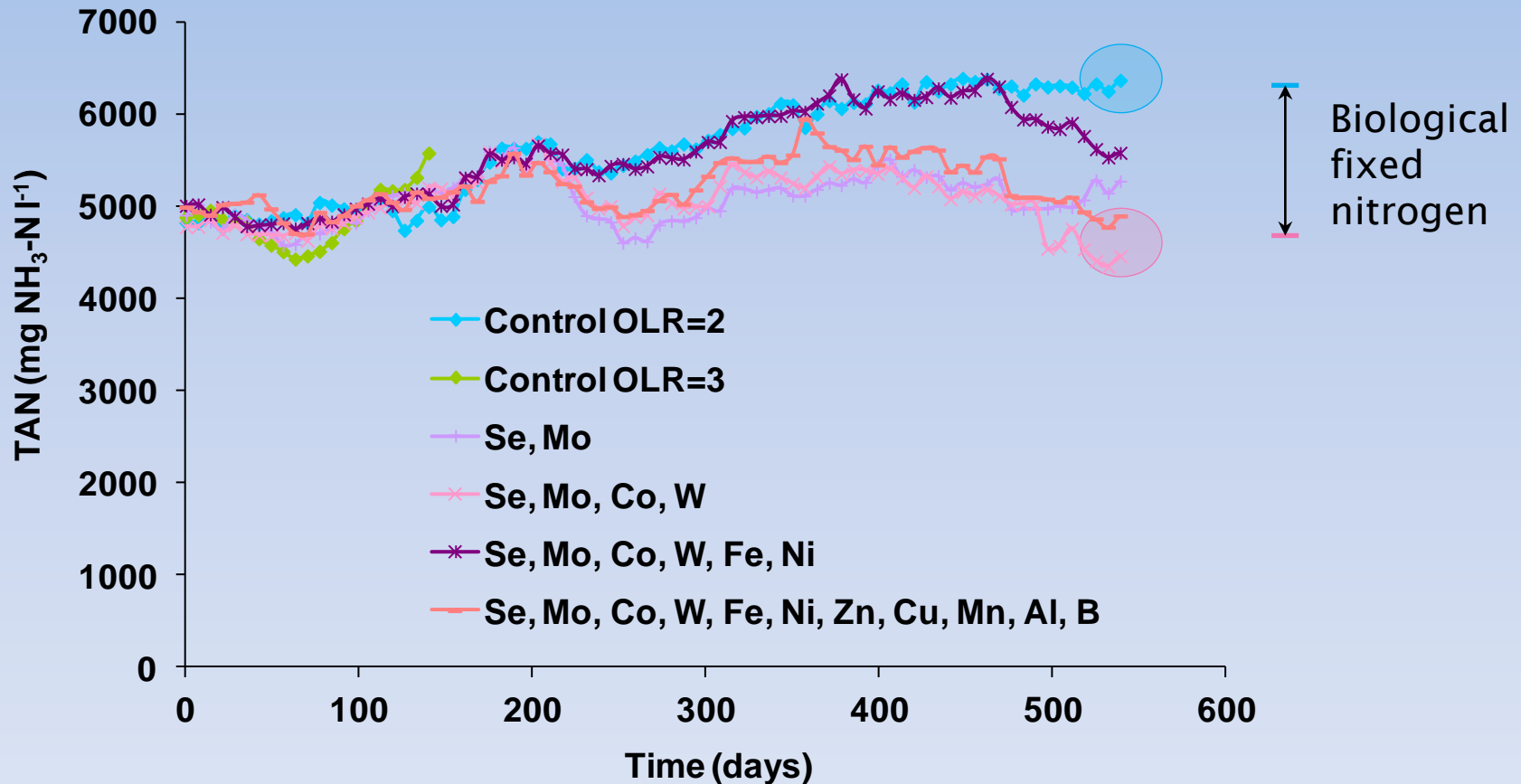
Digestion efficiency



Other digester parameters



Total ammoniacal nitrogen (TAN)

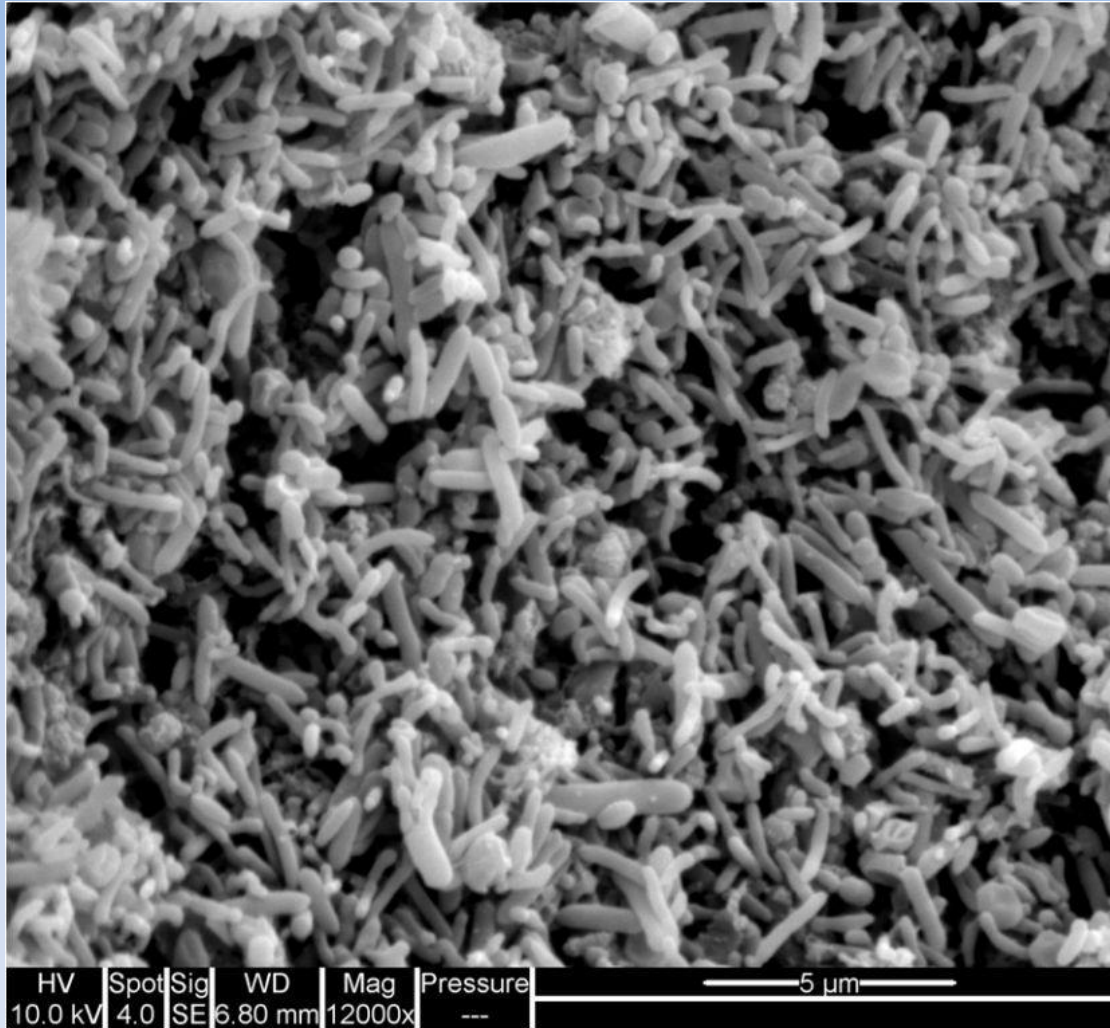


FISH analysis on methanogenic community structure

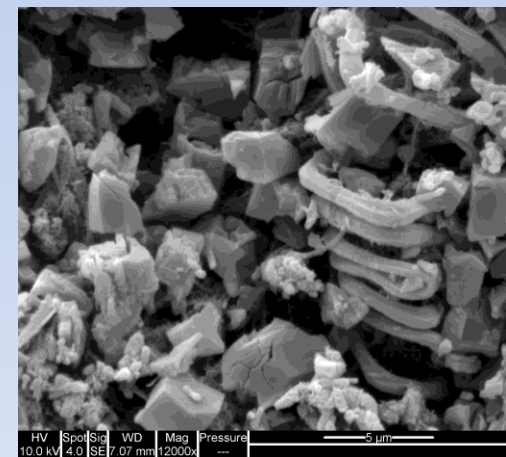
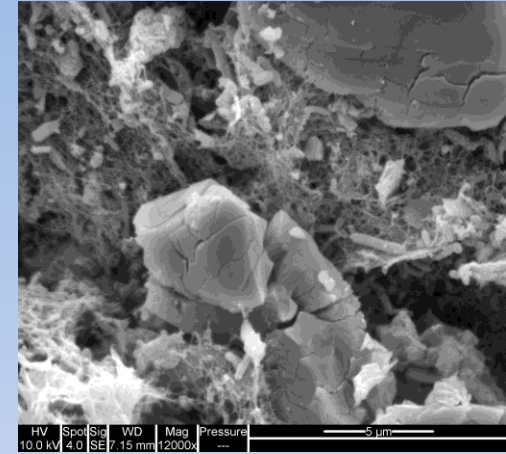
Classification of Methanogens

| Methanogen | Carbon source | |
|---------------------------|----------------------------|------------------|
| <i>Methanobacteriales</i> | CO ₂ / formate | Hydrogenotrophic |
| <i>Methanococcales</i> | CO ₂ / formate | |
| <i>Methanomicrobiales</i> | CO ₂ / formate | |
| <i>Methanosarcinales</i> | | Acetotrophic |
| <i>Methanosarcinaceae</i> | CO ₂ Acetate | |
| <i>Methanosarcinales</i> | | |
| <i>Methanosaetaceae</i> | Acetate | |

SEM images after density gradient centrifugation

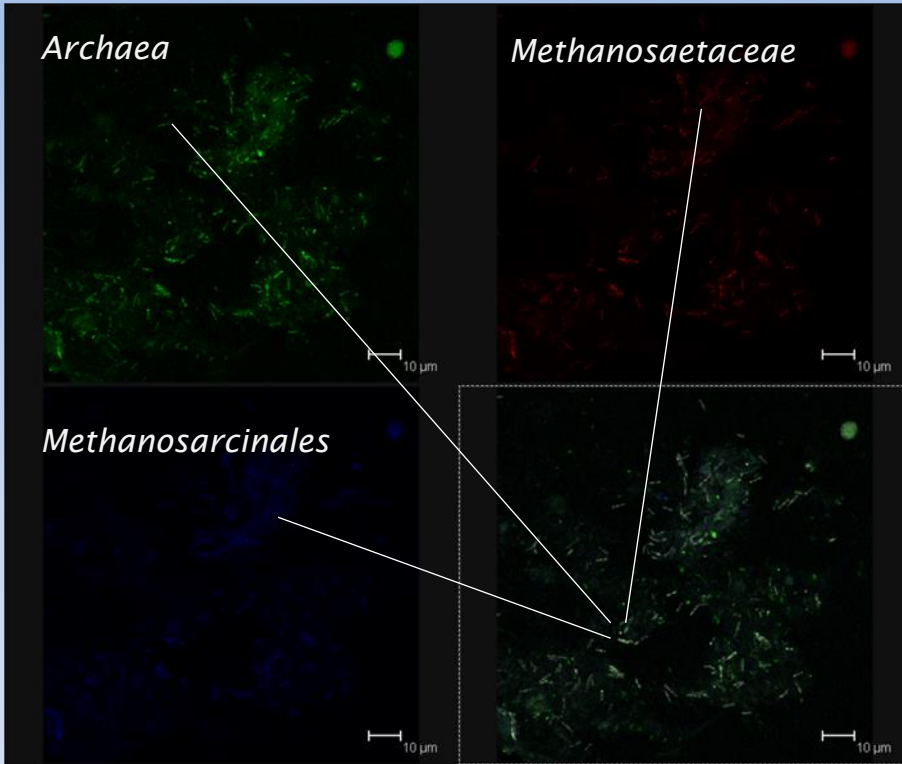


Separated microbial biomass



Food waste residues

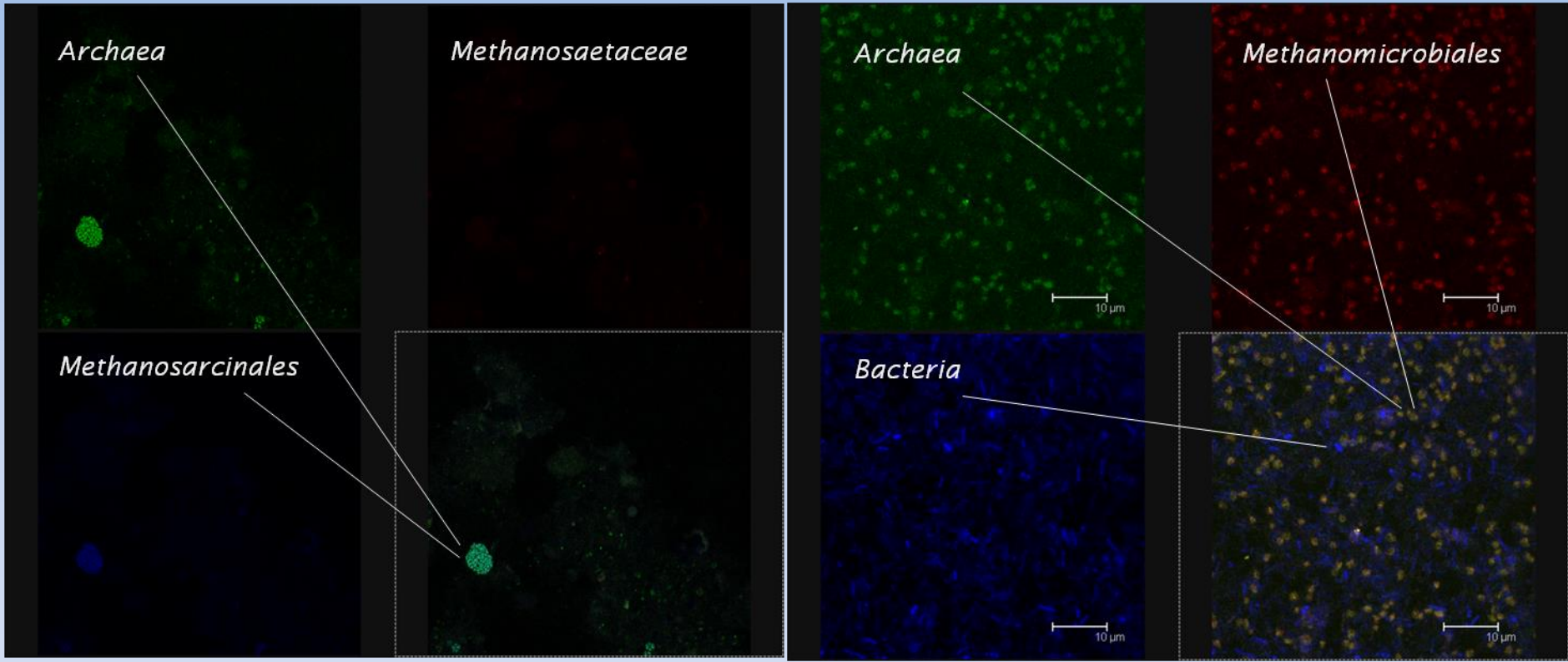
Fluorescence in-situ hybridisation (FISH)



Inoculum – a wide range of methanogens

| Probe name | Target group | Fluoro-chrome | Formamide (%) |
|------------|-----------------------------|---------------|---------------|
| EUB338 | <i>Bacteria (most)</i> | Cy5 | 20~50 |
| EUB338+ | <i>Bacteria (remaining)</i> | Cy5 | 20~50 |
| ARC915 | <i>Archaea</i> | 6-Fam | 20~50 |
| MX825 | <i>Methanosaetaceae</i> | Cy3 | 50 |
| MS1414 | <i>Methanosarcinaceae</i> | Cy3 | 50 |
| hMS1395 | MS1414-helper | - | 50 |
| hMS1480 | MS1414-helper | - | 50 |
| MSMX860 | <i>Methanosarcinales</i> | Cy5 | 45 |
| MG1200 | <i>Methanomicrobiales</i> | Cy3 | 20 |
| MB1174 | <i>Methanobacteriales</i> | Cy3 | 45 |
| MC1109 | <i>Methanococcales</i> | Cy3 | 45 |

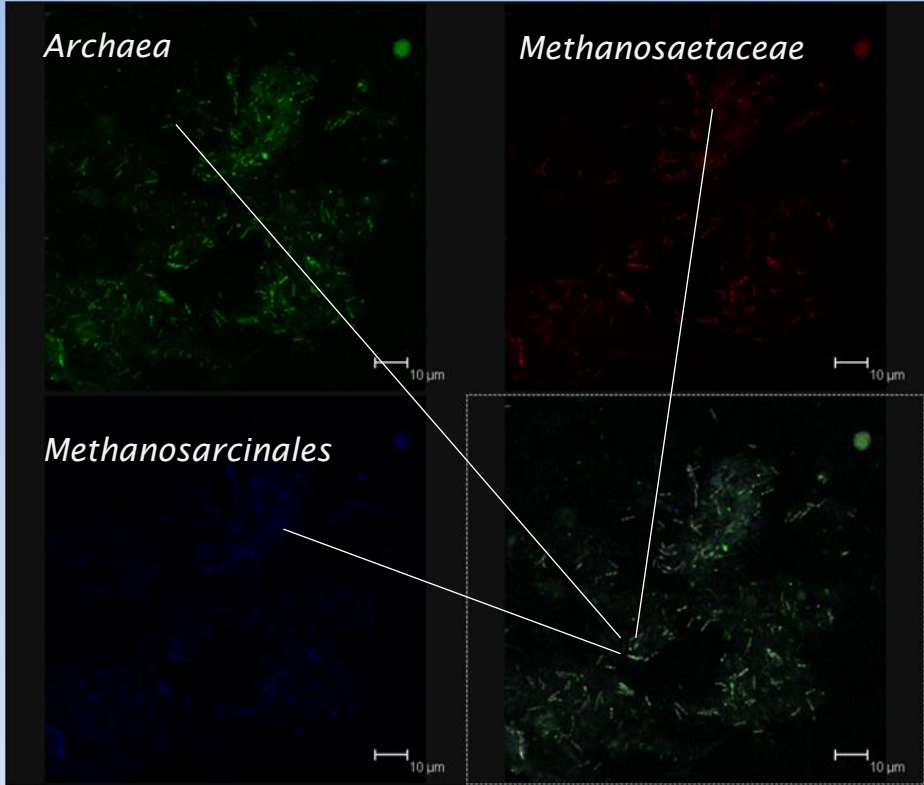
Fluorescence in-situ hybridisation (FISH)



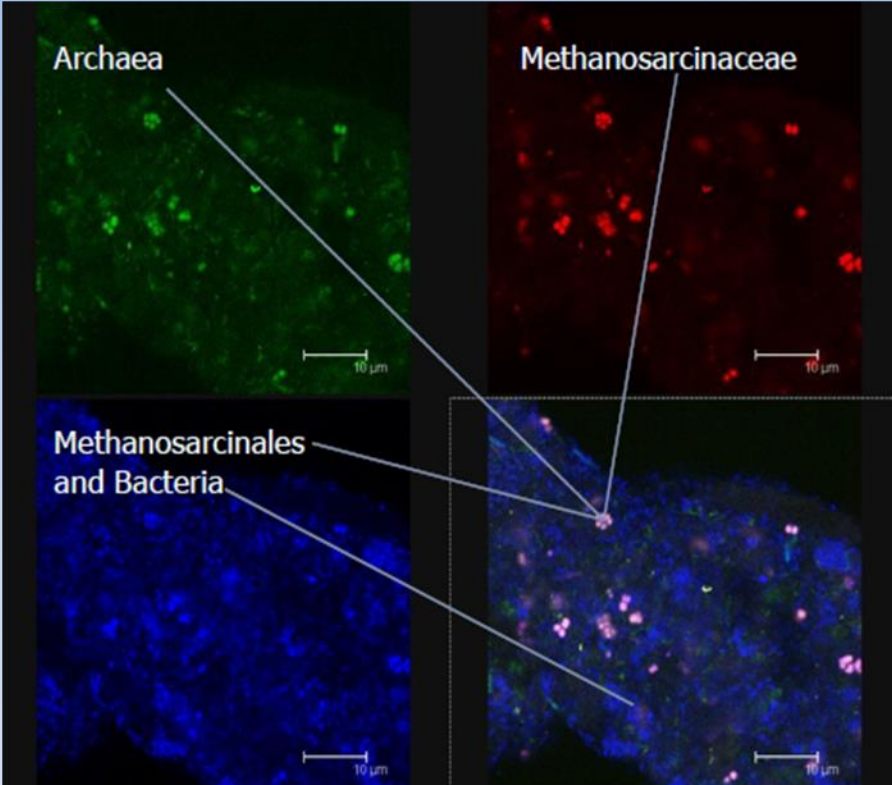
After 3 months

After 1.5 years

FISH images on another digestate sample



Inoculum



Vegetable waste digestate

Conclusions

Conclusions – trace elements

- Selenium and cobalt are the key trace elements needed for the long-term stability of food waste digesters, but are likely to be lacking in the food waste
- The minimum concentrations recommended in food waste digesters for selenium, cobalt are around 0.16, 0.22 mg l⁻¹ respectively, when running at a moderate organic loading rate
- A total selenium concentration greater than 1.5 mg l⁻¹ is likely to be toxic to the microbial consortium in the digester
- Food waste is likely to have sufficient Al, B, Cu, Fe, Mn, and Zn. We are still not sure about Ni, Mo and W

Conclusions – digester operation

- Following proper trace element supplementation strategy, food waste digesters can be operated stably with low VFA concentrations at an organic loading rate of $7 \text{ kg VS m}^{-3} \text{ d}^{-1}$ with a volumetric methane production of $> 3.0 \text{ STP m}^3 \text{ m}^{-3} \text{ d}^{-1}$ and specific methane production of $\sim 0.45 \text{ STP m}^3 \text{ kg}^{-1} \text{ VS}$
- Prevention of VFA accumulation in the digester by trace element supplementation is necessary, as recovery of a severely VFA-laden digester is not a rapid process even when supplements are added

Further consideration

- The concept of the use of essential trace elements has been accepted by AD operators
- The species and quantity of trace elements supplemented are often determined by experiments or trial-and-error approach
- Lack of insight knowledge on the impact of trace elements in AD under different process conditions
- The fate of trace elements (heavy metals, selenium) once they leave the digesters
- COST Action: European network on ecological functions of trace metals in anaerobic biotechnologies

Anaerobic digestion challenges

Expectation on anaerobic digestion

- Robust
- Efficient
- Flexible

Future research needs

- Have insight into the microbial activities
- Better control and diagnose the digestion system
- Improve the conversion efficiency of organic materials